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# Effects of *Lupinus angustifolius* (cv. Arabella and Kalya) on the growth and serum profiles in broilers

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## Abstract

This work examined the effects of incorporation of exogenous enzyme preparation on growth and performance traits in broilers fed diet containing 400 g/Kg of *L. angustifolius*(cv. Arabella) of Europe and *L. angustifolius (*cv. Kalya) obtained from Australia. A maize- soy (MS) based diet served as a control diet and acted as positive, while non-protein control diet acted as a negative control (NPC) and fed ad *libitum* for 18 days. Growth rate, apparent digestibility of N, fat and excretion of essential minerals were assessed. In comparison to broilers fed maize-soy (MS) diet, broilers fed the *L. angustifolius* of European origin (EL) diet, consumed less feed, had considerably lower body weight gain, as well as lower apparent digestibility. Addition of Rena phytase to the EL (ELPH) and *L. angustifolius of Australian origin* (ALPH) increased feed intake, however, the feed gain ratio was found to be decreased. . This study indicates that a diet containing high level of lupin meal is detrimental to feed intake broilers and thus affected their performance. However, when lupin meal diet was supplemented with Rena phytase, performance parameters were not different from those obtained from raw meal. The inclusion of lupin meal with or without exogenous enzyme had significant effect on the mineral content of the faeces and on mineral balance. The results suggest that lupin protrein is involved in the elevated loss of minerals associated with long-term effects of this seed as protein source in poultry. Compared to those of control, the N excretion was in the range of 14.9% (EL), 15.6% (ELPH) and 13.9% (ALPH). On the other hand, digestibility was found to be in the range of 78% (EL) and 73% (ELPH) than the corresponding control fed on (MS) diet. The fat excretion was found to be highest in (ALPH) fed diet 19.1% as compared to those of control. True fat digestibility was found to be at the lowest level n ALPH group. The present work has revealed a very remarkable property of *L. angustifolius* of Australian lupin, that has caused high level of fat excretion in the droppings of broilers fed this as compared to those of its European counterpart. It is highly likely that Australian lupin due to its gel forming character may have caused this kind of response in fecal lipid excretion.

**Key words:** broiler, *Lupinus angustifolius,* performance

 

## Introduction

*Lupinus angustifolius*(sweet lupin) seeds are widely used in Australia as a source of protein and energy in livestock feeds, and hence, their nutritive value has been evaluated in various feeding studies in pigs and poultry. Overall, nutritional studies in pigs and chickens indicate that lupin feeds are generally well tolerated (Olkowski et. al., 2005; Zraly et al., 2007); however, due to the generally low levels of both methionine and lysine in lupin (Petterson, 1998), feeds for pigs and poultry are more beneficial when they include multiple sources of protein, or supplemental amino acids (Edwards and van Barneveld, 1998). Seed meal was analyzed for its content of starch, non-starch polysaccharides (NSP) and oligosaccharides. Lupin seed meal contained negligible amounts of starch and contained high levels of NSPs; most of which were insoluble in aqueous solvents and were polymers of arabinose, xylose, galactose, glucose and uronic acids. Raffinose and stachyosee were found in the undialysed fraction. The level of verbascose was found to be higher in undialysed fraction than in the lupin meal. Tow unidentified peaks were also detected between raffinose and stachyose and stachyose and verbascose on GLC (Rahman, 1994). Rahman*et al*. (1997) conducted metabolic balance studies in growing rats to investigate the effect of *Lupinus angustifolius* (sweet lupin) seed meal and its fractions on the balance of calcium, phosphorus, magnesium, potassium, sodium and apparent absorption of zinc. However, there is little information in broilers when they are fed on sweet lupin based diets. Thus, the

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present work was aimed to determine the effects of phytase fed on *Lupinus angustifolius*cv. Arabella and Kayla and to estimate total cholesterol, BUN and triglyceride levels in the serum after being fed on the diets based on sweet lupin.

## Materials and Methods

**Seeds and chemicals**

Sweet lupin (*Lupinusangustifolius*) cv. arabella seeds were obtained as donation from Dr. Peter Roemer, SuedwestdeutscheSaatzucht GmbH & Co. Kg, ImRheinfeld 1-13, D-76437 Rastatt, Germany and Michael D. Unruh, President of Shasta Foods International, Inc. California, USA. *Lupinusangustifolius* cv. Kalya was obtained from Dr. Mark Withington of the Department of Agriculture, Perth, Western Australia. Maize starch and soybean meal, oil, vitamin and minerals were purchased from local sources. Rena phytase was obtained as gift from Renata Bangladesh. All other chemical used were of Analar grade.

**Diets**

The feed was prepared by incorporating the ingredients listed in Table. 1. Raw lupin seeds were ground into a fine meal with a grinding machine and the diet was prepared according to Rahman, (2000).

**Experimental designs and feeding protocol**

***Pre experimental period***

Twenty day old Cobb 500 hundred broiler chicks were obtained from local hatchery through a local agent in Mymensingh and were housed on rice husk unit 14 day of age. They were fed commercial broiler starter diet *ad libitum* and reared on husk floor. Water was supplied *ad libitum* in plastic water trough. The body was monitored daily

***Bird husbandry and sample collection***

A total of 20 growing broilers, 4 birds in each group were weighed on day 15 (weighing 555.3±5.03g) and randomly allocated to cages. Two broiler bird were placed in each cage. There were a total of four experimental and two control (positive and negative non-protein control) diets.

**Table 1.** Composition of diet (g/Kg)

| Ingredients | Diet supplemented with NPC (Non-protein control) | Diet Supplemented with Maize-Soyabean | Diet supplemented with European Lupin (EL) | Diet supplemented with European lupin+RenaPhtase (ELPH) | Diet supplemented with Australian lupin+Renaphytase (ALPH) |
|---|---|---|---|---|---|
| Maize crush | 10.5.0 | 576.0 | 376.0 | 376.0 | 376.0 |
| Maize starch | 714.0 | 0 | 0 | 0 | 0 |
| Rice polish | 0 | 0 | 140.0 | 140.0 | 140.0 |
| Lupin meal cv. | 0 | 0 | 400.0 | 400.0 | 0 |
| Lupin meal cv. Kalya | 0 | 0 | 0 | 0 | 400.0 |
| Soy bean meal | 0 | 358 | 0 | 0 | 0 |
| DCP | 28.0 | 12.0 | 12.0 | 10.5 | 10.5 |
| Vit-Mineral mix | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Maize oil | 147.0 | 42.0 | 60.0 | 60.0 | 60.0 |
| Methionine | 0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Lysine | 0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Salt (iodized) | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Rena phytase | 0 | 0 | 0 | 1.5 | 1.5 |
| Total | 1000 | 1000 | 1000 | 1000 | 1000 |

Diets were fed *ad libitum* from day 15 for a period of 18 days (conventional slaughter weight). Body weight, feed intake, feed refusal and fecal weights were recorded every day. Cumulative feed efficiency per bird was calculated as the ratio of weight gained to feed consumed. Dry matter of excreta was determined from the last 10 d of feeding trial by drying aliquots of every day's droppings for 6 h at $105^0$C. The number of chicks with sticky droppings adhering to the cloacae was noted on the same days of excreta collection. Morality throughout the trial did not occur. Water was available *ad libitum* throughout the study, via plastic water trough. On the 19$^{th}$ day of the experiment, birds were slaughtered and blood samples were collected and serum was prepared and kept at-$20^0$C**.**

***Serum collection and biochemical parameters analysis***

On the 19$^{th}$ day of the experiment, birds were killed by severing the jugulars and blood samples were collected in tightly rubber stoppered glass test tubes and serum samples were collected. Serum samples were contrifuged at 3, 000 xg for 15 min in a bench top centrifuge. Clear serum samples were analysed for cholesterol,triglycerides and blood urea nitrogen (BUN) using commercially available test kits.

## Results and Discussion

When the broilers fed on the diet containing raw lupin of European origin (EL) as the sole source of dietary protein, their food intake was similar as compared to those of the broilers fed on control fed on maize-soy (MS) diet.

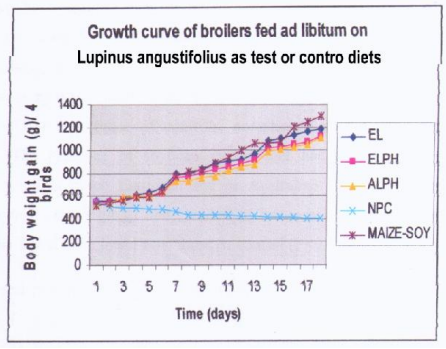


**Figure 1.** Growth curve of broilers fed *Lupinus angustifolius* of Australian and European origins fed with or without phytase

However, body weight gain was found to be lower in broilers fed raw EL. The food intake failed to pick up by the addition of phytase to the diet thus the body weight gain failed to show any improvement when compared with the growth data of EL group.

Compared to those of broilers, fed *L. angustifolius* of Australian origin fortified with Rena phytase (ALPH) grew slower that the corresponding broilers fed on phytase fortified European lupin (ELPH). As a whole, broilers from ALPH grew at a slower pace than any other groups involved in feeding trials. Lupin meal was deficient in a number of essential amino acids including methionine, and wascorrected by addition during feed preparation. Indeed, food intake and growth rate was lowest in broilers fed on non-protein control.

The N intake of broilers on the EL and ELPH was much similar to those of control birds fed on MS diet. On the other hand, N intake was much lower in group fed on ALPH. However, despite this, the N digestibility value obtained from this group was higher than the control and other dietary groups. Considering that the amount of added phytase (1.5 g/Kg diet) was comparatively small, these should not have increased apparent N excretion which was even higher in ELPH than those of positive control broilers fed on MS diet. Compared to those of *L. anugustifolius* cv. Arabella, the N digestibility value for *L. angustifolius* for Australian orgin was much higher (81%). Therefore, the higher N digestibility value obtained was probably a consequence of the comparative lower level of anti-nutritional factor(s) in Australian lupin than the corresponding European lupin. . Final body weight and feed intake of broilers fed diets containing whole lupin meal of European origin (400 g/kg) (Table 1) were lower ($P<0.05$) than those of controls, nonetheless gain feed ratios were not different. The presence of soybean in the diet had no effect on productive parameters. Birds given European and or Australian lupin with added enzyme (Rena phytase @ 1.5 g/Kg) diets tended to increase feed intake however, final weight was found to be lower than those of control ($P<0.05$).

Over the test period, the true lipid digestibility was reduced by broilers given diet based on ALPH and thus the lipid absorption by broilers fed on this diet was only 57% and was estimated to be 21% less than those of broilers given the MS diet. This was because of the very high faecal loss from broilers of this group. In contrast, with the MS diet, the true fat digestibility is above 70% by broilers and thus faceal loss was found to be reduced 16.9% which was the lowest as compared to those

calculated from the broilers fed on test diets. As found from the Table 1, inclusion of ALPH in the diet caused an increase in feed intake and faecal N output was found to be decreased as compared to those of MS fed control indicating that N excretion from the absorbed N by the broilers fed on this diet are not contributing to the growth since, broilers fed on this diet lost 43% of its ingested fat through droppings. Therefore, it is possible that the effect is due in part to malabsorption of dietary lipid.

Weight gain was lower in whole lupin of European origin seed meal fed birds than in controls; this was most likely due to depressed feed intake in chicken given whole seed meal, as gain: feed values were not affected. This suggests that the presence of large amount of insoluble lupinfibre in the seed meal affected feed intake. Previous studies (Rahman*et al.* 1996) have shown that non-starch polysaccharides comprised 327 g/Kg seed meal (on air dry basis) and 64.6% of the residue in a dry-matter basis after having extracted with water and McIlvaine's buffer at pH 7. The monomer sugar of polymers in the residue in greatest amount was glucose, followed by galactose, arabinose, uronic acid, xylose and a small amount of mannose. As previously shown, degradation of lupin fibre in the small intestine is very limited in birds, which leads to accumulation of undigestible material in the intestine is very limited in birds, which lead to accumulation of undigestible material in the intestine and lower feed intake (Berne*set al.*, 1992). However, the inclusion of Rena Phytase-400 in whole European and Australian lupin meal improved feed intake although body weight gain was found to be depressed. As there is no declared carbohydrase activity in this product, the effect should not be directly due to increased polysaccharide degradation. Kocher *et al.* (2000) showed that the addition of a commercial enzyme preparation to a lupin based diet resulted in a significant increase in the ileal digestibility of NSP, related to the digestibility of glucose, xylose and arabinose.

Previous data from rats pings suggest that proteins, and not the presence of anti-nutritional factors , are the main factors influencing the nutritional value of sweet lupin seeds for monogastrics. Although, the explanation for this is still not clear, it is highly likely that the effects of growth depression in broilers fed enzyme treated lupin meal are mainly related to the absorption and metabolism of the protein (Rubio, 2000). It was suggested that phytase and carbohydrate combinations might be used in the diet to improve broiler performance and to have maximum benefit. Feed intake and feed conversion broiler performance and to have maximum benefit.

**Table 2.** Effect of sweet lupin (*Lupinus angustifolius*) on performance of broilers (mean value ± Standard deviation)

| **Parameters** | **Dietary groups** | | | | |
|---|---|---|---|---|---|
| | **Non-protein control (NPC) (4)** | **Maize-soya (MS) (4)** | **European Lupin (EL) (4)** | **European Lupin+ Phytase (ELPH) (4)** | **Australian Lupin+ Phytase (ALPH) (4)** |
| Starting body wt. (gm) | 555.3 ± 5.03 | 522.3 ± 45.03 | 562.3 ± 18.9 | 552.75 ± 3.3 | 536.3 ± 19.3 |
| Final body wt. in av. (gm) | 424.2 ± 22.0 | 1294.50 ± 45.5 | 1181.0 ± 24.1 | 1120.5 ± 46.2 | 1109.7 ± 26.7 |
| Body weight gain as% of initial weight (g) | -23.6 | +147.8 | +110.0 | +102.7 | +106.1 |
| Total feed intake (gm)/4 birds | 2443 | 8078 | 8024 | 8304 | 8387 |
| Protein efficiency ratio (PER) | | 1: 0.64 | 1: 0.59 | 1: 0.54 | 1: 0.53 |
| Nitrogen intake % | 3.30 | 23.19 | 22.10 | 21.04 | 19.96 |
| Fecal nitrogen excretion% | - | 75 | 78 | 73 | 81 |
| Fat intake % | 6.5 | 23.9 | 27.1 | 23.2 | 21.03 |
| Fat excretion % | 10.1 | 16.90 | 18.06 | 18.3 | 19.1 |
| True fat digestibility | - | 72 | 71 | 64 | 57 |

**Table 3.** *Lupinus angustifolius* (sweet lupin) seed meal added with enzyme on faecal weight and water content of dropping of broilers (mean values ± Standard deviation)

| Parameters | Dietary groups | | | | |
|---|---|---|---|---|---|
| | **Non-protein control (NPC) (4)** | **Maize-soya (MS) (4)** | **European Lupin (EL) (4)** | **European Lupin+ Phytase (ELPH) (4)** | **Australian Lupin+ Phytase (ALPH) (4)** |
| Total fecal wt. (gm) in last 10 days/bird | Not determined | Not determined | 486.5 | 500.8 | 507.0 |
| Total dry droppings (g per 10 days)/ bird | Not determined | Not determined | 114.4 ± 3.8 | 115.7 ± 4.6 | 132.9 ± 6.3 |
| Water in droppings (%) | Not determined | 65.3 ± 2.3 | 76.5 ± 5.4 | 76.9 ± 3.8 | 73.8 ± 4.5 |
| Water in droppings (g per 10days) | Not determined | Not determined | 372.1 ± 3.9 | 385.2.9 | 374.1 ± 6.2 |
| Fecal nitrogen excretion (%) | 10.00 | 15.58 ± 3.2 | 14.86 ± 2.9 | 15.634 ± 3.1 | 13.86 ± 5.6 |

Digestibility values reflect the disappearance of amino acids from the intestinal lumen, but they do not provide any indication of as to how this protein is handled by the enterocytes of general protein metabolism. In this respect, it is interesting to notice that liver metabolism together with serum cholesterol and triglycerides were probably affected by lupin inclusion in the diet. It is worth mentioning here the works of Batterham *et al.* (1986), who found that even though amino acid digestibility, including that of lysine was high, lysine availability for lupin seedmeal was lower than for soybean and that this effect is not due to the presence of heat labile ANFs. They suggest that either there is another unidentified ANF present in lupin seeds, or the lysine may be in a form that is absorbed but not efficiently utilized. The present experiment (Table 2) has shown that the birds fed on raw European lupin excreted high level of N in the faeces while faecal fat excretion was found to be elevated in broilers fed on Australian lupin. It is generally believed (Widdowson, 1947) that fiber decreases energy availability by hastening transport through the gut and hence increases the nitrogen and fat in the feces. Mechanism for the decreased digestibility of fat and nitrogen seen with ingestion of some fiber sources are not well understood. However, the additional fecal fat could represent bacterial lipids (Cummings *et al.,* 1979) or a decreased absorption of dietary fat (Losowsky, 1979). Most evidence supports the idea that fiber causes in increase in unabsorbed dietary fat. The present evidence on lipid profile is very similar to those observed by Rubio *et al.* (2003). In fact, very little is known about the regulation of lipoprotein synthesis and secretion in avian liver, at least in growing birds.

**Table 4.** Serum concentration of cholesterol, triglycerides and blood urea nitrogen in growing broilers fed on raw *L. angustifolius* of European and Australian orgin and incorporation of Rena phytase in the diet

| Parameters | Dietary groups | | | | |
|---|---|---|---|---|---|
| | **Non-protein control (NPC) (4)** | **Maize-soya (MS) (4)** | **European Lupin (EL) (4)** | **European Lupin+ Phytase (ELPH) (4)** | **Australian Lupin+ Phytase (ALPH) (4)** |
| Cholesterol (mmol/ l) | 5.45 | 1.40 | 0.56 | 1.54 | 1.46 |
| BUN (mmol/ l) | 2.41 | 2.81 | 2.38 | 2.55 | 2.08 |
| Triglycerides (mmol/ l) | 2.12 | 5.05 | 1.96 | 1.99 | 1.39 |

As shown in Table 3. inclusion of lupin seed meal (EL, ELPH and ALPH) in the diet caused a significant high in both wet weight and dry faecal weight. In broilers fed on these diets, the water level in the faeces was significantly increased as compared to those of control. Compared to those of *L. angustifolius* of European origin, the faecal dry weight was increased significantly in broilers fed on *L. angustifolius* of Australian origin. Moreover, Table 4 showed that serum cholesterol concentration was about 2.5 times lower in broilers fed on European lupin as compared to those of control fed on maize-soya. In contrast, broilers fed on lupin diets showed an increase in cholesterol as compared to those of control. However, BUN level was found to be decreased in these birds and triglycerides level was recorded to be at its lowest level among the groups. A matter requires further studies.

## Conclusion

In conclusion, this study showed four completely new features of deleterious effects on *Lupinus angustifolius*:

a) Depression of feed intake and growth rate in broilers fed lupin meal of Europe and/or Australian origin.

b) Although there had an increase in feed intake due to incorporation of Rena-phytase, however, the feed gain ratio was poor as compared to those of control.

c) Despite having low phytate in the meal, mineral excretion in the droppings were very high.

d) *L. angustifolius* of Australian origin excreted highly significant amount of both N and fat in the droppings.

The scale of these changes could be directly correlated with the very high level of non-starch polysaccharides. Thus, only exogenous enzyme like addition of phytase alone in the lupin may not be appropriate to improve the broiler's performance.

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