試験プラントでルーサンから生産した緑葉蛋白質濃縮物及びそれを添加した小麦粉あるいは子牛代用乳の栄養価

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Nutritive Value of Leaf Protein Products from Lucerne Prepared at Pilot Plant Scale : Leaf Protein Concentrate, Enriched Wheat Flour and Calf Milk Replacer

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Synopsis

OHSHIMA, M., V.A. SAVANGIKAR and C.V. SAVANGIKAR (1986) Nutritive value of leaf protein products from lucerne prepared at pilot plant scale: Leaf proetin concentrate, enriched wheat flour and calf milk replacer. J. Japan. Grassl. Sci., 32, 205-210.

Lucerne leaf protein concentrate (LP) was prepared at the pilot plant at Bidkin in India by the following procedure. Extracted lucerne juice was heated to 90°C, poured into filter tubes and pressed to recover LP. The LP was resuspended in water. The suspension was adjusted to pH 4 to coagulate the LP again and filtered as before. The wet LP was dried in the shade.

Nutritive values of LP, 1 : 3.8 mixture of LP and a wheat flour (LP : WF) and a calf milk replacer containing LP at 10% (MR) were studied using rats.

True digestibility of protein, nitrogen retention and biological value of LP were 83, 44 and 71%, respectively. By supplementing methionine, the nitrogen retention and the biological value of LP were significantly improved to 60 and 89%, respectively. Supplementation of lysine in combination with methionine was not effective in improving the nutritive value of LP. These results are superior to those reported on LP prepared by oven-drying without washing and similar to those reported on freeze-dried LP suggesting that the nutritive value of LP is affected by the remaining brown juice rather than the drying method.

True digestibility of protein in LP : WF and MR was better than that of LP. Biological value was similar in the three preparations. The supplemetary effect of methionine alone was significant only in LP. Supplementation of lysine alone was effective only in LP : WF. The nutritive value of MR was significantly improved by adding both methionine and lysine. The improved biological value was the highest in LP (89) followed by LP : WF (85) and MR (81) in that order. While improved nitrogen retention was the highest in LP : WF (69) followed by LP (61) and MR (58).

The Ca and P contents of MR were a little shorter than those recommended by NRC. LP contained more than a half of the reguirements of Ca and P of monogastric animals. But, LP was a good source of Mg. The concentration of Na and K in LP was very

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low. The high Fe content of LP might be derived from rust of plantmachine and/or water.
Key words: Leaf protein-wheat flour mixture, Milk replacer, Nutritive value.

Introduction

Fodder fractionation is now attracting attention all over the world for its various economic benefits.[14] JOSHI *et al.*[7] are working in India to evolve technologically and economically viable-village based industry for production of leaf protein concentrate (LP). They feel that this technology will benefit various sections of rural population, like landless labourers, small farmers etc.[7]. Preparations made by them from LP include "enriched wheat flour" and calf milk replacer[7]. Value of these preparations will depend upon the nutritive value. Work done earlier on LP produced on laboratory scale or small scale has shown good nutritive value for human consumption[13]. However, when large scale production is considered, it is the type of machinary and processing conditions which will decide the nutritive value of the product. Hence, in a collaborative study, products prepared at the pilot plant at Bidkin in India were studied at Kagawa University for *in vivo* nutritional evaluation and chemical analysis.

Materials and Methods

LP was prepared at the pilot plant at Bidkin in India by the method reported by JOSHI *et al.*[7]. Freshly harvested lucerne was well washed and excess water removed from it. The washed crop was disintegrated and dejuiced in a single pass using a screw press. The throughput of the screw is 120 to 150 kg crop per hour. The juice was strained over a vibrating screen, heated suddenly to 90°C by dropping into preheated water, and then filtered through a filter tube made with a closely woven cloth. The filter tube was laid between two strips corrugated rubber and pressed at about 2.5 kg/cm^2 for at least four hours. The coagulum was then hard and contained about 65% moisture. It was then dispersed in water. The suspension was adjusted to PH 4 to coagulate the LP again, then poured into filter tubes and the same routine as before was followed for draining and pressing. The wet cake was granulated to pass through 10-12 mesh sieve and dried in the shade to about 25% moisture when it was just fit for grinding without forming a paste. This material was then milled to pass through 100 mesh screen and drying is completed to equilibrate with ambient moisture in the shade.

Enriched wheat flour (LP : WF) was prepared with wheat flour available in Japan. LP and WF were taken in such a way as to contribute 50% each of the total crude protein content of the mixture. The proportion was about 1 : 3.8.

Calf milk replacer (MR) is a calf feeding product prepared at the plant using LP as an ingredient at 10% level[7].

Ten percent protein diets were prepared using one of the preparations as the sole protein source. All the preparations were tested for methionine and lysine supplementation. N-free diet was also prepared to determine metabolic fecal and endogenous urinary nitrogen excre-

Table 1. Composition of the diets

	LP[1]				LP : WF[2]			MR[3]				N free
		+Met	+Lys	+Met +Lys		+Met	+Lys		+Met	+Lys	+Met +Lys	
	%	%	%	%	%	%	%	%	%	%	%	%
LP[1]	19.3	18.3	18.3	18.3	—	—	—	—	—	—	—	—
LP : WF[2]	—	—	—	—	48.1	48.1	48.1	—	—	—	—	—
MR[3]	—	—	—	—	—	—	—	39.4	39.4	39.4	39.4	
Premix[4]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Cornstarch	62.7	63.3	63.3	62.9	33.9	33.5	33.5	42.6	42.2	42.2	41.8	82.0
Corn oil	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
L-Methionine	—	0.4	—	0.4	—	0.4	—	—	0.4	—	0.4	—
L-Lysine • HCl	—	—	0.4	0.4	—	—	0.4	—	—	0.4	0.4	—

1. Leaf protein concentrate. 2. 1 : 3.8 mixture of LP and wheat flour. 3. Calf milk replacer containing LP at 10% level. 4. One hundred grams contain; 50 g glucose, 25 g cellulose powder, 5g vitamin mixture[4)] and 20 g mineral mixture[4)].

Table 2. Chemical composition of LP, LP : WF and MR on DM basis[1,2]

	Crude protein	Ether extract	Crude fiber	Total ash	Ca	P	Fe	Mg	Na	K	Available lysine
	%	%	%	%	%	%	%	%	%	%	g/16gN
LP	55.4	14.7	4.41	7.69	0.532	0.307	0.449	0.304	0.065	0.203	6.93
Wheat flour	14.9	2.2	0.82	0.49	0.055	0.177	Trace	0.117	Trace	0.164	(4.80)
LP : WF	23.5	(5.0)	(1.60)	2.73	0.144	0.204	0.123	0.181	0.018	0.163	5.29
MR	26.5	17.6	2.64	6.14	0.530	0.380	0.088	0.251	0.211	1.055	5.50

1. See footnotes 1, 2 and 3 of Table 1.
2. Figures given in parentheses were calculated from its components.

Table 3. Nutritive value of LP, LP : WF and MR in growing rats

	LP[1]				LP : WF[1]			MR[1]				Pooled standard error
		+Met	+Lys	+Met +Lys		+Met	+Lys		+Met	+Lys	+Met +Lys	
	%	%	%	%	%	%	%	%	%	%	%	
True digestibility[2]	83.0^{a}	83.6^{a}	83.6^{a}	84.2^{a}	88.6^{b}	89.5^{bc}	91.2^{c}	89.3^{bc}	90.5^{bc}	91.6^{bc}	90.2^{bc}	0.49
Biological value[3]	71.0^{ab}	89.2^{e}	67.5^{a}	88.9^{e}	70.1^{ab}	71.1^{ab}	84.8^{de}	73.7^{ab}	75.6^{bc}	69.7^{ab}	81.0^{cd}	1.42
Nitrogen retention[4]	44.2^{a}	60.3^{de}	42.8^{a}	61.4^{de}	48.2^{ab}	49.8^{ab}	64.0^{e}	51.9^{bc}	54.7^{bcd}	49.2^{ab}	58.0^{cde}	1.46

1. See footnotes 1, 2 and 3 of Table 1.
2. $\left(1-\frac{\text{Fecal N}-\text{Metabolic fecal N}}{\text{N intake}}\right)\times 100.$
3. $\left(1-\frac{\text{Urinary N}-\text{Endogenous urinary N}}{\text{N intake}-(\text{Fecal N}-\text{Metabolic fecal N})}\right)\times 100.$
4. $\left(1-\frac{\text{Fecal N}+\text{Urinary N}}{\text{N intake}}\right)\times 100.$
5. Figures with different superscript letters were significantly different at 5% level.

tions of rats. Composition of the diets are shown in **Table 1.** Each diet was restrictedly fed to four growing rats weighing about 50 g for 10 days. The feeding procedure was the same to that reported by OHSHIMA and UEDA[11] except for N-free diet. The rats of N-free group were given the diet with 10% casein for first three days and then switchd over to the N-free diet. After the switching, four days preliminary feeding was followed by three days collection period.

True digestibility (TD) of crude protein, biological value (BV) and nitrogen retention were determined using the fomulae shown in the footnotes of **Table 3.**

Crude protein, ether extract, total ash, crude fiber, moisture and P were determined according to AOAC[6]. Ca, Mg, Fe, Na and K were determined with a two wavelength atomic absorption spectrophotometer Model Hitachi 308. Available lysine was determined by the method of BOOTH[1]. The results are analysed by the Tukey's multiple range test.

Results and Discussion

Chemical composition of LP, WF, LP : WF and MR is given in **Table 2.** The results show that there is still further scope for reducing the content of crude fiber. Because LP prepared at laboratory scale contains only lower than 1% crude fiber[5]. The composition of MR compares well with the NRC recommendations[3] except for a slight shortage of Ca and P. Ca and P contents of LP was about a half of the requirements of monogastric animals. LP was a good source of Mg. The concentration of Na and K of LP was very low. The high Fe content of LP might be derived from contamination of rust of plant machine and/or from plenty of water used.

Table 3 gives TD, BV and nitrogen retention of LP, LP : WF and MR. Results show that LP was adequate in lysine but marginally deficient in methionine. This agrees well with the best samples prepared in laboratory scale[2,10]. Nitrogen retention of LP supplemented with methionine resembled that of freeze-dried LP prepared by OHSHIMA[10] and OHSHIMA and UEDA[11]. OHSHIMA[10] showed that oven-drying at 70°C reduced both digestibility and available lysine content of LP and consequently reduced nitrogen retention of rats fed on the LP. The present sample was left in the shade until drying up but contained as high available lysine as the freeze-dried preparations. The difference between the present and the previous studies[10,11] is attributable to the different brown juice content of wet LP. The LPs of the previous studies[10,11] were oven-dried immediately after being separated from green juice, while the present LP was washed before drying to reduce the browning reactions between LP and reactive substances in brown juice such as sugars, phenols etc[12]. Compared with the nutritive value of casein reported previously,[8,9] LP is lower in TD but higher in BV and nitrogen retention when supplemented with methionine[8].

TD of LP : WF and MR was better than that of LP. BV of LP : WF and MR were similar and comparable with that of LP. Thus these preparations can very well be used to replace milk protein in food as well as feed preparations. The LP : WF combination has served the purpose of enhancing the nutritive value of wheat protein. BV of wheat protein in rats is known to be far lower than LP[15]. While BV of LP : WF was similar to that of LP. So, the objective of the present study was partially achieved. But it was signicantly improved by the

lysine supplementation. Therefore, the incorporation of LP to WF at the rate of 1 : 3.8 was not enough to complete the lysine deficiency of WF. Further improvement in nutritive value of LP : WF will be possible by increasing the rate of LP. The nutritive value of MR was slightly improved by the methionine supplementation and was significantly improved by the simultanious supplementation of methionine and lysine. The improved value of MR is superior to that of casein but further balance test with calves is necessary.

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試験プラントでルーサンから生産した緑葉蛋白質濃縮物及びそれを添加した小麦粉あるいは子牛代用乳の栄養価

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要 約

本実験に供した緑葉蛋白質濃縮物（LP）は，インドのマラスワダ大学で次の工程により調製した。

ルーサン搾汁液を 90℃ に加熱後，布袋に入れて圧搾した。得られた凝固物（LP）を水に懸濁させ，pH 4 にして再凝固させたのち上記の方法で回収することにより，水溶性成分を除去した。LP の乾燥は日陰にひろげて行った。

この LP は，子供の食料あるいは子牛の代用乳の成分として，すでに高い効果が認められている。しかし，その詳細な栄養価は明らかにされていない。そこで LP，LP と小麦粉の 1：3.8 混合物（LP：WF）及び LP を 10% 含有する子牛代用乳（MR）の蛋白質の栄養価について，蛋白質含量 10% の半精製飼料を生長中のラットに給与することにより検討した。

LP の蛋白質の真の消化率，N 保留及び生物価はそれぞれ 83，44 及び 71% であった。メチオニンの添加により N 保留及び生物価は 60 及び 89% に向上した。さらにリジンを添加しても効果は認められなかった。以上の結果は，従来報告されている無洗滌の LP を加熱乾燥したものより優れ，凍結乾燥したものと等しい。よって LP の栄養価保持には，乾燥法よりも乾燥前の水溶性成分の除去が重要であることが明らかとなった。

LP：WF 及び MR の蛋白質の消化率は等しく，ともに LP よりも優れていた。生物価は 3 者の間に差がなかった。

LP の制限アミノ酸がメチオニンであったのに対し，LP：WF のそれはリジンであった。一方 MR は，メチオニンとリジンを同時に添加して，はじめて有意な栄養価の改善がみられた。アミノ酸添加の結果得られた生物価は LP（89），LP：WF（85）及び MR（81）の順であった。それに対し N 保留は消化率の差を反映して LP：WF（69），LP（61）及び MR（58）の順となった。

MR の成分含量は，Ca と P がやや低いほかは NRC の推奨値とよく合致した。LP は Mg 含量に富んでいたが，Ca 及び P 含量は豚，ヒナ等の飼料中の必要量の約 1/2 であり，また Na 及び K 含量も著しく低かった。鉄含量は高かったが，これは機械の錆，水等からの混入によるものであろう。

キーワード：緑葉蛋白質・小麦粉混合物，子牛代用乳，栄養価．

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