The relative nutritive value of lucerne leaf protein concentrate (LPC) coagulated by means of steam or hot water

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Two types of lucerne LPC were prepared by either adding the juice to hot water (85°C) or by injecting steam until the juice reached a temperature of 85°C after which the coagulum was freeze-dried. The resulting water and steam-coagulated LPC had a protein content of 54,5 and 49,9% and a lysine content of 3,22 and 2,92% respectively on an air-dry basis. The quality of the protein concentrates was determined by using the relative nutritive value (RNV) assay as well as *in vivo* true protein digestibility (PTD) with male Wistar rats. Body protein accretion was used as the measure of response to dietary protein and lactalbumin was used as the reference protein. The RNV's of lactalbumin, water-coagulated and steam-coagulated lucerne LPC were respectively 1,0; 0,573 and 0,397, whereas the PTD was respectively 97,6; 80,9 and 76,7%. All the rats on the water-coagulated lucerne LPC diet showed symptoms of photosensitivity.

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Twee soorte lusern-blaarsapproteïenkonsentraat (BPK) is berei deur lusernsap óf by warm water (85°C) te voeg, óf deur stoom daardeur te borrel tot die sap 'n temperatuur van 85°C bereik het, waarna die koagulaat gevriesdroog is. Die resulterende water- en stoomgekoaguleerde BPK's het proteïeninhoude van 54,5 en 49,9% en lisieninhoude van respektiewelik 3,22 en 2,92% op 'n lugdroë basis gehad. Die kwaliteit van die proteïenkonsentrate is deur middel van die relatiewe voedingswaarde-tegniek asook in terme van *in vivo* ware proteïenverteerbaarheid met manlike Wistar-rotte bepaal. Die toename in liggaamsproteïen is as die responsparameter op dieetproteïen gebruik terwyl laktalbumien as verwysingsproteïen gebruik is. Die relatiewe voedingswaardes van laktalbumien, water- en stoom-gekoaguleerde lusern-BPK was respektiewelik 1,0; 0,573 en 0,397 terwyl die ware proteïenverteerbaarhede respektiewelik 97,6; 80,9 en 76,7% was. Al die rotte wat water-gekoaguleerde BPK ontvang het, het simptome van fotosensitiwiteit getoon.

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Methods of producing lucerne LPC, in which the protein fraction is separated from the fibrous part, have been described by Kohler, Chrisman & Bickhoff (1973) and Donnelly, McDonald & Rattray (1983). The purpose of the present study was to determine the effect of a patented procedure for the coagulation of lucerne LPC by means of hot water (International classification number A 23J, A 23K) on the protein quality of lucerne LPC. This procedure consists essentially of the addition of lucerne juice to two parts (v/v) of hot water (85°C).

According to Pirie (1975) both temperature and the rate of heating have an effect on the quality of the resulting LPC. Sudden heating minimizes enzymatic changes, whilst heating to 70 or 80° does not inactivate all the enzymes and they may cause changes in the protein during prolonged storage. Another factor which may affect the quality of the LPC is the presence of water-soluble growth depressants and reducing sugars which react with lysine undergoing the Maillard reaction (Pirie, 1975). The hot-water method dilutes these water-soluble substances and could, therefore, yield a LPC with a better protein quality than the steam-treatment method. Furthermore, the temperature of the steam at the point of injection may be high enough to damage some of the protein whereas the maximum temperature which the juice can reach with the hot-water method, is never higher than that of the water.

Materials and Methods

Fresh lucerne was cut at about 10% bloom stage and pulped by passing it through a Wolf King carcass grinder without a screen after which an equal mass of water was added to facilitate manual pressing. The resulting juice was heated by either injecting steam during agitation until it reached a temperature of 85°C or by adding the juice to two parts of water (v/v) at 85°C. The coagulum was separated from the deproteinized juice by passing it through a centrifugal separator (Westfalia SA 01/076 self-desludging separator). The wet lucerne LPC was cooled and stored at 4°C and subsequently dried in a laboratory freeze-drier (Specht Scientific mod. SS-FD-5S).

The protein quality was determined by means of a multi-point slope ratio assay as developed by Hegsted, Neff & Worcester (1968) and used by Donnelly, *et al.* (1983). Fifty-five male Wistar rats were divided into 11 groups of equal mass at 27 days of age and fasted for 24 h after which the initial slaughter group was asphyxiated, their intestinal contents removed, and their chemical composition determined according to the method described by Bell & Stern (1977). One group

Table 1 Composition of the experimental diets on an air-dry basis (%)

Component	Control	Lactalbumin			Lucerne LPC		
	0	3% CP	6% CP	9% CP	3% CP	6% CP	9% CP
Maize starch	85	80,3	77,2	72,4	76,9	71,5	63,4
Maize oil	10	10	10	10	10	10	10
Mineral & vitamin premix*	5	5	5	5	5	5	5
Lactalbumin	–	4,7	7,8	12,6	–	–	–
Lucerne LPC	–	–	–	–	8,1	13,5	21,6
Determined protein content	0,28	3,75	5,88	9,36	4,16[a] 4,28[b]	6,54 6,11	10,10 9,50

[a]Water-coagulated LPC; [b]Steam-coagulated LPC; *Supplied per kilogram feed: Vitamin A, 2,0 IU; Vitamin D, 1 000 IU; Vitamin E, 35 mg; Vitamin K, 50 µg; Thiamin hydrochloride 1,25 mg; Riboflavin 2,5 mg; Vitamin B6, 7 mg; Vitamin B12, 5 µg; Calcium pantothenate, 8 mg; Niacin, 15 mg; Choline chloride 750 mg; Cu, 5 mg; Mn, 50 mg; Zn, 12 mg; I, 0,15 mg; Fe, 35 mg; Se, 0,04 mg; Mg, 0,4 g; P, 4,0 g; K, 1,8 g; Na, 0,5 g; Ca, 5,0 g

received a protein-free diet whilst the remaining nine groups were randomly allocated to the other treatments, namely diets with 3, 6 or 9% protein with lactalbumin (Sigma), water-coagulated lucerne LPC, or steam-coagulated lucerne LPC as the protein source. The experimental diets (Table 1) were fed *ad libitum* from 28 to 49 days of age whereafter the rats were fasted again for 24 h and then slaughtered and treated similarly to the initial slaughter group. Hegsted, *et al.* (1968) stated that measurement of body protein is usually presumed to be the measurement of choice but that it is expensive and relatively difficult to determine. The technique of Bell & Stern (1977) is, however, both relatively inexpensive and simple to perform and, therefore, body protein was chosen as the measure of response to the dietary protein. The initial body protein contents of the final slaughter groups were calculated from a linear regression equation that was fitted to the data of the initial slaughter group. The response was estimated as the gain of body protein by subtracting initial body protein from final body protein.

The experimental diets are described in Table 1. Maize starch was replaced by the experimental protein sources and each diet contained 10% maize oil and 5% of a vitamin and mineral mixture.

The rats were individually housed in metabolism cages at 20°C and a 12 h light-dark cycle. Faeces and spillage were collected daily. The faeces were frozen for subsequent analysis.

The efficiency of use of protein for growth was estimated as the coefficient (b) of the linear regression ($y = a + bx$) of body protein gain (y) on protein intake (x). Data from rats fed the protein-free diet were included in the regression calculation. A common intercept was fitted for all the protein sources. Relative nutritive value (RNV) is the ratio of the regression coefficient (b) for the test protein to that for the reference protein (lactalbumin) (Hegsted, *et al.* 1968; Donnelly, *et al.* 1983).

Protein true digestibility (PTD) was also estimated by a regression procedure (Donnelly, *et al.* 1983). The faeces were freeze-dried, milled and analysed for nitrogen. Total faecal protein was related to protein intake according to the same technique employed to calculate the efficiency of protein use, and the coefficient of the relationship, which defined in-digestibility, was subtracted from 1,00.

The regression lines were statistically analysed by analysis of covariance (Snedecor & Cochran, 1980).

Results and Discussion

The chemical compositions of the two lucerne LPC samples prepared in the present study are given in Table 2.

Table 2 Chemical composition of experimental lucerne leaf protein concentrates[a]

Component	Steam-coagulated	Water-coagulated
Dry matter	93,91	93,87
Protein	49,9	54,5
Ether extract	13,2	12,7
Crude fibre	<0,5	<0,5
Ash	9,1	8,0
Lysine	2,92	3,22

[a]On an air-dry basis

The two experimental LPC's do not differ markedly in chemical composition, although there is a tendency for the water-coagulated LPC to have a higher protein content together with a lower ash content. The latter is possibly due to the dilution effect of the added hot water. Both products compare favourably with a traditional protein source such as soya bean oilcake meal. The regression equations describing the chemical compositions of the rats in the initial slaughter group are presented in Table 3.

The slope ratio response curves of protein intake against protein accretion are presented in Figure 1 whilst the slopes of the respective lines together with RNV values are given in Table 4.

Although the response curves seem to deviate from linearity (Figure 1) regression analysis revealed that they were best described by linear regression. According to Hegsted, *et al.* (1968) a prime consideration in the assay is that the regression lines between response and protein consumed must be linear. They stated that deviations from blanks were most common indicating that animals fed a protein-free diet may respond in an atypical manner and that there is not always a linear

Table 3 Chemical composition of the initial slaughter group (28 days of age) expressed using regression equations

Component (y)	Equation	r^2	Syx
Protein (g)	$y = 1{,}5677 + 0{,}14893x$[a]	0,984	0,143
Ether extract (g)	$y = -2{,}9669 + 0{,}10064x$	0,910	0,241
Moisture (g)	$y = 1{,}5171 + 0{,}71752x$	0,996	0,354
Ash (g)	$y = 0{,}5761 + 0{,}02409x$	0,936	0,048

[a]x = Empty bodymass (g)

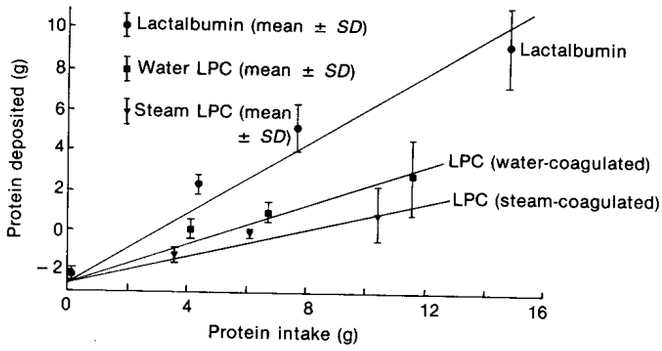

Figure 1 Response curves of the rats on the experimental protein sources where cumulative protein intake is the independent variable and protein accretion the dependent variable.

Table 4 The relative nutritive value of lucerne leaf protein concentrate (LPC) coagulated by means of steam or hot water (Intercept = −2,533)

Protein source	Slope ± *SD*	RNV ± *SD*
Lactalbumin	0,845 ± 0,03	1,0
Water-coagulated	0,484 ± 0,02[a]	0,573 ± 0,03
Steam-coagulated	0,336 ± 0,03[a]	0,397 ± 0,04

[a]Slopes differ significantly ($P \leq 0,05$)

response from the zero dose to higher doses. This suggests that some caution should be exercised in relying upon the usual measure of net protein use (NPU). Donnelly, *et al.* (1983) did not report any deviation from linearity of the response curves, either because it was not considered to be important or because it was not found. By omitting the data on the protein-free rats, somewhat smaller slopes were found but the relative nutritive values remained the same. This suggests that the slope ratio technique is insensitive to changes in absolute slope and that relative values remain constant. The procedure of using a common intercept ensures that all the variation between treatments, even in the presence of slight curvature, is expressed in the differences between slopes.

From Table 4 it is evident that the relative nutritive value of the water-coagulated lucerne LPC (0,573) was significantly ($P < 0,05$) higher than that of the steam-coagulated lucerne LPC (0,397). This difference was most probably due to the lower temperature used during coagulation and consequently less heat damage to the protein. A further reason for less damage to the protein might be that the reducing sugars were sufficiently diluted by the additional water to inhibit the Maillard reaction. The results of the PTD (Table 5) suggest that although significant differences were obtained, protein digestibility was not solely responsible for the lower nutritive value of the steam-coagulated lucerne LPC.

Table 5 True protein digestibility (PTD) of the experimental diets containing lactalbumin and lucerne leaf protein concentrate (LPC)

Protein source	Slope ± *SD*	PTD ± *SD*
Lactalbumin	0,024 ± 0,01	97,6 ± 0,5%
LPC (water-coagulated)	0,191 ± 0,01[a]	80,9 ± 1,2%
LPC (steam-coagulated)	0,233 ± 0,01[a]	76,7 ± 1,3%

[a]Slopes differ significantly ($P \leq 0,05$)

The protein of the steam-coagulated LPC was four percentage units less digestible (76,7 vs 80,9%) than that of the water-coagulated LPC. Donnelly, *et al.* (1983) found the PTD of lactalbumin to be 92,7% which is slightly lower than the 97,6% found in the present study. This is possibly due to the fact that a chemically pure (Sigma) preparation was used in the present study whereas Donnelly, *et al.* (1983) used commercially prepared lactalbumin (New Zealand Co-operative Dairy Co Ltd). The PTD of lucerne LPC found by Donnelly, *et al.* (1983) was exactly the same as the value found for the water-coagulated LPC in the present study, namely 80,9%.

Conclusion

It can be concluded that the steam-coagulation process damaged the protein to such an extent that the relative nutritive value was 17 percentage units lower than that of the water-coagulated LPC.

Unfortunately certain undesirable proteins also escaped damage during the water-coagulation process. All the rats on the water-coagulated LPC diet showed signs of photosensitivity namely loss of hair and skin lesions in the form of blackening of the ear rims despite the fact that the animal room was windowless. Three rats even sloughed their ears. This phenomenon was studied by Lohrey, Tapper & Hove (1974) and Tapper, Lohrey, Hove & Allison (1975) who found that the sensitivity is caused by chlorophyllides and pheophorbides which are derived from chlorophyll. At 80°C the enzyme chlorophyllase, in which lucerne is rich, converts chlorophyll to chlorophyllides (Arkcoll & Holden, 1973). This enzyme is inactivated by briefly heating the juice to 90°C (Tapper, *et al.* 1975).

Despite the fact that the water-coagulated LPC was nutritionally superior to the steam-coagulated LPC, steam coagulation should remain the process of choice until it can be established that unpigmented farm animals (pigs and poultry) are insensitive to pheophorbides and chlorophyllides.

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