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Short communication. Effect of enzyme addition on the nutritive value of six lupin cultivars with different alkaloid content

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Abstract

An experiment was conducted to study the effect on chick performance of crude enzyme preparations when added to diets containing six different cultivars of lupin (*Lupinus albus* L.) seed grown in Canada. The lupin cultivars studied were Pnognus, NL, LAD, Hutterite, Amiga and Brandon. The total alkaloid content of these cultivars was 0.01, 0.02, 0.01, 0.04, 0.01 and 1.42%, respectively. The weight gain and feed consumption of the birds fed the high alkaloid content were reduced significantly ($P < 0.0001$; up to 43 and 31%, respectively) and the feed to gain ratio increased significantly ($P < 0.0001$; up to 21%) in comparison to the other cultivars. The addition of enzymes (0.1% each of Energex, Bio-Feed-Pro and Novozyme) increased significantly weight gain ($P < 0.0033$) and feed consumption ($P<0.0116$) by 5 and 1%, respectively, and reduced ($P < 0.0055$) feed to gain ratio by 4%. This was only seen in the low alkaloid cultivars. These results suggest that the enzyme addition improve the nutritional value of lupin cultivars with low content of alkaloid and that lupin alkaloids depress chick performance.

Additional key words: bird nutrition, chicken, *Lupinus albus.*

Resumen

Efecto de la adición de enzimas sobre el valor nutritivo de seis diferentes variedades de altramuz

Se ha realizado una prueba experimental en pollos con el objetivo de estudiar el efecto sobre los índices productivos de la inclusión de un complejo enzimático en raciones que contenían seis variedades distintas de altramuz (*Lupinus albus* L.). Se estudiaron las variedades canadienses Pnognus, NL, LAD, Hutterite, Amiga y Brandon, con un contenido total en alcaloides de 0,01, 0,02, 0,01, 0,04, 0,01 y 1,42%, respectivamente. La inclusión en las raciones de variedades con alto contenido en alcaloides producía una reducción ($P < 0{,}0001$) de la ganancia de peso y del consumo de alimento de hasta el 43 y 31% respectivamente y un incremento ($P < 0{,}0001$) en el índice de transformación de hasta un 21%. La inclusión de un complejo enzimático producía un incremento de la ganancia de peso (5%; $P < 0{,}0033$) y del consumo de alimento (1%; $P < 0{,}0116$) y una reducción en el índice de transformación (3%; $P < 0{,}0055$) solamente en las variedades con bajo contenido en alcaloides. Estos resultados demuestran que la adición de enzimas mejora el valor nutritivo de las variedades de altramuz bajas en alcaloides, siendo estos alcaloides los responsables de los malos índices productivos de las aves.

Palabras clave adicionales: alcaloides, alimentación de aves, *Lupinus albus,* pollos.

Lupin seeds are a source of good quality protein supplements for monogastric animals such as poultry and pigs, however, their use has been limited due to the presence of quinolizidine alkaloids mainly lupanine (Halvorson *et al.,* 1983). Lupanine and other alkaloids affect the human and animal central nervous system causing symptoms such as depression, labored breathing, convulsions and death from respiratory failure (Keeler, 1989; Hill and Patuszewska, 1993). Plant breeders have worked on improving lupin by selecting varieties high in protein and low in alkaloids. Poultry can tolerate 15-25% dietary low alkaloid lupin meal with no adverse effects; at higher levels, feed intake and growth rate are impaired (Karunajeewa and Bartlett, 1985; Ferraz de Oliveira, 1998). Positive production responses from the addition of supplementary enzymes to poultry diets containing whole and dehulled sweet lupin seeds

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have been reported by Roth Maier and Kirchgessner (1994a,b, 1995), Annison *et al.* (1996), Ferraz de Oliveira (1998), and Brenes *et al.* (1993, 2002), while no effect or a negative effect has been reported by Alloui *et al.* (1994), Roth Maier and Kirchgessner (1995), Annison *et al.* (1996), and Eder *et al.* (1996). The objective of this work was to analyse the total alkaloid content of six lupin seeds cultivars, and to determine the influence of lupin cultivars and enzyme additions to the diets on chick performance.

The *Lupinus albus* cultivars selected for this experiment were: Pnognus, NL, LAD, Amiga and Hutterite (they were grown in Saskatchewan, Canada), and Brandon (Manitoba, Canada). The crude enzyme preparation used in this study included: Energex (multienzyme complex that hydrolyses a broad range of carbohydrates produced from selected strain of *Aspergillus niger*), Bio-Feed Pro (proteolytic enzyme produced from selected strain of *Bacillus licheniformis*) and Novozyme SP-230 (alpha-galactosidase activity) from Novo Nordisk S.A., Denmark. The enzymes activities per gram 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; Bio-Feed Pro, 150,000 protease units; Novozyme SP 230, 500 α-galactosidase units. The mixed crude enzyme preparation consisted of Energex, Bio-Feed Pro and Novozyme at 0.1% each. The different lupin cultivars were incorporated at a concentration of 50% in the diet. All diets were given in crumbles form and the birds had free access to water and feed throughout the entire experiment (1-3 weeks). The experimental diets were formulated to meet or exceed the minimum National Research Council (1994) requirements for Leghorn chickens. Chick performance was measured in terms of feed consumption, weight gain and feed to gain ratio.

Three hundred and sixty Leghorn chicks were purchased from a commercial hatchery and raised in Jamesway battery brooders for 7 days. All chicks were fed a commercial chick starter diet during the 7 days pre-experimental period. At 7 day of age, the birds were randomly distributed in Petersime battery brooders among 12 treatments using 6 birds per pen and 5 pens per treatment. The treatments were as follow: 1) Wheat-

Table 1. Composition of experimental diets (%)

Ingredients	Lupin cultivar[1]					
	1	2	3	4	5	6
Wheat (15.4% CP)[2] (%)	33.67	33.81	35.32	40.32	39.59	37.53
Soybean conc. (%)	6.50	6.10	4.90	—	0.40	2.50
Lupin (%)	50.00	50.00	50.00	50.00	50.00	50.00
Sunflower oil (%)	6.00	6.00	6.00	6.00	6.00	6.00
Dicalcium phosphate (%)	1.10	1.10	1.10	1.32	1.32	1.28
Calcium carbonate (%)	1.31	1.27	1.25	0.98	1.25	1.24
DL-Methionine (%)	0.07	0.07	0.08	0.13	0.12	0.10
Vitamins[3] (%)	1.00	1.00	1.00	1.00	1.00	1.00
Minerals[4] (%)	0.35	0.35	0.35	0.35	0.35	0.35
Enzyme mix[5]	±	±	±	±	±	±
Calculated analysis						
Crude protein (%)	23.50	23.47	23.46	23.48	23.45	23.42
ME (kcal kg^{-1})	2,977	2,987	2,984	2,969	2,960	2,969
Lysine (%)	1.13	1.13	1.13	1.01	1.02	1.03
Meth. + cyst. (%)	0.71	0.71	0.70	0.74	0.75	0.72
Calcium (%)	0.80	0.80	0.80	0.80	0.80	0.80
Available phosphorus (%)	0.40	0.40	0.40	0.40	0.40	0.40

[1] Lupin cultivars: 1-Pnognus; 2-NL; 3-LAD; 4-Hutterite; 5-Amiga; 6-Brandon. [2] CP: Crude protein. [3] Vitamin 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}, 12.5 µg; riboflavin, 5.5 mg; Ca panthotenate, 11 mg; niacin, 53.3 mg; choline chloride, 1,020 mg; folic acid, 0.75 mg; biotin, 0,25 mg; delaquin, 125 mg; DL-methionine, 500 mg; amprol, 1 g. [4] Mineral mix supplied the following per kilogram of diet: Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.18 mg; NaCl, 2,500 mg. [5] Enzyme added to the diets were 0.1% each of Energex® + Bio-Feed Pro® + Novozyme®.

Pnognus lupin cultivar (WL1); 2) WL1 + E (0.1% Energex, 0.1% Bio-Feed Pro and 0.1% Novozyme); 3) Wheat-NL lupin cultivar (WL2); 4) WL2 + E; 5) Wheat-LAD lupin cultivar (WL3); 6) WL3 + E; 7) Wheat-hutterite lupin cultivar (WL4); 8) WL4 + E; 9) Wheat-Amiga lupin cultivar (WL5); 10) WL5 + E; 11) Wheat-Brandon lupin cultivar (WL6); 12) WL6 + E. The composition of the diets is given in Table 1. The experimental design was a completely randomized block with a factorial arrangement of treatments: 6 (six lupin cultivars) × 2 (two enzyme doses, 0, 0.3%). Dry matter, crude protein (N × 6.25), ether extract, neutral detergent fiber (NDF), acid detergent fiber (ADF), ash, calcium, phosphorus, and manganese were determined by AOAC methods (1984). Amino acid analyses were performed by the method of Moore and Stain (1963) with a Beckman automatic analyser. Samples were prepared for cystine analyses by the method of Hirs (1967). The gas and thin-layer chromatography methods (Muzquiz *et al.,* 1982, 1989) were used to respectively

Table 2. Chemical composition of different lupin seed cultivars (*L. albus*) (% dry matter).

	Lupin cultivar[1]					
	1	**2**	**3**	**4**	**5**	**6**
Dry matter (%)	94.07	94.57	94.00	94.71	94.92	94.58
Protein (%)	25.78	26.27	27.88	34.62	34.12	31.15
Ether extract (%)	10.96	10.31	9.68	7.32	9.97	10.30
ADF (%)	17.44	17.73	16.96	14.70	13.00	16.65
NDF (%)	22.78	19.99	20.85	22.11	17.93	23.45
Ash (%)	3.44	3.29	3.18	4.14	3.74	2.93
Calcium (%)	0.22	0.25	0.26	0.39	0.19	0.21
Phosphorus (%)	0.36	0.33	0.29	0.37	0.41	0.29
Manganese (mg kg^{-1})	1,412	1,348	1,075	574	1,044	1,335
Hull (%)	16.91	15.70	18.30	17.67	16.02	17.84
Amino acid (%)						
Alanine	0.94	0.92	1.00	1.16	1.18	1.05
Arginine	2.18	2.38	2.43	3.69	3.49	2.84
Aspartic acid	2.67	2.79	3.00	3.71	3.60	3.23
Cystine	0.49	0.57	0.49	0.55	0.58	0.54
Glutamic acid	5.49	6.14	6.09	7.80	7.67	7.29
Glycine	1.11	1.09	1.22	1.44	1.41	1.31
Histidine	0.62	0.62	0.67	0.82	0.80	0.74
Isoleucine	0.93	0.95	1.00	1.25	1.25	1.03
Leucine	1.93	2.02	2.09	2.53	2.56	2.30
Lysine	1.28	1.32	1.43	1.70	1.67	1.49
Methionine	0.31	0.25	0.30	0.31	0.32	0.29
Phenylanine	1.00	1.16	1.04	1.26	1.36	1.30
Proline	1.26	1.25	1.41	1.54	1.61	1.37
Serine	1.44	1.55	1.60	1.91	1.90	1.79
Threonine	1.01	1.03	1.20	1.27	1.29	1.16
Tyrosine	0.89	1.00	0.99	1.28	1.30	1.19
Valine	0.99	0.98	1.05	1.20	0.24	1.04
Ammonia	0.25	0.27	0.26	0.34	0.32	0.30
Total alkaloid[2] (%)						
Lupanine	0.01	0.02	0.01	0.04	0.01	1.34
Isolupanine	ND[3]	ND	ND	ND	ND	0.04
13-OH lupanine	ND	ND	ND	ND	ND	0.01
Angustifoline	ND	ND	ND	ND	ND	0.03
Total alkaloid	0.01	0.02	0.01	0.04	0.01	1.42

Triplicate analysis were carried out on representative values. [1] Lupin cultivars: 1-Pnognus; 2-NL; 3-LAD; 4-Hutterite; 5-Amiga; 6-Brandon. [2] Using gas chromatography method. [3] ND: Non detected.

determine the total alkaloid content and their qualitative distribution in lupin cultivars (lupanine, isolupanine, 13-OH-lupanine and angustifoline).

The data (pen means) were subjected to analysis of variance using the General Linear Models (GLM) procedure of SAS® software (SAS Institute, 1986). The experiment was analyzed by ANOVA in 6 (lupin cultivars) × 2 (enzyme concentration) factorial arrangements of treatments, and single df contrasts were used to separate treatment means in the factorial experiment.

The chemical composition and the amino acid content of the different cultivars of lupin seeds are presented in Table 2. A comparison of the gross composition of the six cultivars of lupin indicated differences among cultivars in percent protein, amino acids, ether extract, NDF, ADF, ash, calcium, phosphorus and manganese. The chemical composition shows good agreement with that quoted by Hill (1977) and Gdala and Buracewska (1996) for other *L. albus* cultivars. The variation in the crude protein content observed in the different cultivars studied was in agreement with the results reported by Petterson *et al.* (1997). The content of essential amino acids in lupin seed protein was found to be relatively balanced except for methionine which is low relative to the requirements of the growing chicks. The amino acid composition was similar to that reported by Hove (1974), Yule and McBride (1976), and Hill (1977). Among the different minerals, the most significant differences is reported in Mn content. These results confirm those showed by Hung *et al.* (1987, 1988). In general, lupin tend to accumulate manganese when grown in acidic soils. Karunajeewa and Bartlett (1985) reported a manganese level in seed of *L. albus* cv. Hamburg grown in Victoria (Australia) that ranged from 900 to 3,920 mg kg^{-1}. The alkaloid content (Table 2) shows that five of the cultivars are low alkaloid lupins (Pnognus, NL, LAD, Hutterite and Amiga), with concentrations ranging from 0.01 to 0.04%. Brandon cultivar had the highest alkaloid content (1.42%). The dominant alkaloid in the Brandon cultivar was lupanine with 1.34%. Isolupanine, 13-OH-lupanine and angustifoline content were only detected in this cultivar. The concentrations of alkaloids in these cultivars are similar to those reported by Hill (1977) using *L. albus* cultivars. In general, the alkaloid content of the *L. albus* exhibits less variation within individual cultivars than *L. angustifolius* cultivars (Harris *et al.,* 1986).

The performance data are presented in Table 3. The weight gain and feed consumption of the birds fed the high alkaloid content (Brandon cultivar) were reduced significantly ($P < 0.0001$; up to 43% and 31%, respectively) and feed to gain ratio increased significantly ($P < 0.0001$; up to 21%) in comparison to the other cultivars. The addition of the enzyme mixture to the diet increased weight gain ($P < 0.0033$) and feed consumption ($P < 0.0116$) by 5 and 1%, respectively, and reduced ($P < 0.0055$) feed to gain ratio by 4% only in the low alkaloid varieties.

Table 3. Effect of enzyme addition on the performance of Leghorn chicks (1-3 weeks) fed six lupin cultivars with different alkaloid content

Main effects	Weight gain (g)	Feed consumption (g)	Feed to gain ratio ($g\ g^{-1}$)
Diets			
Pnognus	116^{b}	234^{bc}	2.02^{de}
Pnognus + Enzyme	122^{a}	235^{ab}	1.93^{f}
NL	112^{c}	224^{e}	2.00^{e}
NL + Enzyme	118^{b}	226^{e}	1.92^{f}
LAD	113^{c}	225^{e}	1.99^{e}
LAD + Enzyme	121^{d}	232^{bc}	1.92^{f}
Hutterite	117^{b}	233^{bc}	1.99^{e}
Hutterite + Enzyme	123^{a}	237^{a}	1.93^{f}
Amiga	108^{d}	229^{d}	2.12^{c}
Amiga + Enzyme	113^{c}	231^{cd}	2.04^{d}
Brandon	69^{e}	160^{f}	2.32^{b}
Brandon + Enzyme	68^{e}	162^{f}	2.38^{a}
Enzyme[1]			
No enzyme	106^{b}	218^{b}	2.07^{a}
Enzyme	111^{a}	221^{a}	2.02^{b}
Pooled SEM	2.24	2.25	0.03
Source of variation		**Probabilities**	
Diets (D)	0.0001	0.0001	0.0001
Enzyme (E)	0.0033	0.0116	0.0055
D × E	0.0012	0.0487	0.0001
Contrast			
Without E *vs* E (LAD)[2]	0.0001	0.0149	0.0001
Without E *vs* E (HAD)[3]	NS	NS	NS

[a,b,c,d,e,f] Means within column having different superscript are significantly different. [1] Enzyme added to the diets were 0.1% each of Energex® + Bio-Feed Pro® + Novozyme®. [2] LAD: Low alkaloid diets (Pnognus, NL, LAD, Hutterite, Amiga). [3] HAD: High alkaloid diet (Brandon). The respective values for weight gain (g), feed consumption (g) and feed/gain ratio being 119, 232, and 1.95 for diets 1 to 5 with enzyme; 113, 229, and 2.02 for diets 1 to 5 without enzyme; 68, 162, and 2.38 for diet 6 with enzyme and 69, 160, and 2.32 for diet 6 without enzyme.

The results of the performance experiment demonstrated that Leghorn chicks fed a 50% of high alkaloid lupin diet performed poorly compared with those fed the low alkaloid lupin cultivars. This significant depression in chick performance was associated with a significant reduction in feed intake. The feed consumption was depressed up to 31% compared with the birds fed with low alkaloid content cultivars. Guillaume *et al*. (1979) found that alkaloids in bitter varieties of lupins depressed the appetite in chickens. In contrast, Buracewska *et al.* (1993) revealed no negative correlation between intake of the diets and their alkaloid content in chickens. This could be due to the use of a low content of alkaloids as they only incorporated 30% of lupin in the diet. In the current study the positive effect on the performance of birds fed enzymes in the low alkaloid cultivars has been previously demonstrated by Brenes *et al.* (1993, 2002) using the Amiga cultivar in the diet. This effect has also been demonstrated by other authors (Annison *et al.,* 1996; Ferraz Oliveira, 1998; Naveed *et al.,* 1999) and corroborated by Kocher *et al.* (2000) and Brenes *et al*. (2002) that also showed that addition of a commercial enzyme preparation to a lupin-based diet resulted in a significant increase in the ileal digestibility of non-starch polysaccharides.

In conclusion, the results of the present study show that lupin cultivars with a high alkaloid content compared to those with a low alkaloid content are poorly utilized by chicks and that enzyme addition to diets with the white lupin low alkaloid cultivars improve their nutritional value.

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