UTILIZATION OF LUPIN (*Lupinus angustifolius*) SEEDS SUPPLEMENTED WITH AMINO ACIDS BY CHICKS

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Summary

The utilization of feeding white sweet lupin (*Lupinus angustifolius* cv. Uniwhite) seeds supplemented with the limiting amino acids were investigated in day-old single comb White Leghorn male chicks. These were fed a commercial chick mash for the first 10 days and on a semi-synthetic protein-free diet for the next 6 days. For the subsequent 6 days of experimental feeding period, the birds were fed on the protein-free diet, basal diet containing 9.31% of lupin seed meal (LSM) protein, diets supplemented with methionine, methionine+tryptophan or methionine+tryptophan+lysine in the basal diet, and diet containing 9.84% of soybean meal (SBM) protein.

When the LSM protein was supplemented with methionine, protein intake, body weight gain, protein efficiency ratio (PER) and net protein ratio (NPR) were increased ($p < 0.05$). The birds excreted lower urinary nitogen and fecal nitrogen per protein consumption, had improved apparent (AD) and true (TD) digestibility but did not alter biological value (BV) of the protein. Metabolizability (MEn/GE) and heat production (HP) per MEn intake (HP/MEn) was lowered while energy retention (ER) was highered ($p < 0.05$) compared with those of the basal diet. Also the body weight gain, PER, NPR and ER was increased but the BV and HP/MEn was lowered compared with those of the SBM protein.

The results indicated that lupin seed supplemented with methionine increase body weight gain and energy rentention but did not alter biological value compared with those of lupin seed and soybean meal.

(**Key Words**: *Lupinus angustifolius,* Protein, Digestibility, Biological Value, Heat Production, Methionine, Antinutrients, Poultry)

Introduction

Pearson and Carr (1977) reported poor growth and food consumption by pigs fed on three different samples of lupin-seed meal (LSM) of *Lupinus albus* cultivar Neuland. And pigs fed on *L. angustifolius* LSM, an alkaloid-free variety, however, grew well at about 70% of the rate of pigs on the control diets. The alkaloid contents of *L. angustifolius* was lower than 0.01% and the major alkaloid components of *L. albus*, a sweet lupin, was identified as DL-lupanine (Ruiz et al., 1977).

Also Payne et al. (1972) showed that the protein of Uniwhite lupin seeds, a sweet variety of *Lupinus angustifolius*, was low in lysine, methionine and tryptophan compared with soybean protein. And the poor growth and food comsumption may be resulted by the limiting amino acid, methionine, deficiency in the LSM protein(Hove, 1974, Hanczakowski et al., 1981, Schoeneberger et al., 1981). Also the protein quality of *L. albus* was definitely low and improved on supplementaion with DL-methionine (Hanczakowski et al., 1981, Schoeneberger et al., 1981).

When birds fed on diets containing graded levels LSM (*Lupinus angustifolius* cv. Uniwhite), diet consumption, weight gain, protein efficiency ratio (PER), biological value (BV) and heat production (HP) were higher in birds fed diets containing 20% of LSM than in that fed 30% of LSM (Nam and Koh, 1991). This will be due to the lupin alkaloid poisonings or the liminting amino acids deficiency of the LSM protein.

The objectives of the present work was to

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study the effect of the supplementation with DL-methionine, L-lysine or L-tryptophan on the biological utilization of the *L. angustifolius* protein in comparison with those of soybean meal in chicks, and the effect of the protein quality on the energy utlization of the diets was also studied.

Materials and Methods

Experimental diets

The composition of a semi-synthetic protein-free (lupin seed 0%), basal and control diets are given in table 1. Whole seeds of *L. angustifolius* were milled by Willey mill to give a meal (LSM). Basal diet containing 30% of the LSM and control diet containing 21.49% of soybean meal (SBM) were prepared by substitution of LSM and SBM, respectively, for the same amount of corn starch in the protein-free diet. And diets substituted with 0.241% of DL-methionine (Met), 0.241% of DL-methionine plus 0.07% of L-tryptophan (Met + Trp), and 0.241% of DL-methionine plus 0.07% of L-tryptophan plus 0.229% of L-lysine (Met + Trp + Lys) for the corn starch in the basal diet were prepared, respectively. The substitution rate of the Met, Trp and Lys were decided, respectively, from the first, 2nd and 3rd limiting amino acids of the LSM protein which were calculated by the reported amino acid distributions for the lupin seed protein (Uzu, 1983, Pearson and Carr, 1976) and the whole egg protein (Scott et al., 1982). The basal, Met, Met + Trp, Met + Trp + Lys and SBM diets are isocaloric (as gross energy). Also the chemical compositions of the experimental diets are shown in table 2.

TABLE 1. COMPOSITION (%) OF PROTEIN-FREE, BASAL AND SOYBEAN MEAL DIETS

Ingredients	Protein-free	Basal	Soybean meal
Corn starch	83.365	53.365	58.875
Cellulose	5.0	5.0	5.0
Lupin seed meal	–	30.0	–
Soybean meal	–	–	21.49
Soybean oil	5.0	5.0	5.0
Vitamin mix.[1]	0.50	0.50	0.50
Choline-HCl (50%)	0.49	0.49	0.49
Ethoxyquine	0.015	0.015	0.015
Mineral mix.[2]	5.63	5.63	5.63

[1] Menge et al. (1965), [2] Nesheim et al. (1962).

TABLE 2. CHEMICAL COMPOSITION (%) OF EXPERIMENTAL DIETS

Diets	Protein-free	Basal[1]	Soybean meal
Moisture	10.9	10.8	10.8
Crude protein	0.64	9.31	9.84
Crude fat	7.68	11.32	11.90
Crude fiber	4.99	9.57	7.99
Ash	4.29	5.49	5.85

[1] Basal diet containing 30% of lupin seed meal.

Animals and procedures

Day-old commercial single comb White Leghorn male chicks were fed on a commercial chick mash for the first 10 days and fed on the protein-free diet for the next 6 days. And then for the subsequent 6 days, the protein-free, basal, Met, Met + Trp, Met + Trp + Lys and SBM diets were used for the experimental feeding of 6 groups of 7 birds each. Birds were raised at electrically heated brooder for the first 10 days and caged individually thereafter at room temperature of 24-29℃. Diet and water was given *ad libitum*.

During experimental feeding period, feed consumption was recorded every day at 24 hr interval and body weights were checked individually when the birds were 16 day-old and 22 day-old. Excreta were picked up every day and blended as soon as possible by Waring blender to avoid decomposition of nitrogenous compounds. An aliquots of the blended mixtures were taken for the extraction of nitrogenous compounds and the analysis of total nitrogen and ammonia. And the remnnant excreta were dried at 60℃ in the mechanically convected dry oven for the determination of combustion energy values. The nitrogenous compounds in excreta were extracted by the saturated lithium carbonate solution (Tasaki and Okumura, 1964). After removing materials in the digestive tracts, whole body with plumage were used for the determination of carcass composition when the birds were 16 day-old and

22 day-old for the calculation of heat production (HP).

Analysis of results

Total nitrogen and crude fat of diets were measured by AOAC (1980), and combustion energy values of diets and excreta were determined by an adiabatic bomb calorimeter. Uric acid (Baker, 1946), total creatinine (Hawk et al., 1954) and urea (Iino and Kameoka, 1969) were determined using the lithium carbonate extracts. And total nitrogen and ammonia contents of the blended excreta and nitrogen of carcass were also analysed by the procedures of AOAC (1980). The protein content of the carcass was the value obtained by multiplying 6.25 to nitrogen content. Total lipids of carcass were extracted by the method of Folch et al. (1957) and measured by gravimetrically.

Urinary nitrogen (UN) (Koh and Oh, 1984; Lim et al., 1986) were estimated on the basis that nitrogen contents of uric acid, ammonia, total creatinine and urea in the excreta are primary UN in poultry (Sturkie, 1976). And the value of fecal nitrogen (FN) was obtained by substracting UN from the total nitrogen of excreta. Metabolic faecal (MFn) and endogenous urinary (EUn) nitrogen were calculated from the nitrogenous compounds of birds fed the protein-free diet. The values for the protein efficiency ratio (PER), net protein ratio (NPR), true digestibility (TD) and biological value (BV) of protein and net protein utilization (NPU) were calculated (Koh and Oh, 1984).

Metabolizable energy (MEn) of diets was measured by the method of Hill and Anderson (1958). Energy retention (ER) was calculated by multiplying 9.5 kcal for a gram lipids retained and 3.84 kcal in the case of negative balance or 5.7 kcal in the positive balance for a gram protein retained (Brouwer, 1965). Then HP is the MEn intake minus ER during experimental feeding period.

All experimental results were subjected to analysis of regression and analysis of variance, followed by multiple comparison procedure (Snedecor and Cochran, 1967).

Results and Discussion

1. PER and NPR

As shown in table 3, chicks fed on the Met, Met + Trp and Met + Trp + Lys diets showed higher ($p < 0.05$) consumptions of diet and protein, weight gain, PER and NPR than those of the basal and SBM diets. Also those values of SBM diet were higher ($p < 0.05$) than those of the basal diet. Among birds fed the protein supplemented with amino acids, birds fed the Met + Trp diets had shown a tendency to decrease diet intake and weight gain, while PER and NPR were similar.

Birds fed on the protein-free, Met, Met + Trp and Met + Trp + Lys diets showed a

TABLE 3. EFFECTS OF DIETS BASED ON THE LUPIN SEED MEAL PROTEIN SUPPLEMENTED WITH METHIONINE, TRYPTOPHAN OR LYSINE ON THE PER AND NPR OF CHICKS

Diets	Protein-free	Basal	Met[1]	Met[1] + Trp	Met[1] + Trp + Lys	Soybean meal	LSD ($p<0.05$)
Diet intake (g/6 days)	39.1[a]	47.3[a]	94.5[c]	72.9[bc]	92.5[c]	67.9[b]	16.2
Protein intake (g/6 days)	0.25[a]	4.40[b]	9.35[d]	6.78[cd]	9.07[d]	6.68[c]	2.29
Initial weight (g/bird)	86.0	81.8	85.7	86.1	82.9	81.5	9.2
Weight gain (g/6 days)	−10.9[a]	−0.8[b]	35.1[d]	22.9[c]	33.9[d]	16.7[c]	8.0
PER[2]	−	−0.17[a]	3.76[b]	3.38[b]	3.74[b]	2.50	3.32
NPR[3]	−	2.07	4.92	4.99	4.94	4.23	3.31

[1] Amino acid diet in which 0.241% of methionine, 0.07% of tryptophan and 0.229% of lysine or combinations of it were supplemented to the basal diet.

[2] Protein efficiency ratio : weight gain/protein intake

[3] Net protein ratio : (weight gain of protein fed-those of protein-free diet fed)/protein intake.

[a-d] Means with different superscripts with the same row are significantly different at $p < 0.05$.

positive linear relationship between body weight gain (y, g) and the protein consumption (x, g) during 6 days of experimental feeding period with an equation of $y = 5.07x - 12.0$ ($r = 0.99^{**}$). By the extrapolation to zero protein consumption, the equation means that birds fed on the protein-free diet will grow- 12.0 g. But bird fed on the protein-free diet which contains 0.64% of protein grew −10.99 g (table 3). When the estimated value, −12.0 g, is applied for the calculation of the NPR, birds fed on the Met, Met + Trp and Met + Trp + Lys-supplemented protein gave 5.04, 5.16 and 5.06, respectively. The average value, 5.07 is in accord with the gradient of the equation which is also called nitrogen growth index (Allison, 1964). But Nam and Koh (1991) found that the NPR of the LSM protein lowered as the LSM intakes increased in birds fed on the graded levels of the LSM.

One of the symptoms of lupin alkaloids poisoning is lack of appetite in livestock (Garner, 1957). And Pearson and Carr (1977) reported poor growth and food consumption by pigs fed on three different samples of LSM of *L. albus* cultivar Neuland. However, pigs fed on *L. angustifolius* LSM grew at the rate of 70% of the pigs on the control diets. It was considered that symptons found in birds fed the basal diet was similar with the reported alkaloid poisoning. But birds fed on the protein supplemented with Met showed a linear relationships between protein consumption and weight gain. The decreased feed consumption and weight gain in birds fed basal diet were repaired completely in birds fed Met-supplemented diets, thus the Met supplementation may detoxicate the alkaloid poisoning. Also, the lowered feed consumption and weight gain could be caused by the Met deficiency of the LSM protein since diet consumption and weight gain of chicks is decreased also in Met deficiencies of protein (Kino and Okumura, 1986).

2. Digestibility and biological value of protein

Digestibilities and biological values of the protein supplemented with Met, Trp or Lys, and SBM protein are given in table 4.

Birds fed on the Met, Trp or Lys-supplemented LSM protein excreted higher ($p < 0.05$) daily urinary and fecal nitrogen than those of birds fed on the basal diet which was similar with those of birds fed on the SBM protein. Uric acid and ammonia were excreted linearly as the nitrogen intake increased (Tasaki and Okumura, 1964). And the UN and FN were excreted proportionally to the protein consumption (Koh and Oh, 1984; Nam and Koh, 1991). Also daily nitrogen intake (x, mg) correlated with the UN (y, mg) as shown in exponential equation of $y = 18.08e^{0.00519x}$ ($r = 0.99^{**}$) and with the FN (y, mg) as a linear equation of $y = 0.2215x +$

TABLE 4. BIOLOGICAL UTILIZATION OF LUPIN SEED PROTEIN SUPPLEMENTED WITH METHIONINE, TRYPTOPHAN OR LYSINE

Diets		Protein-free	Basal	Met[1)]	Met[1)] + Trp	Met[1)] + Trp + Lys	Soybean meal	LSD ($p<0.05$)
UN	(mg/bird/day)	18.6$^{a2)}$	35.9^{b}	70.1^{c}	47.4^{bc}	58.6^{c}	36.4^{bc}	16.9
	(mg/protein)	–	48.9^{a}	45.0^{ab}	41.9^{b}	38.8^{bc}	32.7^{d}	4.7
FN	(mg/bird/day)	5.2$^{a2)}$	33.4^{b}	63.5^{c}	44.4^{bc}	52.2^{bc}	39.4^{bc}	24.1
	(mg/protein)	–	45.5^{a}	40.7^{b}	39.3^{b}	34.5^{c}	35.4	4.5
AD	(%)	–	71.2^{a}	74.5	75.4	78.4^{b}	77.9	7.1
TD	(%)		75.8^{a}	76.6	78.3	86.6^{b}	80.8	7.3
BV	(%)	–	80.5	72.9^{a}	80.1	79.4	87.7^{b}	13.9
NPU	(%)	–	61.2	55.9^{a}	63.2	64.1	70.4^{b}	14.3

1) Amino acid diet in which 0.241% of methionine, 0.07% of tryptophan and 0.229% of lysine or combinations of it were supplemented to the basal diet

2) Endogenous urinary nitrogen (EUn) and metabolic fecal nitrogen (MFn).

UN : Urinary nitrogen, FN : Fecal nitrogen, AD : Apparent digestibility, TD : True digestibility, BV : Biological value, NPU : Net protein utilization (TD × BV).

Means with different supercripts within a row differ significantly at $p < 0.05$.

3.755 (r = 0.99**). It means birds fed on the protein-free diet excretes 18.1 mg of EUn and 3.8 mg of MFn daily per bird at 25-29℃ of room temperature.

However, the excretion of UN per protein consumtion was lower ($p < 0.05$) in birds fed the amino acids-supplemented LSM protein than in birds fed the LSM protein, while it was further decreased in birds fed the SBM protein. Also birds fed the amino acid-supplemented LSM diets and the SBM diet had lower ($p < 0.05$) FN per protein consumption than that fed basal diet. Thus it was found that higher AD and TD in birds fed on the Met, Trp or Lys supplemented protein than those of the LSM protein. The AD and TD were increased in the order of the supplementaion with the Met, Met + Trp and Met + Trp + Lys like the excretion of UN per protein consumption was. Also the AD and TD of the SBM protein were higher compared with those of the basal diet.

And birds fed on the basal diet had 80.5 and 61.2% of the BV and NPU, respectively, which were similar with birds fed the protein supplemented with amino acids and also lower ($p < 0.05$) than those of birds fed on the SBM protein. The BV were not significantly different among diets containing amino acids and NPU were increased in the order of the Met, Met + Trp and Met + Trp + Lys-supplemented protein since the TD were increased in the same order. The BV and NPU of Met-supplemented protein tended to decrease compared with those of the Met + Trp and Met + Trp + Lys-supplemented protein.

When birds were fed on the LSM protein supplemented with Met, Trp or Lys, nitrogen balance (y) correlated linearly with the nitrogen intake (x) and absorbed nitrogen (x) as shown in the equations of $y = 0.5784x - 18.3$ (r = 0.974**) and $y = 0.7228x - 15.3$ (r = 0.989**), respectively. The one hundred magnificance of the gradients are 57.8 with the nitrogen intake and 72.2 with the absorbed nitrogen which are similar with the NPU and BV (table 4) of the amino acid-supplemented LSM protein, which were also similar with the observed NPU and BV (table 4), respectively (Allison, 1964). Thus the BV of LSM protein added with Met, Trp or Lys was tended to be low compared with that of the LSM protein, but the reasons were not studied in this study.

3. Energy utilization

Also an effect of diets based on the LSM protein supplemented with Met, Trp or Lys on the daily energy utilization of birds are shown in table 5. Diets containing the LSM protein supplemented with the Met, Trp or Lys contained 2.612-2.789 kcal of MEn per gram diet, which was lower ($p < 0.05$) than 2.868 kcal of the basal and 3.081 kcal of the SBM diets. Thus the metabolizability (MEn/GE) of basal diet was 74.5% which was decreased in diets supplemented

TABLE 5. EFFECT OF DIETS BASED ON THE LUPIN SEED PROTEIN SUPPLEMENTED WITH METHIONINE, TRYPTOPHAN OR LYSINE ON THE DAILY ENERGY UTILIZATION OF BIRD

Diets	MEn[2]	MEn/GE[2]	ER[2]	HP[2]	HP/MEn
	(kcal/g)	(%)	($kcal/kg^{0.75}$)	($kcal/kg^{0.75}$)	(%)
Protein-free	3.131[a]	85.7[a]	−10.2[a]	145.9	107.5[a]
Basal	2.868[b]	74.5[bc]	15.5[a]	133.6	89.5[a]
Met[1]	2.612[c]	67.1[c]	96.5[c]	133.0	57.9[b]
Met + Trp[1]	2.736[c]	71.9[c]	71.6[c]	120.3	62.7
Met+Trp+Lys[1]	2.789[c]	71.8[c]	95.5[c]	148.5	60.9[b]
Soybean meal	3.081[ab]	80.6[ab]	63.3[bc]	150.5	70.3
LSD ($p < 0.05$)	0.286	7.4	53.6	76.1	27.7

[1] Amino acid diet in which 0.241% of methionine, 0.07% of tryptophan and 0.229% of lysine or combinations of it were supplemented to the basal diet

[2] MEn : Nitrogen-corrected metabolizable energy, GE : Gross energy, ER : Energy retention, HP : Heat production.

Means with different superscripts in the same column differ significantly at $p < 0.05$.

with the Met, Trp or Lys, while that of the SBM diet was 80.6% which was the highst value among the experimental diets.

And daily ER of birds fed on the diets supplemented with Met, Trp or Lys was 72-96 kcal per $kg^{0.75}$, which was higher ($p < 0.05$) than 15.5 kcal of bird fed on the basal diet and tended to be high compared with 63.3 kcal of birds fed on the SBM diet. Daily HP of birds fed on the basal and Met-supplemented diets was 133 kcal, while HP of birds fed Met + Trp-supplemented diet tended to decrease. And birds fed on the Met + Trp + Lys-supplemented and SBM diets produced 148 and 150 kcal, respectively, which were higher than those of the birds fed on other experimental diets.

Birds fed on the LSM protein supplemented with met, trp or lys dissipated 58-63% of heat per MEn consumption (HP/MEn), which were low ($p < 0.05$) compared with 89.5 and 70.3% of birds fed on the basal and SBM diets, respectively. The HP/MEn of birds fed the basal diet was tended to be high compared with that of birds fed the SBM diet.

The MEn value of Met, Trp or Lys-added diets were lower than those of the basal diet and were proportional ($r = 0.863^{*}$) to the AD of the protein, though the MEn contents of diets containing the graded levels of LSM were inversely proportional to the AD of the protein (Nam and Koh, 1991). Also the metabolizability (MEn/GE) of diets was proportional to the BV and NPU of protein showing a regression equations of $MEn/GE = 0.922 \times BV - 0.7$ ($r = 0.982^{**}$, $n = 5$) and $MEn/GE = 19.2 + 0.8566 \times NPU$ ($r = 0.909^{*}$, $n = 5$). However, the effects of the amino acids supplementation on the digestibility and BV of the protein were not found (table 4). It was suggested that the antinutrients affecting feed intake and energy retention was repaired when met was supplemented to the LSM protein.

Birds fed on the Met + Trp diet tended to decrease diet consumption (table 3) and HP (table 5) comparing with those of the Met and Met + Trp + Lys diets. Tryptophan (Trp), the amino acid precursor of 5-HT (serotonin) has been known to decrease food intake (Lacy et al., 1982) and body temperature (Lacy et al., 1985) in the chicken. Because dietary loads of tryptophan have been demonstrated to increase brain levels of 5-HT (Ferstrom, 1986), it was assumed that tryptophan affected feed intake involved changes in central 5-HT concentrations. And the plasma tryptophan to large neutral amino acids (LNAA) ratio correlated with the brain serotonin and 5-hydroxy indolacetic acid (5-HIAA) (Ferstrom, 1986). The results may be attributed to the fact that birds fed on the Met + Trp diet should increase plasma Trp : LNAA as the lupin seed may contain relatively adequate tryptophan although plasma and brain contents of trytophan and serotonin were not determined.

In conclusion, the lupin seed meal protein supplemented with methionine had higher energy retentions compared with that of soybean meal protein, although the alkaloid poisonings and methionine deficeincy of the protein was not elucidated.

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