Nutritive Value of Leaf Protein Concentrates for Young Chicks

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The nutritive value of leaf protein concentrates (LPCs) has been investigated by several workers. The nine LPC samples from tropical plants were prepared and evaluated nutritionally with rats by CHEEKE¹⁾. The LPCs from tropical legumes gave growth rates from 75% to 93% of that obtained with the soybean meal control, and he suggested that tropical legumes may be potential source of good quality LPC for human and animal feeding. HENRY and FORD²⁾ reported that biological value and true digestibility of LPC increased with the maturity of the leaves at harvesting because of increasing availability of methionine in biological tests with rats and by microbiological methods. OHSHIMA and OOUCHI³⁾ found that the first, second and third limiting amino acids of ladino clover LPC for growing rats were methionine, lysine and threonine, respectively. The biological value of the ladino clover LPC was 21, and it was improved to about 90 by supplementing the three limiting amino acids. The true digestibility of protein was a little lower than 80%. LUIZA *et al.*⁴⁾ reported that LPC of cassava (*Manihot utilissima*) leaves contained 38.4% of protein. Amino acid analysis revealed that the cassava LPC was comparable to FAO reference protein concerning essential amino acids, except for a deficiency in methionine and excess in leucine. Protein efficiency ratio for diets containing milk casein or LPC+0.5% methionine was 2.97 or 0.28, respectively, and rats fed on a diet containing LPC as a sole protein source lost weight during the period of 28 days. HORIGOME and UCHIDA⁵⁾ suggested that methionine content in leaf protein was regarded as an important factor causing the difference in nutritional quality among leaf proteins made from different crops. The use of LPC as a rabbit feed was studied by OMOLE and OKE⁶⁾. The daily feed intake and weight gain were significantly higher in the fish meal diet than in the LPC diet. The best result was obtained with the mixture of fish meal and LPC, and it was suggested that amino acid imbalance be responsible for the poor results obtained in the LPC. Nutritive value of the LPC from a mixture of oat and annual meadow grass for growing pigs was investigated by OHSHIMA and UEDA⁷⁾, and they concluded that about 30% of dry matter of a growing pig ration could be substituted by the LPC without any ill-effects in the experiments of 4-week duration.

The order of limiting amino acids in ladino clover LPC to chicks was determined by UEDA and OHSHIMA⁸⁾. The first limiting amino acid in the ladino clover LPC was methionine and the second limiting amino acid was lysine.

The present study was conducted to evaluate the nutritional value of some LPCs and to investigate the supplementary effect of insufficient amino acids when the LPCs were used as a sole source of protein to chicks.

Materials and Methods

Three different LPCs were used in this experiment. LPC A was obtained from a mixture of alfalfa (46%), barnyard grass (50%) and weeds (4%), which were grown at the Nagoya University Farm. LPC B was obtained from a mixture of oat (33%) and annual meadow grass (67%) and LPC C from ladino clover. Both LPCs B and C were provided by Dr. Ohshima of Kagawa University. The making process of the LPCs will be described elsewhere⁹⁾.

Day-old White Leghorn male chicks were placed in electrically heated battery brooders and were given a commercial chick starter diet for 7 days. On day 8, the chicks were individually weighed after 2-hour fasting, and were selected by equal weight to six groups of seven birds each. One chick from each group was killed by neck dislocation and used for initial body composition analysis. Residual 6 birds from each group were kept individually in metabolism cages and the ambient temperature was maintained at $29 \pm 2^\circ\text{C}$ and lighting was provided continuously. Six experimental treatments were allocated so that there were six replicates of one chick each per treatment (initial body weight was 36.7 ± 0.9 g). The experimental diets and water were given

Table 1. Composition of experimental diets (%)

Ingredient	Control	LPC A	LPC B	LPC C	LPC A+ Arg+Met	LPC C +Arg
Soybean protein	21.40	—	—	—	—	—
LPC A	—	70.87	—	—	70.87	—
LPC B	—	—	48.53	—	—	—
LPC C	—	—	—	35.60	—	35.60
L-Arginine	—	—	—	—	0.13	0.29
DL-Methionine	0.15	—	—	—	0.05	—
Corn starch	56.52	13.02	33.97	31.93	13.02	31.93
Corn oil	1.00	5.00	1.00	1.00	5.00	1.00
Cellulose	14.53	4.71	10.10	25.07	4.53	24.78
Choline chloride	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin mixture*	0.08	0.08	0.08	0.08	0.08	0.08
Mineral mixture**	6.17	6.17	6.17	6.17	6.17	6.17
Total	100	100	100	100	100	100
Crude protein***, %	19.5	19.6	19.5	19.1	19.5	20.1
Gross energy***, kJ/g	16.4	15.8	15.4	15.4	16.0	17.8
ME****, kJ/g	12.1	12.1	12.1	12.1	12.1	12.1

* Supplies in mg/kg diet: thiamine · HCl 100, niacin 100, riboflavin 16, Ca pantothenate 20, vitamin B₁₂ 0.02, pyridoxine · HCl 6, biotin 0.6, folic acid 4, inositol 100, p-aminobenzoic acid 2, menadione 5, ascorbic acid 250, vitamin A acetate (1,000,000 IU/g) 10, vitamin D₃ 0.015, vitamin E 0.0005, BHT 0.0156.

** Supplies in g/kg diet: CaHPO₄ 20.7, CaCO₃ 14.8, KH₂PO₄ 10.0, KCl 1.0, NaCl 6.0, MgSO₄ 3.0, FeSO₄ · 7 H₂O 0.3330, MnSO₄ · H₂O 0.3330, KI 0.0026, CuSO₄ · 5 H₂O 0.0167, ZnO 0.062, CoCl₂ · 6 H₂O 0.0017, Na₂MoO₄ · 2 H₂O 0.0083, Na₂SeO₃ 0.0001.

*** Analyzed.

**** Calculated using values of 14.6, 15.3, 36.9, 11.7, 13.6 and 19.6 kJ/g for soybean protein¹⁰⁾, corn starch¹⁰⁾, corn oil¹⁰⁾, and LPCs A, B and C (Unpublished data), respectively.

ad libitum from day 8 to day 18 (10 days). Body weight and feed consumption were recorded every other day for each chick to check the growth and appetite. Droppings for last 4 days of the experimental period were collected in 0.05 M H₂SO₄ and stored till analysis. On day 18, all chicks were killed by neck dislocation and nitrogen and energy content of the carcass were determined.

Composition of experimental diets are shown in Table 1. A control diet was formulated with soybean protein supplemented with methionine as the protein source. Other experimental diets were formulated with LPCs A, B, C, LPC A+Arg+Met and LPC C+Arg, respectively, as the sole source of protein. Vitamins and minerals were supplemented to meet the NRC requirements. Crude protein, gross energy and metabolizable energy of each diet were also shown in Table 1. Among them, crude protein and gross energy were analyzed data, whereas metabolizable energy values were calculated using the data of NRC¹⁰⁾ (soybean protein, corn starch and corn oil) and the authors' unpublished data which were determined with cockerels (11.7 kJ/g for LPC A, 13.6 kJ/g for LPC B and 19.6 kJ/g for LPC C). Amino acid composition of the experimental diets were listed in Table 2 together with the NRC requirements revised in 1977¹⁰⁾. It can be seen from Table 2 that LPC A diet was deficient in arginine and methionine and LPC C diet in arginine when compared to the requirements recommended by NRC. This is the reason why LPC A diet supplemented with arginine and methionine and LPC C diet supplemented with arginine were formulated.

Nitrogen, amino acids and energy of the diets, droppings and carcasses were determined by the methods of A. O. A. C.¹¹⁾. Data were analyzed using the analysis

Table 2. Amino acid composition of experimental diets (% of diet)

	Control	LPC A	LPC B	LPC C	LPC A +Arg+Met	LPC C +Arg	NRC requirement
Crude protein	19.47	19.46	19.48	19.12	19.45	20.11	18.00
Ala	0.88	0.97	1.19	1.02	0.96	1.07	—
Arg	1.65	0.95 (95)	1.09	0.76 (76)	1.07	1.08	1.00
Asp	2.33	1.79	1.61	1.96	1.77	2.06	—
Glu	3.79	1.76	2.05	2.15	1.74	2.26	—
Gly	0.86	0.95	1.06	1.05	0.94	1.10	—
Gly+Ser	1.73	1.60	1.76	1.85	1.59	1.95	0.70
His	0.52	0.57	0.46	0.55	0.56	0.58	0.26
Ile	1.04	0.88	1.10	1.01	0.87	1.07	0.60
Leu	1.72	1.45	1.65	1.70	1.44	1.79	1.00
Lys	1.27	1.16	1.08	1.17	1.15	1.23	0.85
Met	0.34	0.30 (94)	0.45	0.36	0.34	0.38	0.32
Phe	1.09	0.99	1.05	1.28	0.98	1.35	0.54
Phe+Tyr	1.96	1.78	1.82	2.28	1.76	2.39	1.00
Pro	0.99	0.80	1.12	0.78	0.79	0.82	—
Thr	0.73	0.80	0.85	0.99	0.79	1.04	0.56
Val	1.06	1.05	1.11	1.28	1.04	1.34	0.62

Figures in parentheses indicate the degree of satiety (%) of deficient amino acids compared to the NRC requirements.

of variance technique and multiple comparisons among treatment means were made by the Tukey's multiple procedure as described by SNEDECOR and COCHRAN¹²).

Results and Discussion

Crude protein content of the experimental diets were 19-20%. As described before, amino acid composition of Table 2 shows that the LPC A diet was deficient in arginine and methionine, and the LPC C diet in arginine when compared to the NRC requirements. The LPC B diet did not show any amino acid deficiency.

Body weight gain, feed intake and feed efficiency indicated as gain per feed ratio are shown in Table 3. Body weight gain of the LPC treated birds are significantly lower than that of the soybean protein control ($P < 0.05$). Among the LPC treatments, LPC B gave the highest body weight gain ($P < 0.05$), followed by LPC A and LPC C, and the difference between LPCs A and C was not significant. Feed intake of the LPC A diet was not different from the control, while that of the LPC B diet was significantly higher ($P < 0.05$) and that of LPC C diet was significantly lower ($P < 0.05$) than the control. Feed efficiency of LPC treatments was significantly lower than that of the control, and among LPC treatments LPC B was higher than the others ($P < 0.05$). Even

Table 3. Body weight gain, feed intake and feed efficiency from 8 to 18 days of age

Treatment ¹⁾	Body weight gain (g)	Feed intake (g)	Gain/Feed
Control, soybean protein	81.8 ^d	152.0 ^b	0.54 ^c
LPC A	17.2 ^{a,b}	151.0 ^b	0.11 ^a
LPC B	54.5 ^c	178.3 ^c	0.31 ^b
LPC C	10.2 ^a	95.0 ^a	0.11 ^a
LPC A+Arg+Met	22.2 ^b	150.5 ^b	0.15 ^a
LPC C+Arg	11.0 ^a	102.5 ^a	0.11 ^a
SD	3.7	9.2	0.09

1) Six birds were used in each treatment.

2) Values in the same column with different superscript letters differ significantly at $P < 0.05$.

Table 4. Nitrogen economy for 10 days

Item	Nitrogen intake (g)	Nitrogen retention (g)	Nitrogen retention rate ¹⁾
Control	4.74 ^b	2.75 ^a	0.58 ^a
LPC A	4.75 ^b	0.54 ^{c,d}	0.11 ^c
LPC B	5.56 ^a	1.79 ^b	0.32 ^b
LPC C	2.91 ^c	0.34 ^c	0.11 ^c
LPC A+Arg+Met	4.68 ^b	0.72 ^d	0.15 ^c
LPC C+Arg	3.30 ^c	0.40 ^c	0.12 ^c
SD	1.75	0.79	0.14

Values in the same column with different superscript letters differ significantly at $P < 0.05$.

1) N retention/N intake

when the LPCs A and C diets were supplemented with the deficient amino acids, no significant improvement in body weight gain, feed intake and feed efficiency was observed.

In order to discuss the efficiency of protein utilization, nitrogen economy was measured and the result is indicated in Table 4. Nitrogen retention for the experimental period of 10 days was calculated by reducing the initial nitrogen content from the nitrogen contents of carcasses of all treatments, and nitrogen retention rate was calculated by dividing the retained nitrogen with the nitrogen intake. It was apparent from this table that the soybean protein control was always superior significantly to the other treatments. Among the LPC treatments, protein utilization was significantly higher in the LPC B than in the other LPCs, and again no improvement was observed in amino acid supplementation.

OHSHIMA and OOUCHI⁸⁾ reported that the order of limitation of amino acids in the ladino clover LPC (same as LPC C in this experiment) for growing rats was methionine, lysine and threonine. In the present experiment, however, the first limiting amino acid of the same LPC for chicks was arginine as far as chemical analysis data indicated. OHSHIMA and OOUCHI⁸⁾ reported that the supplementation of the limiting amino acids in the LPC diet improved growth of rats, whereas the present experiment showed that the amino acid supplementation did not give any beneficial effect. The reason why such a difference occurred was not clear, but one of the reasons would be that the level of the supplement was not adequate, because biological availability of some amino acids in LPC might be very low.

CHEEKE *et al.*¹⁾ found that the LPC from the tropical legumes gave growth rates from 75% to 93% of that obtained with the soybean meal control in a rat experiment, and with LPC from *Macroptilium lathyroides* the gain was 41.7% of the control. They suggested that the presence of inhibitory substances might affect the rat growth. In the present experiment, the growth rates were 21.0% for LPC A, 66.6% for LPC B and 10.4% for LPC C when compared to the control. From this result, the existence

Table 5. Energy economy for 10 days

Item	Gross energy intake (GE) kJ	Metabolizable energy (ME) intake, kJ	Metabolizability %	Retained* energy (NE) kJ	Retained fat g	NE/ME %
Control	2492.8 ^{a b}	1998.8 ^a	80.1 ^a (73.8)**	656.4 ^a (652.8)	6.3 ^a	32.9 ^a
LPC A	2385.8 ^b	1031.7 ^{c d}	43.4 ^e (76.6)	171.1 ^c (156.5)	2.0 ^b	16.4 ^b
LPC B	2746.3 ^a	1727.3 ^b	62.8 ^b (78.6)	509.2 ^b (507.6)	6.2 ^a	29.1 ^a
LPC C	1463.0 ^d	679.7 ^e	46.4 ^{d e} (78.6)	77.5 ^d (74.4)	0.6 ^b	11.3 ^{c d}
LPC A+Arg+ Met	2408.0 ^b	1150.5 ^c	47.7 ^d (75.6)	164.4 ^c (158.9)	1.1 ^b	14.3 ^{b c}
LPC C+Arg	1834.7 ^c	970.9 ^d	53.1 ^c (68.0)	91.0 ^{c d} (78.1)	0.7 ^b	9.4 ^d
SD	143.6	83.6	1.5	43.5	2.7	2.2

Values in the same column with different superscript letters differ significantly at $P < 0.05$.

* Values were obtained by direct carcass energy determination. Values in parentheses were calculated from the data of protein and fat retention using energetic values of 39.12 kJ/g fat and 23.98 kJ/g protein¹³⁾.

** Calculated using the ME values listed in Table 1.

of inhibitory substances could be considered, though these substances were not determined yet.

It was found in this experiment that LPCs were significantly lower in nutritive value than the soybean protein, while HORIGOME and UCHIDA⁵⁾ reported that there was no difference in weight gain and protein efficiency ratio in rats between ladino clover and oat leaf protein, and this parameters were not different between leaf protein and soybean protein both supplemented with methionine.

In Table 5 metabolizability of energy and energy economy of each treatment are indicated. It could be seen from this table that energy metabolizability of soybean protein diet was 80%, whereas that of the LPC diets showed only 43-63%. When the metabolizability of each diet was calculated using the ME values listed in Table 1, they were not so much different from each other, being between 68.0% and 78.6%. This means that chicks metabolize dietary energy less efficiently than cockerels. In fact, droppings/feed intake ratios on air dry basis in chicks fed the LPC diets were much higher than that in chicks fed the soybean protein diet, being 31%, 77%, 53%, 63%, 74% and 60% for soybean protein, LPC A, LPC B, LPC C, LPC A+Arg+Met and LPC C+Arg, respectively. The metabolizable energy of soybean protein used here was referred to the NRC, being 14.6 kJ/g, however, when used the authors' unpublished data (18.7 kJ/g) metabolizability of the soybean protein diet was calculated as 79.6%. This value was almost the same as that obtained in the present experiment, being 80.1%. Supposed that the metabolizability of corn starch, corn oil and cellulose were 99%, 100% and 0%, respectively¹⁰⁾, the metabolizability of soybean protein, LPCs A, B, C, LPC A+Arg+Met and LPC C+Arg could roughly calculated as 94%, 25%, 49%, 25%, 32% and 54%, respectively. Metabolizability of LPC C seemed to be increased by supplementation of arginine, however, the authors could not find the reason of this phenomenon. As shown in Table 5, chicks fed the LPC B diet retained large amount of energy, being 77% that of the soybean protein fed chicks. When compared the LPC diets, LPC B diet showed the highest energy retention followed by LPC A diet and LPC C diet. Relative values were 78%, 26% and 12% that of the control diet. From Tables 3 and 4, relative values of body weight gain and of nitrogen retention in LPC B, LPC A and LPC C diets were 67%, 21%, 12% and 65%, 20%, 12%, respectively, of the values of the control diet. When compared these values, it could be considered that chicks retained more fat by feeding LPC B diet than by LPC A and LPC C diets. In fact, fat retention by LPC B diet was almost the same as that by control diet, and LPC C diet gave the smallest fat retention. This is quite reasonable because retained energy values determined calorimetrically were almost the same values calculated from the data of retained protein and fat. The net utilization of metabolizable energy (NE/ME) of the LPC B diet was almost the same as that of the control diet, whereas the other LPC diets showed significantly low values. In conclusion, it could be necessary to take steps to improve not only protein utilization but also energy utilization of LPCs.

Summary

Eight-day old chicks were fed the diets containing leaf protein concentrates (LPCs) with or without supplementation of deficient amino acids as a sole protein source to supply 19-20% crude protein in the diet. The performance of the chicks fed the LPC diets was compared with that fed the methionine supplemented soybean protein control diet. The LPCs used here were obtained from the mixture of alfalfa+barnyard grass (LPC A), from the mixture of oat+annual meadow grass (LPC B) and from ladino clover (LPC C). Chemical analysis showed that the LPC A diet was deficient in arginine and methionine and the LPC C diet in arginine, when compared to the NRC requirements for chicks. Feed intake of the birds fed the LPC A or LPC A+Arg+Met were not significantly different from that of the control diet, while the birds received the LPC B consumed significantly more diet than the control. Feed intake of the birds fed the LPC C or LPC C+Arg were significantly lower than that of the control. Body weight gain and feed efficiency were significantly lower in the LPCs than in the control. Even when the deficient amino acids were supplemented to the LPC diets no significant improvement of both body weight gain and feed efficiency was observed. Nitrogen retention rate was significantly lower in the LPC diets than in the soybean protein control diet even when the LPCs were supplemented with deficient amino acids. Energy metabolizability was extremely low in the LPC diets compared to that of the soybean protein diet. Among the LPCs including those supplemented with deficient amino acids, LPC B (oat+annual meadow grass) showed the highest protein utilization and energy metabolizability. The inferiority of LPCs in protein and energy utilization might lead the low nutritive value of LPCs for poultry.

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緑葉蛋白濃縮物のニワトリヒナに対する栄養価

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三種の緑葉蛋白濃縮物 (LPC A: アルファルファ+イヌビエ, LPC B: エンバク+スズメノカタビラ, LPC C: ラジノクロバ) の栄養価を, 8日齢のニワトリヒナに10日間給与して検討した。LPC飼料のアミノ酸含量を測定すると, A飼料ではアルギニンとメチオニンが, またC飼料ではアルギニンがNRC要求量に比し欠乏していたので, これらを添加したものとしないものを用いてCP 19~20%の飼料を配合し, 大豆蛋白とその栄養効果を比較した。飼料摂取量についてみると, A飼料およびこれにアミノ酸を添加したものは対照の大豆蛋白飼料と差がなかったが, B飼料は対照より多く, またC飼料とそれにアルギニンを添加したものは有意に対照

より低かった。ヒナの増体ではLPC飼料はいずれも対照より著しく劣ったが, LPCのなかではB飼料が他のものより優れていた。なおAおよびC飼料にアミノ酸を補足してNRC標準のレベルにしてもその効果はみられなかった。

窒素の蓄積率, エネルギーの代謝率および蓄積率も増体とその傾向が全く一致し, LPC飼料は対照に比して著しく低かった。これらの利用性が悪いことがLPCの栄養価を低下させる原因と考えられるので, 利用性を向上させる方策を究明する必要がある。

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