Effect of Enzyme Supplementation on the Nutritional Value of Raw, Autoclaved, and Dehulled Lupins (*Lupinus albus*) in Chicken Diets

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ABSTRACT The effects of adding crude enzyme preparations to diets containing raw, autoclaved, and dehulled lupin seeds on the performance of broiler and Leghorn chicks (7 to 21 days) were evaluated in five experiments. In the first experiment, enzyme addition (combination of Energex®-carbohydrase, Bio-Feed Pro®-protease, and Novozyme®-α-galactosidase) to a diet containing 70% raw lupins improved the weight gain and feed to gain ratio of broiler chicks by 18 and 10%, respectively. The relative intestinal length and gizzard weight also were reduced by enzyme treatment. In the second experiment, the optimum concentration of enzymes was determined in diets containing 50% raw lupins. Bio-Feed Pro® at 3% increased weight gains by 24% and the feed to gain ratio by 11%, whereas a combination of three enzymes at .10% of each yielded respective improvements of 18 and 9%. In Experiment 3, the AME and protein digestibility of dehulled lupin seeds were increased by 18 and 7% compared with those for raw seeds. Autoclaving (20 min) significantly ($P < .05$) improved chick performance and AME and protein digestibility of raw seeds. In the fourth experiment, autoclaving (15 min), dehulling, and a combination of both improved weight gains by 11, 15, and 8% and feed to gain ratios by 4, 11, and 6%, respectively. Enzyme addition improved the performance of birds fed raw, but not autoclaved lupin diets. In the fifth experiment, dehulling and enzyme treatment but not autoclaving (20 min) improved the performance of birds fed diets containing 50% lupins. The relative weight of the gizzard was reduced by both treatments but that of the pancreas was affected only by enzyme treatment. Overall, enzyme supplementation of raw lupin diets considerably improved chick performance. Dehulling of lupins also improved chick performance with results for autoclaved lupins being inconsistent.
(*Key words*: lupins, autoclaving, dehulling, enzymes, chicken)

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INTRODUCTION

Lupins (*Lupinus albus*) have been available in many parts of the world as a feeding source for animals, but their use has been limited due to the presence of toxic and bitter alkaloids (Cheeke and Kelly, 1989). Recently, genetic selection by plant breeders has resulted in the release of a number of low alkaloid varieties that are attracting interest as substitutes for soybean or meat meals in the animals' diets. Limited amounts of data are available concerning the feeding value of the new low alkaloid varieties of sweet *L. albus* seeds. However, the nutritional value of low alkaloid (<.01%) lupins has been evaluated for pigs (Aguilera *et al.*, 1985; Batterham *et al.*, 1986a,b) and poultry (Yule and McBride, 1976; Halvorson *et al.*,

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1983; Perez-Escamilla *et al.*, 1988; Watkins *et al.*, 1988). Chickens and pigs can tolerate up to 25 and 30% low-alkaloid lupin seeds without adversely affecting growth provided that adequate amounts of lysine and methionine are supplied.

Low alkaloid lupins also appear to be free of other major antinutritional factors such as trypsin inhibitors and hemaglutinins (Hill, 1977; Schoeneberger *et al.*, 1983) and can be fed without heat processing. Heat treatment of *L. albus* lupin seed, however, has been shown to produce variable effects on hen performance (Watkins and Mirosh, 1987) and nutrient utilization (Molina *et al.*, 1983; Boldaji *et al.*, 1986). The lupin seed, however, has a high level of α-galactosides (Mercier, 1979; Brenes *et al.*, 1989; Saini, 1989), which are not hydrolyzed in the duodenum due to the inability of the chick to secrete α-galactosidase. These undigested carbohydrates could affect the productive performance of chicks fed diets containing lupin, as they may produce an osmotic effect in the intestinal tract and promote the growth of a population of microorganisms in the large intestine and cecum. Supplementation of diets with enzymes that hydrolyze these carbohydrates may increase the energy value of lupin meal and also improve the nutritive value of other dietary components. Another factor that may affect the nutritional value of lupin seed is its high content of testa or hulls, which consists mainly of cellulose, hemicellulose, and lignin (Hove, 1974; Withers *et al.*, 1975).

Little or no research has been published on the effects that dehulling and enzyme supplementation have on the nutritional value of lupins. The present experiments were conducted to determine whether dehulling and heat treatment of lupins and the addition of a combination of carbohydrase, protease, and galactosidase enzymes to diets containing lupin would improve the performance of Leghorn and broiler chicks. The energy value and digestibility of protein of raw, dehulled, and autoclaved lupin seeds were also determined.

MATERIALS AND METHODS

General Experimental Procedures

The lupin (*L. albus*) cultivar, Amiga, used in the experiments (Table 1) was grown in the province of Saskatchewan[4] and did not contain any of the lupin alkaloids (analyzed according to Musquiz *et al.*, 1989). The experimental diets (Experiments 2, 4 and 5) were formulated to meet or exceed the minimum NRC (1984) requirements for Leghorn chickens. The broiler diets were formulated on the basis of calorie to nutrient ratio similar to NRC (1984) (Experiment 1) but below requirements for the balance trial (Experiment 3) because high levels of lupins were incorporated into these diets to facilitate accuracy in the AME assay. All diets were given in mash form, and the birds had free access to water and feed throughout the entire experiment. The crude enzyme preparations (referred to as enzymes) that were used were: Energex®[5] (from selected strains of *Aspergillus niger*), Bio-Feed Pro®[5] (from selected strains of *Bacillus licheniformis*), and Novozyme® SP-230®[5] and Cellulase Tv[6] concentrate (from controlled fermentation of *Trichoderma viride*). The enzyme activities per gram of crude product as determined by the manufacturers were as follows: Energex®, 75 fungal β-glucanase units, 150,000 hemicellulase units, 10,000 pectinase units, and 400 endoglucanase units; Biofeed Pro®, 150,000 protease units; Novozyme® SP 230®, contained an unknown α-galactosidase activity and 2,500 units inulinase; Cellulase Tv, 23,880 cellulase units.

One-day-old male broiler (Arbor Acres × Ross) and Leghorn chicks were obtained from a commercial hatchery and raised in Jamesway battery brooders[7] for 7 days. All chicks were fed a commercial chick starter crumbles (21% CP) during the 7-day pre-experimental period. At 7 days of age, the birds were randomly distributed in Petersime battery brooders.[8] Chick performance was measured in terms of feed consump-

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[5]Novo Nordisk/A/S, Bagsvaerd, Denmark.

[6]Miles Laboratories Inc., Elkhart, IN 46515.

[7]Jamesway Manufacturing Co., Fort Atkinson, WI 53538.

[8]Petersime Incubator Co., Gettysburg, OH 45328.

tion, weight gain, and feed to gain ratio, and overall values were reported for each experiment. All variables are reported on a per bird basis. Experiments 1, 2, 4, and 5 were conducted over a period of 14 days (Days 7 to 21 of age). Feed consumption and bird weights were determined on Days 7 and 14 of the experiment. Experiment 3 was conducted over a 7-day period (Days 10 to 17).

Experiment 1

The effects of enzyme addition (Energex®, Bio-Feed Pro®, Novozyme®, and Cellulase Tv and combinations of them) to the lupin-based diets (Table 2) on chick performance were evaluated. Two hundred and fifty-two broiler chickens were randomly distributed among nine treatments using four birds per pen and seven pen replicates per treatment. The treatments were as follows: 1) corn-lupin control diet (CL); 2) CL plus .1% Energex®; 3) CL plus .1% Bio-Feed Pro®; 4) CL plus .1% Novozyme®; 5) CL plus .1% Cellulase Tv; 6) CL plus .1% Energex® plus .1% Bio-Feed Pro®: 7) CL plus .1% Energex® plus .1% Novozyme®; 8) CL plus .1% Bio-Feed Pro® plus .1% Novozyme®; and 9) CL plus .1% Energex® plus .1% Bio-Feed Pro® and 1% Novozyme®. The weight of the gizzard and length of the different sections of small intestine and ceca were determined in 14 randomly selected birds (2 birds per replicate) from each of Treatments 1 and 9 (CL and CL plus a combination of three enzymes). The feed and feces were analyzed for nitrogen [Association of Official Analytical Chemists (AOAC), 1984] and chromic

TABLE 1. Chemical composition of the different fractions of lupin seeds

Composition	Lupin seed		
	Whole seed	Cotyledon	Hull
	(% DM)		
DM	94.43	94.49	94.71
Protein (N × 6.25)	31.45	37.76	3.98
Fat	10.89	12.60	1.01
ADF[1]	16.06	4.51	65.38
NDF[2]	18.50	5.00	74.74
Ash	3.60	3.77	2.71
Ca	.21	.12	.65
P	.41	.47	.02
Hull	16.02	. . .	. . .
Total alkaloid	.01	0	0
Amino acid			
Alanine	1.08	1.26	.15
Arginine	3.01	3.78	.14
Aspartic acid	3.31	4.00	.48
Cystine	.34	.44	.03
Glutamic acid	6.66	8.36	.40
Glycine	1.36	1.56	.19
Histidine	.74	.87	.08
Isoleucine	1.13	1.15	.12
Leucine	2.29	2.71	.25
Lysine	1.54	1.74	.25
Methionine	.24	.28	.00
Phenylalanine	1.26	1.49	.19
Proline	1.32	1.62	.18
Serine	1.73	2.12	.27
Threonine	1.20	1.38	.16
Tyrosine	1.29	1.59	.11
Valine	1.17	1.19	.19
Ammonia	.32	.37	.04

[1]Acid detergent fiber.

[2]Neutral detergent fiber.

oxide (Williams *et al.*, 1962). In addition, excreta samples from Treatments 1 and 9 were analyzed for uric acid content (Marquardt, 1983), and the apparent protein digestibilities (APD) of these diets were calculated as described by Rotter *et al.* (1989).

Experiment 2

The objectives of this experiment were to determine the optimum concentration of three combined enzyme preparations and the effect of individual crude enzymes (.3% each) when added to a lupin diet. Two hundred and fifty-two Leghorn chicks were randomly distributed among seven treatments using six birds per pen and six pen replicates per treatment. The treatments were as follows: 1) corn-lupin diet (CL); 2) CL plus .05% of each enzyme (Energex®, Bio-Feed Pro® and Novozyme®); 3) CL plus .1% of each enzyme; 4) CL plus .2% of each enzyme; 5) CL plus .3% Energex®; 6) CL plus .3% Bio-Feed Pro®; 7) CL plus .3% Novozyme®.

Experiment 3

This study was conducted to determine the AME of raw, autoclaved, and dehulled lupins. Raw lupin meal that was autoclaved was spread to a depth of approximately 1 cm on stainless steel pans and heated to 121 C for 20 min in a standard laboratory sterilizer. Dehulling was carried out in a commercial pea splitter. The process involved the cracking of the seed with a plate grinder followed by mechanical removal of the hulls from the cotyledon fraction (dehulled lupins). Hulls for chemical analysis were further purified by air classification. The dehulled lupins and the hulls were estimated to be more than 95% pure (Table 3).

One hundred and twenty broiler chicks were randomly distributed among five treatments using four birds per pen and six pen replicates per treatment. The treatments were as follows: 1) corn-soybean basal diet (CS); 2) raw-lupin basal diet (LD); 3) CS plus LD diet (50:50 mixture); 4) CS plus autoclaved-lupin diet (50:50 mixture); and 5) CS plus dehulled-lupin diet (50:50 mixture). High concentrations of lupins were included in the raw-lupin basal diet to facilitate accuracy in the AME assay. All chicks were fed commercial chick starter crumbles (21% CP) during the 10-day preexperimental period. The balance trial consisted of a 4-day pretrial (adaptation) and a

TABLE 2. Composition of experimental diets, Experiments 1 and 2

Ingredients and analysis	Experiment 1	Experiment 2
	(%)	
Corn	17.51	39.43
Lupins[1]	70.00	50.00
Tallow	7.00	6.00
Dicalcium phosphate	1.59	1.22
Calcium carbonate	1.25	1.04
DL-methionine	.42	.16
Lysine	.10	. . .
Other ingredients[2]	2.15	2.15
Calculated analysis		
Crude protein (N × 6.25)	21.63	19.32
ME, kcal/kg	2,928	2,970
Lysine	1.11	.90
Methionine + cystine	.89	.60
Ca	.94	.80
P available	.45	.40

[1]Protein content 31.5% (N × 6.25).

[2]Other ingredients were: vitamin mix, 1%; mineral mix, .35%; chromic oxide, .30%; and enzyme mix (premix), .50%. The vitamin mix supplied per kilogram of diet: vitamin A, 8,250 IU; cholecalciferol, 991 IU; vitamin E, 11 IU; vitamin B_{12}, 11.5 μg; vitamin K, 1.1 mg; riboflavin, 5.5 mg; Ca-pantothenate, 11.0 mg; niacin, 53 mg; choline chloride, 1,020 mg; folic acid, .75 mg; biotin, .25 mg; delaquin, 125.0 mg. The mineral mix supplied per kilogram of diet: Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, .1 mg; I, .18 mg.

TABLE 3. Composition of experimental diets, Experiments 3, 4, and 5

Ingredients and analysis	Experiment 3 Control	Experiment 3 Lupins[1]	Experiments 4 and 5 Corn-whole lupins	Experiments 4 and 5 Corn-dehulled lupins
	(%)			
Corn	64.84	. . .	39.43	39.43
Whole lupins[1]	. . .	95.00	50.00	. . .
Dehulled lupins	. . .	. . .	. . .	50.00
Soybean meal	30.00	. . .	. . .	. . .
Tallow	. . .	. . .	6.00	6.00
Dicalcium phosphate	2.20	1.44	1.22	1.22
Calcium carbonate	1.00	1.49	1.04	1.04
DL-methionine	.31	.42	.16	.16
Enzyme mix (premix)	. . .	. . .	.50	.50
Other ingredients[2]	1.65	1.65	1.65	1.65
Calculated analysis				
Crude protein (N × 6.25)	20.10	29.88[3]	19.32	23.33
ME, kcal/kg	2,917	. . .	2,970	3,053
Lysine	1.11	1.52	.90	.96
Methionine + cystine	.90	.90	.60	.60
Ca	.82	1.09	.80	.80
P, available	.59	.49	.40	.40

[1]Protein content of lupins and dehulled lupins were 31.5 and 39.5% (N × 6.25), respectively.

[2]Other ingredients were: vitamin mix, 1%; mineral mix, .35%; chromic oxide, .30%. The vitamin mix supplied per kilogram of diet: vitamin A, 8,250 IU; cholecalciferol, 991 IU; vitamin E, 11 IU; vitamin B_{12}, 11.5 μg; vitamin K, 1.1 mg; riboflavin, 5.5 mg; Ca-pantothenate, 11.0 mg; niacin, 53 mg; choline chloride, 1,020 mg; folic acid, .75 mg; biotin, .25 mg; delaquin, 125.0 mg. The mineral mix supplied per kilogram of diet: Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, .1 mg; I, .18 mg.

[3]High protein due to the 95% level of lupins in this diet.

3-day collection period (10 to 17 days of age). Feed consumption, weight gain, and the feed to gain ratio were determined in this period. Feed and excreta samples were analyzed for gross energy using a Parr adiabatic oxygen bomb calorimeter.[9] The AME values were determined as outlined by Rotter *et al.* (1990). Apparent protein digestibility was determined as described by Rotter *et al.* (1989).

Experiment 4

This experiment was carried out to determine the effect of adding enzymes to diets containing raw, autoclaved (15 and 30 min), and dehulled lupin on chick performance. Three hundred and sixty Leghorn chicks were randomly distributed among 10 treatments using six birds per pen and six pen replicates per treatment. The experiment was designed with 2 × 5 factorial arrangements of treatments as follows: 1) corn-lupin (CL); 2) CL plus E (Energex®, Bio-Feed Pro®, Novozyme® .1% each enzyme); 3) corn-autoclaved (15 min) lupin (CAL1); 4) CAL1 plus E; 5) corn-autoclaved (30 min) lupin (CAL2); 6) CAL2 plus E; 7) corn-dehulled lupin (CDL); 8) CDL plus E; 9) corn-autoclaved (15 min) dehulled lupin (CADL); 10) CADL plus E.

Experiment 5

This experiment was conducted to confirm the result obtained in Experiment 4 with autoclaved and dehulled lupin seeds. Two hundred and eighty-eight Leghorn chicks were randomly distributed among eight treatments using six birds per pen and six pen replicates per treatment. The experiment was designed with 2 × 4 factorial arrangement of treatments as follows: 1) corn-lupins (CL); 2) CL plus E (Energex®,

[9]Parr Instrument Co., Moline, IL 61265.

Bio-Feed Pro®, Novozyme® at .1% each enzyme); 3) corn-autoclaved (20 min) lupins (CAL); 4) CAL plus E; 5) corn-dehulled lupins (CDL); 6) CDL plus E; 7) corn-autoclaved dehulled lupins (CADL); 8) CADL plus E. Immediately after the randomly selected chicks (2 birds per replicate) had been killed by cervical dislocation (12 birds per treatment), the weights of the crop, gizzard, and pancreas were determined.

Other Chemical Analyses

The alkaloid content of lupins was determined by the method of Muzquiz *et al.* (1989). Dry matter, ash, protein (N × 6.25), acid detergent fiber (ADF), neutral detergent fiber (NDF), fat, Ca, and P were determined by standard methods (AOAC, 1984). Amino acids were analyzed as described by Andrews and Baldar (1985).

Statistical Analysis

The data (pen means) were subjected to analysis of variance using the General Linear Models (GLM) procedure of SAS® software (SAS Institute, 1986). Experiments 4 and 5 were analyzed by ANOVA in 2 × 5 and 2 × 4 factorial arrangements of treatments, respectively, and single df contrasts were used to separate treatment means in the factorial experiments. Significant differences among means of Experiments 1, 2, 3, and 5 were determined using Tukey's studentized range (HSD) test (Steel and Torrie, 1980).

RESULTS

The proximate analyses of the whole seed as compared with the hull and cotyledon portions of the seed (Table 1) show that lupin hulls were high in fiber and Ca and low in protein, whereas cotyledons had the opposite pattern (low fiber, high protein, and P). The amino acid composition of lupin fractions indicated a marked difference in the amounts and proportion of amino acids in the hulls compared to the cotyledons. The total alkaloid content of all fractions was low.

The results of the growth trial (Experiment 1) showed that some enzyme preparations increased the weight gain and improved feed to gain ratio of broiler chicks fed the lupin-containing diets as compared with the control (Table 4). Among the four enzyme preparations added alone, Bio-Feed Pro® (high protease activity) had the most pronounced effect, yielding a 13% increase in weight gain and a 6% improvement in the feed to gain ratio ($P < .05$). Further improvements ($P > .05$) in performance occurred when an enzyme combination (Energex®, Bio-Feed Pro®, and Novozyme®, each at .1%) was added to the diet. In comparison to the control diet, an 18% increase in weight

TABLE 4. Performance of broiler chicks (7 to 21 days) fed enzyme-supplemented 70% lupin seed diets, Experiment 1

Enzyme	Feed consumption[1]	Weight gain	Feed to gain ratio
	(g)	(g)	(g:g)
No enzymes	630	367[c]	1.72[a]
Energex® .1%	638	394[abc]	1.62[abc]
Bio-Feed Pro® .1%	670	416[ab]	1.61[bc]
Novozyme® .1%	637	386[bc]	1.65[ab]
Cellulase Tv .1%	647	377[bc]	1.72[a]
Energex® + Bio-Feed Pro® (.1% each)	669	418[ab]	1.60[bc]
Energex® + Novozyme® (.1% each)	674	414[ab]	1.63[abc]
Bio-Feed Pro® + Novozyme® (.1% each)	628	403[abc]	1.56[bc]
Energex® + Bio-Feed Pro® + Novozyme® (.1% each)	671	433[a]	1.55[c]
SEM	12	9	.02

[a–c]Means within a column with no common superscript differ significantly ($P < .05$).

[1]No significant differences were observed ($P > .05$).

TABLE 5. Relative intestinal length, gizzard weight, and apparent protein digestibility (APD) of broiler chicks fed 70% lupin seed diets with (+) and without (–) enzyme supplementation, Experiment 1

Enzymes	Duodenum	Jejunum	Ileum	Cecum	Gizzard	APD
	(cm/100 g)				(g/100 g)	(%)
–	8.08^{A}	15.78^{A}	15.10^{A}	4.57^{A}	2.45^{A}	86.7
+[1]	6.39^{B}	11.86^{B}	11.71^{B}	3.86^{B}	2.11^{B}	88.9
SEM	.221	.472	.554	.128	.06	1.12

A,BMeans within a column with no common superscript differ significantly ($P < .01$).

[1]Contain .1% each of Energex®, Bio-Feed Pro®, and Novozyme®.

gain and 10% improvement in feed to gain ratio was observed. This treatment also reduced ($P < .05$) the length of the duodenum (13%), jejunum (18%), ileum (15%), and cecum (7%) and the relative weight of the gizzard (13%) but did not affect ($P > .05$) apparent protein digestibility (Table 5).

The dose-response experiment (Experiment 2) with three enzyme preparations (Energex®, Bio-Feed Pro®, and Novozyme®) at concentrations of .05, .1, and .2% of each demonstrated that although there were numerical differences ($P > .05$) due to enzyme levels, maximum growth and the best feed to gain ratio were obtained at a concentration of .1% of each enzyme with the improvements being 18 and 10%, respectively, compared with those obtained with the unsupplemented diet ($P < .05$, Table 6). The .2% level of enzyme resulted in a depressed feed intake compared with the lower levels of supplementation, with a numerically improved feed to gain ratio. When the same enzyme preparations were added individually to the corn-lupin diet at a concentration of .3%, the greatest improvements in weight gain (19%) and feed to gain ratio (11%) were obtained, with the enzyme having high protease activity (Bio-Feed Pro®; Table 6).

In the third experiment, chicks fed a diet containing 95% lupins had considerably poorer ($P < .05$) performance values than those fed the control CS diet (Table 7). However, the feeding of an equal mixture of the LD diet and the CS diet to give a dietary concentration of lupins of 48% resulted in greater improvement ($P < .05$) in weight gains and feed to gain ratios than those obtained with the LD. The feed to gain ratio and APD (Table 8) obtained with the 50:50 mixture of the two diets were not different ($P > .05$) from values obtained with the CS diet. Autoclaving

TABLE 6. Performance of Leghorn cockerels (7 to 21 days) fed enzyme-supplemented 50% lupin seed diets, Experiment 2

Treatment	Feed consumption	Weight gain	Feed to gain ratio
	(g)		(g:g)
Corn-lupin (CL)	239^{bc}	106^{d}	2.24^{a}
CL + .05% E[1]	251^{ab}	118^{cd}	2.12^{b}
CL + .10% E	253^{ab}	125^{ab}	2.02^{bc}
CL + .20% E	235^{c}	118^{cd}	1.99^{c}
CL + .3% Energex®	240^{bc}	118^{cd}	2.03^{bc}
CL + .3% Bio-Feed Pro®	261^{a}	131^{a}	1.99^{c}
CL + .3% Novozyme®	251^{ab}	122^{bc}	2.06^{b}
SEM	2.9	1.8	.02

a–dMeans within a column with no common superscripts differ significantly ($P < .05$).

[1]E = Energex® + Bio-Feed Pro® + Novozyme® added at .05, .1, or .2% each to the diet.

TABLE 7. Performance of broiler chicks (10 to 17 days) fed raw lupin diets and their fraction, Experiment 3

Treatment	Feed consumption	Weight gain	Feed to gain ratio
	(g)		(g:g)
Corn-soy (CS)	258[a]	156[a]	1.66[b]
Lupin diet (LD)[1]	183[b]	59[c]	3.32[a]
CS + LD (50:50)	239[a]	135[b]	1.78[b]
CS + autoclaved[2] lupin (50:50)	243[a]	150[ab]	1.62[b]
CS + dehulled lupin (50:50)	250[a]	151[ab]	1.66[b]
SEM	7.1	4.9	.14

[a–c]Means within a column with no common superscript differ significantly ($P < .05$).

[1]95% lupin content in the diet.

[2]Autoclaved 20 min.

and dehulling of lupins (50:50 mixture) numerically improved performance but these differences were not significant ($P > .05$, Table 7). Dehulling, however, increased the AME (11%) and APD (7%) values ($P < .05$, Table 8). A similar improvement was also observed for autoclaved lupins ($P > .05$). Overall weight gains obtained with diets containing 48% autoclaved or 48% dehulled lupins were the same as those obtained with the CS diet ($P > 05$).

The significant treatment by enzyme interaction for feed consumption in Experiment 4 (Table 9) indicated that enzyme supplementation of the corn-raw lupin diet increased feed intake (8%, $P < .001$) but that it did not affect feed intake of the other diets. The treatment by enzyme interaction for weight gain indicated that enzyme addition only improved weight gains of chicks fed the diet containing the untreated lupins (18%, $P < .001$) and that among the different treatments the greatest improvements relative to the untreated lupins without enzyme addition occurred with the dehulled raw lupins (15%, $P < .001$), followed by whole lupins when autoclaved for 15 min (11%, $P < .001$). Autoclaving for 30 min only improved weight gains by 6% ($P < .04$). Analysis of variance demonstrated that there was also a treatment by enzyme interaction for the feed to gain ratio, but

TABLE 8. Apparent metabolizable energy and apparent protein digestibility (APD) of broilers fed raw lupin diets and their fraction, Experiment 3

Treatment	AME		APD
	Whole diet[1]	Lupin[2]	
	(kcal/kg)		(%)
Corn-soy (CS)	3,144[a]	. . .	89.0[ab]
Lupin diet (LD)[3]	2,488[d]	. . .	73.1[c]
CS + LD (50:50)	2,721[c]	2,368[b]	87.0[b]
CS + autoclaved[4] lupin (50:50)	2,806[bc]	2,468[b]	88.1[ab]
CS + dehulled lupin (50:50)	2,928[b]	2,784[a]	93.5[a]
SEM	36.3	76.7	1.8

[a–c]Means within a column with no common superscript differ significantly ($P < .05$).

[1]Average of six replicates.

[2]ME whole diet – (ME basal/2).

[3]95% lupin content in diet.

[4]Autoclaved 20 min.

that this effect was relatively insignificant as it only accounted for 6% of the variance when the treatment sum of squares were partitioned according to Little (1981), whereas diet and enzyme accounted for 69 and 25% of the variation, respectively. Overall enzyme addition improved the feed to gain ratio by 3.5% (1.98 for heated versus 2.03 for untreated), dehulling by 5% (1.92 versus 2.03), and autoclaving for 15 min by 1.5% (2.00 versus 2.03). Autoclaving for 30 min in the presence and absence of enzyme had a negative effect on the feed to gain ratio (6% increase, 2.15 versus 2.03%).

In Experiment 5, dehulling but not autoclaving of lupins reduced feed consumption (3%, $P = .002$) and the feed to gain ratio (5%, $P < .001$) but enzyme addition improved the overall weight gain (3%, $P = .01$) and feed to gain ratio (3%, $P = .001$, Table 10). The relative weights of the gizzard (9%) and the pancreas (10%) were reduced with enzyme addition while dehulling reduced the size of the gizzard (5%).

DISCUSSION

Chemical analysis demonstrated that the lupin seed was alkaloid-free and had a high content of protein (31%), fat (11%), and fiber (18% NDF). The concentration of methionine relative to the requirements of the growing chicks (NRC, 1984) is low and that of lysine is high. The amino acid composition was similar to that reported by Ballester *et al.* (1980), Halvorson *et al.* (1983), and Aguilera *et al.* (1985). The testa or hull portion of lupin makes up a large portion of the seed (16%) and is primarily responsible for the high content of ADF and NDF in the whole seed. Other researchers reported similar values (Hove,

TABLE 9. Performance of Leghorn chicks (7 to 21 days) fed enzyme-supplemented, raw, autoclaved, and dehulled lupin seeds, Experiment 4

Diet	Enzyme[1]	Feed consumption	Weight gain	Feed to gain ratio
		(g)	(g)	(g:g)
Corn-whole raw lupins[2]	–	226	106	2.12
	+[4]	245 (8.4***)[5]	125 (17.9***)	1.97 (–9.1***)
Corn-whole autoclaved (15[3]) lupins	–	240	118	2.03
	+	241 (.4, NS)	119 (.8, NS)	2.03 (0, NS)
Corn-whole autoclaved (30[3]) lupins	–	238	112	2.15
	+	240 (.8, NS)	112 (0, NS)	2.14 (–.5, NS)
Corn-dehulled lupins	–	229	122	1.88
	+	223 (–2.6, NS)	122 (0, NS)	1.83 (–2.7, NS)
Corn-dehulled autoclaved (15[3]) lupins	–	230	115	2.00
	+	223 (–3, NS)	115 (0, NS)	1.95 (–2.5, NS)
SEM		3	2	.02
Source of variation		Probabilities		
Treatment		<.001	<.001	<.001
Enzyme		NS	.005	<.001
Treatment × enzyme		.001	<.001	.016

[1]With (+) or without (–) enzyme supplementation.

[2]All diets contain 50% lupins.

[3]Autoclaved 15 or 30 min.

[4]+ = Energex® + Bio-Feed Pro® + Novozyme® at .1% each.

[5]Values in parentheses represent percentage increase or decrease for chicks fed enzyme-treated diets relative to those fed the corresponding untreated diet.

***$P < .001$.

1974; Withers *et al.*, 1975). Cerning and Filiatre (1980) determined that the hull fraction (24.1%) in sweet yellow lupins consisted mainly of cellulose, hemicellulose, and lignin. Removal of the hull from the seed greatly reduced its content of fiber and increased that of protein and fat.

The results as discussed above suggest that the high content of hulls can reduce the nutritive value of lupins but possibly can be compensated for, in part, by its high fat content. The AME of raw lupin as determined in this study (2.4 kcal/g) was similar to the ME value of 2.3 and 2.5 kcal/g reported by Guillaume *et al.* (1979), and the 2.2 kcal TME value reported by Watkins *et al.* (1988). The APD of the diets containing 47% lupins were relatively high, being similar to that of the basal diet (87 versus 89% for the CS diet).

In the current study, weight gains, the feed to gain ratio, and APD of broiler chicks fed a diet that contained 48% of autoclaved or dehulled lupins were the same as those obtained with the control diet. Perez-Escamilla *et al.* (1988) and Karunajeewa and Bartlett (1985) reported that up to 30 to 35% raw or autoclaved sweet lupins and up to 22% white seed lupins, respectively, could be included in the diet of broiler chicks with no adverse effect on growth, whereas Watkins *et al.* (1988) reported that 10% extruded lupins when fed to broilers yielded performance values superior to the control diet. In all of the above studies the inclusion into the diets of greater amounts of lupins than that indicated above resulted in a reduced level of performance.

Similar depressions were also obtained in the current study with regards to weight gain at the 47.5% inclusion level of the raw lupins (CS + LD; 50:50) and in all performance values at the 95% inclusion level. The reason for the depression in growth observed in the current study was not established but probably was not caused by the alkaloid content of the lupins as their concentrations were 140 times lower than in the alkaloid-containing cultivars (Brenes *et al.*, unpublished data). Alkaloids in lupins, nevertheless, can cause severe growth depression (Hill, 1977; Pearson and Carr, 1977; Ruiz *et al.*, 1977).

Part of the effect observed at the high lupin concentration may have been related to the high fiber content found in the hulls

TABLE 10. Performance and relative organ weights of Leghorn chicks (7 to 21 days) fed enzyme-supplemented, raw, dehulled, and autoclaved (20 min) lupin seeds, Experiment 5[1]

Treatment	Feed consumption	Weight gain	Feed to gain ratio	Relative weight: Gizzard	Relative weight: Pancreas
	(g)	(g)	(g:g)	(g/100 g BW)	(g/100 g BW)
Diet					
Corn-lupin	290[ab]	143	2.03[a]	2.66[ab]	.37
Corn-autoclaved lupin	296[a]	145	2.05[a]	2.71[a]	.36
Corn-dehulled lupin	284[b]	148	1.93[b]	2.59[ab]	.37
Corn-autoclaved dehulled lupin	284[b]	147	1.94[b]	2.51[b]	.35
Enzyme[2]					
–	287	143	2.01	2.75	.38
+[3]	290	148	1.97	2.49	.34
SEM	3.7	2.4	.02	.07	.01
Source of variation	Probabilities				
Diet	.002	NS	<.001	.020	NS
Enzyme	NS	.010	.001	<.001	<.001
Diet × enzyme	NS	NS	NS	NS	NS

[a,b]Means within columns with no common superscript differ significantly ($P < .05$).

[1]Treatment did not affect crop weight ($P > .05$). The average weight was .42 g/100 g BW.

[2]With (+) or without (–) enzyme supplementation.

[3]+ = Energex® + Bio-Feed Pro® + Novozyme® at .1% each.

of the lupin, the different amounts and type of soluble factors within the cotyledon of the lupin seed, or possibly its content of Mn, which tends to be very high. Karunajeewa and Bartlett (1985) have suggested that the high content of Mn in lupin seeds (2,300 versus 38 mg/kg for soybean meal) may contribute to depressed growth. These concentrations, however, should not affect chick performance, as chicks can tolerate 3,600 mg/kg of Mn (Vohra and Kratzer, 1968).

As previously shown by Carre and Leclercq (1985), cotyledons have a high percentage of cell wall material (20%) composed of a high concentration of pectin-like substances and a low level of cellulose plus lignin. The pectin-like substances are composed of branched β (1→4) galactans, which are quite labile and probably highly susceptible to fermentative break-down (Carre *et al.*, 1985). These carbohydrates, however, may escape hydrolysis and absorption from the small intestine as the enzyme α-galactosidase is not found in the tissues of mammals and other animals (Cummings *et al.*, 1986). Thus, the cell-wall material and other carbohydrates of white lupin cotyledon may contribute to their antinutritive effects and may also be the target substrate for hydrolysis by enzymes added to the diet. α-Galactosides, however, may be a contributing but not be the principal antinutritional factor in lupins, as Brenes *et al.* (1989) showed that diets containing an oligosaccharide dried extract obtained from lupin seeds did not have a detrimental effect on chick performance.

The supplementation of lupin-based diets with crude enzyme preparations resulted in substantial improvement of their nutritive value. Enzyme addition to various diets in the different experiments improved weight gains and feed efficiency by as much as 18 and 11%, respectively. The most effective enzyme preparations had high protein hydrolyzing activity (Bio-Feed Pro®). Enzyme addition to diets containing lupin also affected the size of the gastrointestinal tract and the pancreas. Overall, in Experiment 1 there were reductions as much as 13% in the length of the duodenum, 18% for the jejunum, 15% for the ileum, and 7% in the cecum when compared with an untreated diet. In Experiment 3 enzyme treatment reduced pancreas size by 10%. Although the factor(s) that were affected by the enzyme treatment were not identified, it is conceivable that they may have effected the hydrolysis of the different complex carbohydrates. The type of fiber in the diet has been shown to affect pancreas size in rats (Sheard and Schneeman, 1980; Schneeman *et al.*, 1982; Isaksson *et al.*, 1983).

Enzymes appeared to also increase APD (Experiment 1) and probably would have increased AME values. In contrast to these results, Perez-Escamilla *et al.* (1988) reported that incorporation of 1% hemicellulase into a diet that contained lupins did not significantly improve body weight gains of chicks. An inspection of their data, however, indicated that weight gains in chickens fed two lupin-containing diets was numerically increased by 6% with enzyme addition. Conceivably the number of replicates were not sufficiently high to demonstrate a significant effect.

Other treatments also affected the nutritive value of lupins, including autoclaving and dehulling. Chicks fed diets that had been autoclaved for 15 min (Experiment 4) showed improved performance compared with those fed raw lupins. Autoclaving lupins for 20 min (Experiments 3 and 5) or 30 min (Experiment 4), however, did not result in improved performance. Also, in comparison to results obtained with chicks fed raw lupins, no benefit was obtained by the addition of enzyme to either the 15- or the 30-min autoclaved diets. These observations would indicate that the nutritive value of lupins may be improved when subjected to short (15 min) but not long (30 min) autoclaving treatments. The reason for this effect was not established. Short autoclaving periods (less than 15 min) may predominantly inactivate an antinutritional factor, whereas longer heat treatment may also have reduced availability of nutrients, with the overall net effect being negative. Other researchers also examined the effect of autoclaving on the nutritive value of lupins for poultry and have showed no beneficial effects (Watkins and Mirosh, 1987; Perez-Escamilla *et al.*, 1988). However, Molina *et al.* (1983) and Boldaji *et al.* (1986) found that autoclaving lupin meal increased the AME and TME values.

In contrast to the variable results obtained with autoclaving, dehulling had a positive effect on several performance factors. Chicks fed dehulled compared with raw lupins had increased weight gains, improved feed to gain ratios, and increased AME and APD values. Dehulling of lupins had an effect on the size of the gizzard but not on that of the crop or the pancreas. The effects of dehulling on organ size appeared to be less pronounced than that obtained when diets containing whole lupin seeds were supplemented with enzymes. However, the effect of enzyme addition to dehulled lupin diets on feed to gain ratio was inconsistent as an improvement was observed in Experiment 4 but not in Experiment 5.

Overall, the results of this study demonstrated that the nutritive value of alkaloid-free lupins can be increased by supplementation of lupin-containing diets with an appropriate enzyme, or by dehulling or autoclaving. Chicks fed diets containing high concentrations of autoclaved or dehulled lupins (47.5%) are able to support weight gains and feed to gain ratios that are similar to those obtained in the corn-soybean diet. Enzyme supplementation in addition to improving the nutritional value of lupins also reduced the length and size of various sections of the gastrointestinal tract and the size of the pancreas of chickens. The benefits of enzyme addition, however, were not observed when diets are autoclaved or dehulled. Additional research is required to identify the nature of the antinutritive factor in alkaloid-free seeds, and to establish whether there is more than one factor present in lupins, the location of these factors (testa or cotyledons), the nature of the interaction among dehulling, autoclave treatment, and enzyme treatment in different lupin fractions, and finally the means by which the nutritive value of lupins can be most effectively improved. It is conceivable that the fiber associated with the hull fractions produces an effect and that another factor in the cotyledon produces a second effect. It may be concluded that properly treated lupins can be incorporated into poultry diets at relatively high concentrations without affecting performance.

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