JOURNAL OF AGRICULTURAL SCIENCE IN FINLAND
Maataloustieteellinen Aikakauskirja
Vol. 57: 117—123, 1985

Leaf protein from green pulse crops and nutritive value of legume protein concentrates for poultry

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Abstract. Leaf protein concentrate (LPC) samples were prepared from four pulse crops, goat's rue, pea, field bean and Persian clover. Their proximate composition was 42.5—53.4 % crude protein, 2.1—7.9 % ether extract and 1.0—3.0 % crude fibre. The lysine content was 4.1—4.8 g/16 g N, that of sulphur amino acids 2.0—2.8 g and that of threonine 4.5—4.8 g. The contents of tannins varied from 2.2 to 5.5 %. The nutritional values of the LPC samples were assesed in digestibility and balance trials with male chickens of 16—18 weeks, LPC composing 25 % of the diets. LPC from pea had the highest digestibilties of organic matter (70.3 %), crude protein (77.6 %) and carbohydrates (54.9 %), while LPC from Persian clover had the lowest ($P < 0.01$). The true digestibilities of crude protein for goat's rue, pea, field bean and Persian clover were, respectively, 70.0, 82.2, 69.7 and 56.8 % ($P < 0.01$). The digestibilities of nutrients in the present LPC samples were rather low compared to their minimal crude fibre content. A reason for this may be the contents of tannins. The AME_N values for the LPC's of goat's rue, pea, field bean and Persian clover were, respectively, 10.79, 13.15, 9.80 and 9.18 ($P < 0.01$). The gross energy metabolization ranged from 42.6 to 57.0 %.

Introduction

The increasing cost of nitrogenous fertilizers and the need to develop domestic sources of protein for animal consumption have renewed interest in pulse crops in Finland. Peas and field beans have been found to be good sources of protein for pigs and poultry (ALAVIUHKOLA 1979, KIISKINEN 1982). The use of legumes in silages has also been investigated, but the effluent losses have been marked (SYRJÄLÄ-QVIST et al. 1984). These losses could be avoided and the protein in plant tissues utilised for monogastrics by combining silage making with leaf protein production by extracting protein from the crop prior to ensiling. The true digestibility and biological value of the leaf protein concentrates (LPC) obtained from some green crops have been found to be high when the

Index words: Leaf protein, protein supplement, poultry nutrition, digestibility and metabolizable energy value

LPC was prepared under optimal conditions (MORRIS 1977, PIRIE 1978). LPC from lucerne has given performance equal to that obtained with soybean meal with poultry (KUZMICKY and KOHLER 1977) and pigs (CHEEKE et al. 1977). In diets for poultry LPC has value as a source of pigment (YOSHIDA and HOSHII 1981), since its xanthophyll content is high.

The objective of the present study was to investigate the leaf protein production of various legumes and the nutritive value of leaf protein concentrate in poultry nutrition.

Materials and methods

Four green pulse crops were used as raw material for LPC production. Goat's rue (*Galega orientalis*), pea (*Pisum sativum*), field bean (*Vicia faba*), and Persian clover (*Trifolium resupinatum*) were cut with a harvester on experimental plots. Goat's rue had been sowed two years before and the other crops in the same growing season. The fertilizer application was 500 kg trace PK per ha.

Extraction involved pulping the green material, pressing out the juice, and separating the leaf protein from the juice by heat coagulation, the techniques being similar to those described by NÄSI (1983 a). For the feed evaluation tests, the curd was dried in an oven at 65°C. The analytical methods were the same as used in previous leaf protein experiments (NÄSI 1983).

The feed evaluation was made with 36 WL male chickens, age 16—18 wk and initial weight 1610—1676 g, kept in individual cages. Each treatment comprised six cocks. Besides the basic and experimental groups, there was a fasting group, which was kept without feed for 1.5 days, after which collection was performed for three days. The daily allowance of feed was 100 g and the feed intake was measured daily. The basic diet (No 1) was composed of barley meal 96 % and premix 4 %. The premix included dicalcium phosphate 39 %, calcium carbonate 20 %, sodium chloride 10 %, trace element and vitamin premixes 25.0 %, L-lysine 3 % and DL-methionine 3 %. The leaf protein concentrates were included in the basic diet at the level of 25 %. The birds were allowed to adapt to the diets for five days, after which the excreta were collected daily, for five days. Details of the procedures are presented by KIISKINEN and HUIDA (1984). The nutrient digestibilities of the test feeds and their ME values were calculated from the differences between the values obtained for the basic and test diets by the indirect method of total excreta collection. ME was corrected for the nitrogen balance ME_N using 36.55 kJ/g retained nitrogen (TITUS 1959). The results were evaluated statistically using the analysis of variance. The significance of differences between the test ingredients was assessed by the t-test.

Results and discussion

The protein yields of the goat's rue and pea crops were high, 760—790 kg/ha while field bean and Persian clover gave 330—500 kg protein/ha (Table 1). The dry matter and protein yields corresponded to the figures given by SYRJÄLÄ-QVIST et al. (1984). On average, 57 % juice was expressed from the green crop. The crude protein and true protein extraction ratios averaged 37 and 33 %; the ratios of the pea crop exceeding the others (Table 1). The values were higher than the extractions for grass or clover (NÄSI 1983 a). The crop moisture content has the greatest effect on the recovery of juice and its components (HOUSEMAN and JONES 1978). By heating the juice in leaf protein concentrate could be precipitated 58 % of the crude protein and 85 % of the true protein of the juice. These values accord with those of previous experiments with clover and pea, exceeding the values of grass (NÄSI 1983 a).

The chemical composition and *in vitro* digestibilities of the crop and pulped pressed crop are compared in Table 2. The DM content was almost as twice high in the pulped

Table 1. Outline of the experiments, extraction ratios of plant juice and its components from various crops and separation ratios of leaf protein concentrates and its components precipitated from plant juice.

	Goat's rue	Pea	Field bean	Persian clover
Outline of the experiments				
Cutting date	21. 6. 1982	20. 7. 1982	22. 7. 1982	28. 7. 1982
Fresh yield tn/ha	18.6	19.0	21.2	12.6
DM yield kg/ha	3704	3610	2967	2000
Protein yield kg/ha	760	786	499	329
Pressed amount, kg	435	651	531	302
Extraction ratios, %				
Juice	45.9	58.4	65.6	57.1
Dry matter	19.1	32.3	31.6	28.8
Ash	35.6	38.3	41.9	39.7
Crude protein	28.6	49.3	36.8	32.8
True protein	25.9	46.3	28.0	30.5
Separation ratios, %				
LPC	19.0	21.2	8.9	17.7
Dry matter	35.9	45.5	16.4	29.2
Ash	20.4	31.6	10.7	18.5
Crude protein	60.8	73.2	38.6	66.1
True protein	77.0	110.0	64.1	88.6

Table 2. Compositions and *in vitro* -digestibility of crop and pulped pressed crop (% of DM).

	DM	Ash	Crude protein	True protein	Ether extract	Crude fibre	NFE	Water soluble carbo-hydrate	Pepsine HCl soluble protein	In vitro DOMD
Goat's rue										
crop	19.9	8.2	20.5	15.6	2.3	23.5	45.4	7.3	71.8	71.3
pulp	30.3	5.8	18.0	14.0	2.1	30.2	43.9	4.7	68.5	66.4
Pea										
crop	15.1	9.4	21.8	13.1	3.2	22.8	42.8	19.5	87.2	77.8
pulp	27.3	8.0	16.5	10.9	3.1	30.4	42.0	9.1	80.5	73.1
Field bean										
crop	14.0	9.9	16.8	12.0	2.1	24.4	46.8	19.0	83.9	77.6
pulp	28.7	6.3	13.4	11.1	2.3	33.7	44.2	8.3	73.6	69.3
Persian clover										
crop	15.9	11.7	16.5	11.8	3.1	17.8	51.0	15.6	83.5	80.2
pulp	26.7	8.9	16.0	12.8	3.2	23.6	48.4	8.1	77.3	75.0
Mean										
Crop	16.2	9.8	18.9	13.1	2.7	22.1	46.5	15.4	81.6	76.7
Pulp	28.3	7.3	16.0	12.2	2.7	29.5	44.6	7.6	75.0	71.0

pressed crop and crude fibre one third higher, while the protein content was reduced by only 15 % compared with the original crop. The pulp could be considered suitable material for ensiling and its use would eliminate effluent losses, which have been found to be rather high in the case of pea and field bean used directly after cutting (SYRJÄLÄ-QVIST et al. 1984). The plant juices contained DM 6.8—8.3 % and the crude protein content was 31—33 % in goat's rue or pea and 19—20 % in bean and clover (Table 3). True protein composed on average 61 % of the crude protein in the juices. The sugar content of the juices was high. NÄSI (1983 b) reported that pea juice deteriorated

Table 3. The composition of plant juices extracted from various legumes (in percentages).

	Goat's rue	Pea	Field bean	Persian clover
Dry matter	8.31	8.33	6.84	7.99
Ash	1.27	0.93	0.90	1.29
in DM	15.3	11.2	13.2	16.2
Crude protein	2.55	2.77	1.34	1.50
in DM	30.6	33.2	19.6	18.7
True protein	1.75	1.56	0.73	1.00
in DM	21.1	18.7	10.7	12.5
Water soluble carbohydrates	1.70	3.57	3.14	2.79
in DM	20.5	42.9	45.9	34.9

very rapidly, pulse crop juices thus being difficult to use in animal feeding.

Goat's rue and pea LPC had a protein content of 52—53 %, while LPC derived from field bean or Persian clover had a lower value, 42—46 % of DM. The crude fat content was found to be higher when the ether extraction was preceded by hydrolysis with HCl; Table 5 presents the values of the latter method. There were no great differences in amino acid composition between the sources of LPC. The contents of essential amino acids of the presents LPC samples were lower than the values reported for lucerne LPC (CONNELL and FOXELL 1976, HANCZAKOWSKI and SKRABA 1984), but correspond to the values for alfalfa given by CHEEKE et al. (1977). In all the samples *in vitro* digestibility and solubility in pepsin HCl were high, 84—89 %. KUZMICKY and KOHLER (1977) reported *in vitro* crude protein digestibilities for four lucerne LPC (Pro-Xan) samples ranging from 87.8 to 97.2 %.

The chemical composition and gross energy values of the leaf protein concentrates and the diets are shown in Table 5. GE was higher in pea LPC than in the other concentrates. Diet no. 1, the basic diet, had a slightly lower energy content than the others.

The group of cocks receiving diet no. 3, containing of pea LPC, consumed less ($P < 0.05$) feed mixture than the others (Table 6). The reason for this is uncertain, but the animals were a little lighter than those in other treatments. The digestibility of the crude protein of the pea LPC was 77.6 %, which was significantly higher than in the other LPC products ($P < 0.05$). Carbohydrates and organic matter were also more digestible in pea LPC ($P < 0.05$). Persian clover LPC had the lowest nutrient digestibilities ($P < 0.05$). In the in field bean and Persian clover products the tannin contents were higher (5.5—3.0 %) than in pea or goat's rue LPC (2.7—2.2 %) and this is one probable reason for the differences in digestibility. With peas and field beans (tannins 1.8—2.3 % of DM) LINDGREN (1975) found a strong correlation between the digestibility

Table 4. The chemical composition and *in vitro* digestibility of leaf protein concentrates from various legumes.

	Goat's rue	Pea	Field bean	Persian clover
Chemical composition, % of DM				
Dry matter	15.7	15.0	12.7	13.2
Ash	8.7	7.8	8.6	10.2
Crude protein	52.0	53.4	46.1	42.5
True protein	45.2	45.3	41.7	38.0
Ether extract	4.6	7.3	2.1	7.9
Crude fibre	1.0	2.4	3.0	2.5
NFE	33.7	29.1	40.2	36.9
Tannins	2.2	2.7	5.5	3.0
Amino acids g/16 g N				
Alanine	5.1	5.2	5.3	5.6
Arginine	5.6	5.5	5.6	5.9
Aspartic acid	13.4	9.4	13.1	10.8
Cystine	0.9	1.2	1.0	1.1
Glutamic acid	10.4	10.0	10.6	10.7
Glycine	4.5	4.6	4.8	5.1
Histidine	1.9	2.1	2.1	2.3
Isoleucine	3.9	4.0	4.1	4.2
Leucine	7.9	8.3	8.5	8.9
Lysine	4.8	4.3	4.1	4.4
Methionine	1.1	1.6	1.5	1.2
Phenylalanine	4.8	5.1	5.2	5.6
Serine	4.4	4.0	4.1	4.3
Threonine	4.7	4.5	4.6	4.8
Tyrosine	3.4	3.9	3.8	4.0
Valine	5.2	5.2	5.0	5.5
In vitro digestibility				
Organic matter, %	89.5	83.5	86.4	87.2
Pepsin HCl soluble protein, % of CP	86.4	88.6	87.1	85.3

Table 5. The chemical composition of the LPC's and diets used in the feed evaluation (% of DM).

	Dry matter	Crude protein	Crude fat	Crude fibre	Ash	GE MJ/kg DM
Leaf protein concentrate						
Goat's rue	97.5	53.9	8.1	0.6	9.0	22.33
Pea	93.2	54.6	10.7	1.0	8.0	23.08
Field bean	91.7	46.8	7.7	0.9	8.6	22.00
Persian clover	92.9	40.5	10.1	0.6	11.2	21.55
Experimental mixtures						
Mix no 1 (basic)	86.3	11.4	3.3	4.1	4.6	18.00
Mix no 2	89.3	23.2	4.7	2.9	6.2	19.16
Mix no 3	88.1	22.0	5.1	3.2	5.9	19.22
Mix no 4	87.8	20.4	4.5	3.2	6.7	18.84
Mix no 5	88.1	19.2	5.0	3.4	7.3	18.79

coefficient of crude protein and the percentage of tannins in crude protein. Leguminous seeds have given protein digestibilities from 73.3 % (KIISKINEN and HUIDA 1984) to 80—88 % (LINDGREN 1975, ASKBRANT and HAKANSSON 1984), but they have a higher

Table 6. Dry matter intake, nitrogen balance, digestibilities of nutrients and calculated ME values (mean + s.d.).

	Goat's rue	Pea	Field bean	Persian clover	Basic diet
Dry matter	393.3a	292.9b	395.1a	376.4a	385.8
intake, g/d	66.4	56.7	19.9	26.5	26.2
N balance	9.8	7.4	7.3	8.6	6.8
mg/kg W	1.8	1.3	2.1	0.9	1.1
Apparent digestibility, %					
Crude protein	68.0e	77.6d	67.6e	54.0f	67.1
	1.6	4.6	3.5	3.5	2.6
Ether extract	70.5	72.2	68.0	66.6	57.3
	2.9	2.9	4.5	4.0	1.2
Carbohydrates	38.6b	54.9a	39.7b	43.5b	81.2
	6.5	6.2	7.2	4.3	0.73
Organic matter	58.8e	70.3d	56.4e	50.9f	77.0
	2.1	4.2	3.4	3.1	0.44
True digestibility, %					
Crude protein	70.0e	82.2d	69.7e	56.8f	75.5
	2.1	4.6	3.7	3.2	2.2
Metabolizable energy contents, MJ/kg DM					
Apparent					
AME class	11.42e	13.48d	10.12ef	9.72f	13.68
	0.09	0.62	0.61	0.45	0.07
AME_N	10.79e	13.15d	9.80ef	9.18f	13.43
	0.27	0.55	0.55	0.56	0.09
True					
TME	12.04e	14.83d	10.67ef	10.39f	14.27
	0.46	0.54	0.58	0.43	0.08
TME_N	11.41e	14.50d	10.35ef	9.89f	14.03
	0.29	0.54	0.52	0.50	0.10
AME_N % of GE	48.3	57.0	44.5	42.6	74.6

Means with different letters were significantly different, a—c ($P < 0.05$); d—f ($P < 0.01$).

crude fibre content, 7—9 % of DM as compared to 1—3 % in the LPC samples.

The lower digestibility values of LPC products compared with those of seeds of pulse crops are evidently connected with some properties of the leaf proteins or some antinutritional substances in the products.

From a survey of numerous protein quality trials, MORRIS (1977) concluded that LPC had a satisfactory digestibility and gave biological values and protein efficiency ratios which were consistent with its known amino-acid composition. From rat trials SAUNDERS et al. (1973) and BICKOFF et al. (1975) reported *in vivo* protein digestibilities in rat trials to ranging from 81—91 and 86 to 94 % for lucerne LPC and the true digestibility values obtained by HANCZAKOWSKI and SKRABA (1984) for the crude protein of LPC samples prepared by different treatments ranged from 76 to 88 %. These data are in agreement with the values received for the present pea LPC but exceed the values for the other LPC products. Methionine supplementation have improved the biological value of LPC lucerne protein in a study with chicks (HANCZAKOWSKI et al. 1981).

The metabolizable energy values for pea LPC exceeded those for the other LPC products ($P < 0.01$; Table 6). The AME_N values of the various pulse crop LPC's, 9.2—13.2 MJ/kg DM, are sufficiently high to make these products suitable for poulty feeding. The values for pea LPC are promising. The ME values reported for some leguminous seeds are of the same size order as those for the present LPC products (LINDGREN 1975, KIISKINEN and HUIDA 1984, ASKBRANT and HAKANSSON 1984).

The results presented in this paper indicate that leaf protein from pulse crops can be utilized as a protein supplement in poultry rations, since the amino acid composition is rather promising and the digestibility and metabolization are fairly good, especially in the case of pea LPC. The protein yields and juice extraction from pea were higher than from the other crops.

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Ms received January 22, 1985

SELOSTUS

Vihantapalkokasveista saatu lehtivalkuaistiiviste siipikarjan rehuna

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Tutkimuksessa selvitettiin lehtivalkuaisen eristämistä vuohenherneen, herneen, härkäpavun ja persianapilan vihantakasvustoista ja määritettiin saatujen palkokasvitiivisteiden sulavuus ja muuntokelpoinen energia 16—18 viikon ikäisillä kukoilla kokonaiskeruumenetelmällä. Vesipitoisista vihermassoista saatiin keskimäärin 57 % mehua tuorepainosta. Palkokasvimehut sisälsivät kuiva-ainetta 7.9 % keskimäärin sekä kuiva-aineessa 25.5 % raaka-valkuaista ja 36.1 % sokereita. Lehtivalkuaisissa oli keskimäärin valkuaista 42.6 %, rasvaa 5.5 % ja raakakuitua 2.2 %. Lysiiniä oli 4.1—4.8, rikkipitoisia aminohappoja 2.0—2.8 ja treoniiniä 4.5—4.8 g/16 g N. Valkuaisen pepsiini-HCl liukoisuus oli korkea, 85.3—88.6 %. Hernelehtivalkuaisen sulavuudet olivat merkitsevästi korkeampia kuin muiden, kun taas persianapilan lehtivalkuainen suli huonommin kuin toiset. Raakaproteiinin todelliset sulavuudet olivat: 70.0 % vuohenherne, 82.2 % herne, 69.7 % härkäpapu ja 56.8 % persianapila. Tanniinipitoisuudella oli vaikutusta sulavuuteen. Näennäiset muuntokelpoisen energian (AME_N) arvot olivat 10.79, 13.15, 9.88 ja 9.18 MJ/kg ka., vastaavasti. Hernelehtivalkuaisen sulavuus ja ME -arvo ovat tasoltaan sitä luokkaa, että sen käyttö olisi mahdollista siipikarjanrehuissa. Lehtivalkuaisen tuotantokustannukset ovat kuitenkin korkeammat kuin muiden nykyisin käytössä olevien proteiinirehujen.