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Growth of young rats on diets based on fish silage with different degrees of hydrolysis

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The effect of storage for up to 1 year on the nutritional value of ensiled cooked and non-cooked minced capelin was determined in growth experiments with young rats. Minced fresh capelin of the same batch was also included. Cooked silage came out best after the storage of 1 year showing both better growth and protein efficiency ratio (PER). There were no significant differences in apparent digestibilities. Rats given the uncooked silage accumulated more glycogen in the liver than the rats given the cooked silage diet ($p < 0.01$). The hepatosomatic index and the liver fat content were higher in the rats given cooked silage. No differences were found in hepatic ash and protein contents.

The rats given raw minced capelin performed worse than the rats given the silages of up to 180 days of storage, but they performed better than those with 1 year of storage. This was probably due to the procedure used for mixing the diet.

INTRODUCTION

The fish silage used in Norway is ordinarily made by adding enough formic acid to minced fish and fish offal to bring pH below 4·2. Such a silage is stable to bacterial degradation, but endogenous proteolytic enzymes will, upon storage, hydrolyse the protein to short peptides and free amino acids (Raa & Gildberg, 1976; Hall *et al.*, 1985; Stone & Hardy, 1986). In a previous experiment with lyophilized herring silage as the sole protein source for young growing rats, protein utilization was poorer with stored than with freshly prepared silage (Espe *et al.*, 1989). These results were confirmed in a nitrogen balance experiment with young rats given saithe offal silage as the sole source of protein (Espe *et al.*, 1990).

The present experiment was carried out to test whether arresting the autolysis of fish silage by cooking prior to acid addition would result in a stable product which would not lose nutritional value upon storage. To expose any difference between a cooked and a non-cooked silage in nutritional value, the diets were prepared with silage as the sole source of protein.

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MATERIALS AND METHODS

Silage production

A silage was produced from fresh, frozen capelin (*Mallotus villosus*) caught off the coast of Iceland, February 1988. Partly thawed capelin was minced and raw silage (RS) was produced by adding 22 g kg^{-1} formic acid (85%), 2 g kg^{-1} potassium sorbate and 250 mg kg^{-1} ethoxyquin (EMQ–RALUQUIN, Roche, 66%) to the mince. Another batch of frozen capelin was minced and heated to 90°C for about 30 mins. After chilling to room temperature, formic acid, potassium sorbate and ethoxyquin were added as above (cooked silage (CS)). The silages were kept in tight plastic containers and stored at 12 ± 2°C. Raw capelin was stored frozen. Samples for chemical analysis were taken after 0, 1, 4, 7, 14, 30, 42, 90, 180 and 360 days of storage. The raw material (RM) was analysed at the start of the first growth experiment.

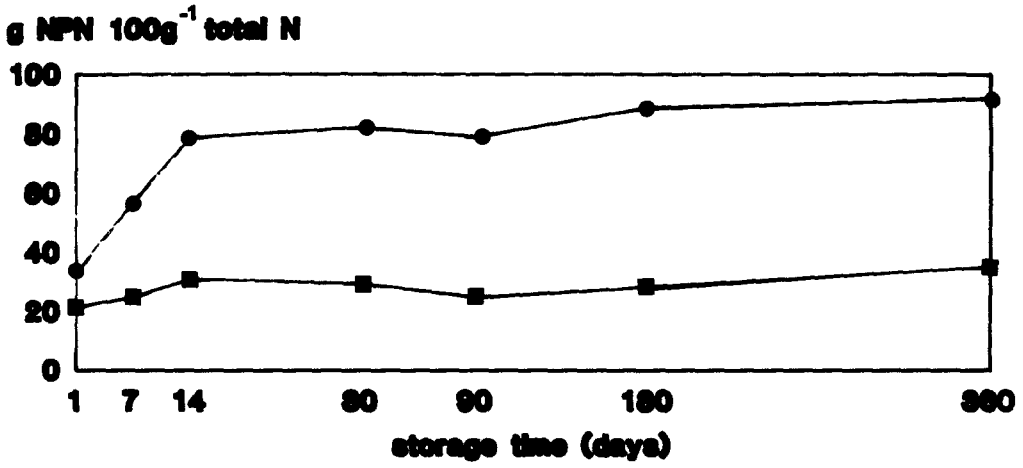

Fig. 1. Non-protein nitrogen (NPN) as g soluble N 100 g^{-1} total N for raw silage (●) and cooked silage (■) stored for 360 days at 12 ± 2°C.

Chemical methods

The silages and the minced capelin were analysed for total protein (N × 6·25), total fat, ash, dry matter and non-protein nitrogen (NPN) as described earlier (Espe *et al.*, 1989). Total volatile N (TVN) and ammonium (NH_3-N) were determined in the silages as described by Haaland and Njaa (1988). Glycogen in the rat livers was determined by the method used by Hemre *et al.* (1989). Chromium was determined in the feed and the faeces according to Rosenlund and Njaa (1982).

Biological methods

Male albino rats (Wistar Møll, Køge, Denmark) weighing about 75 g were used in five growth experiments. The experiments were run when the silages had been stored for 7, 42, 90, 180 and 360 days. The rats were kept individually in wire cages, the photoperiod was 12 h light and 12 h dark. Each diet was given to five rats in all experiments. The diets were prepared every 3rd or 4th day in Expt, 1–4 and every 2nd day in Expt 5, from a dry basal mixture, a lipid source and the protein sources to be tested. The dry basal mixture contained (g kg^{-1}): sucrose 100, cellulose 20, vitamins 10 and minerals 40. It was mixed with 10 g soya bean oil kg^{-1} and silage or minced capelin was added to give 100 g protein kg^{-1} final feed on a dry weight basis (1·6% N). The diets were balanced to 1 kg by adding dry precooked potato starch. The mineral and vitamin mixtures were as described previously (Espe *et al.*, 1989). The diets were fed *ad libitum* for 24 days and the rats had free access to water. The rats were weighed when the experiments started and after 7, 14, 21 and 24 days. From day 14 to 21 faeces was collected individually and the digestibility was determined as described by Rosenlund and Njaa (1982). The rats were killed by intraluminal injections of mebumal (50 mg ml^{-1}). The livers were dissected, weighed and analysed individually for dry matter, fat, protein, ash and glycogen.

The protein efficiency ratio (PER = g total weight gain/g total protein intake) was calculated for each rat.

Statistical methods

Data for weight gain (ΔW), feed intake (feed), hepatosomatic index (HI), protein efficiency ratio (PER), apparent digestibility (AD), liver fat (LF), liver ash (LA), liver protein (LP) and liver glycogen (LG) were evaluated by two-way or one-way analyses of variance with diets and experiments as the variables.

The sum of squares of n treatments was then subdivided into $n - 1$ parts, each corresponding to a single degree of freedom, and these parts were tested individually against experimental error (Snedecor, 1946).

RESULTS AND DISCUSSION

Chemical quality of the protein sources used

The silages kept well throughout the 360 days of storage. The pH was constant at 3·8. The pH of minced capelin was 6·6. The protein content of the latter was 146 g kg^{-1}, total fat was 111 g kg^{-1}, total ash was 20 g kg^{-1} and dry matter was 276 g kg^{-1}. During storage of the non-cooked silage there was a slight increase in dry matter, as reported earlier by Jackson *et al.* (1984), Espe *et al.* (1989) and Haaland *et al.* (1990). Jackson *et al.* (1984) assumed that the increase in dry matter might be due to loss of water by evaporation, but it may also be due to binding of water during autolysis of protein, as suggested by Espe *et al.* (1989). The latter

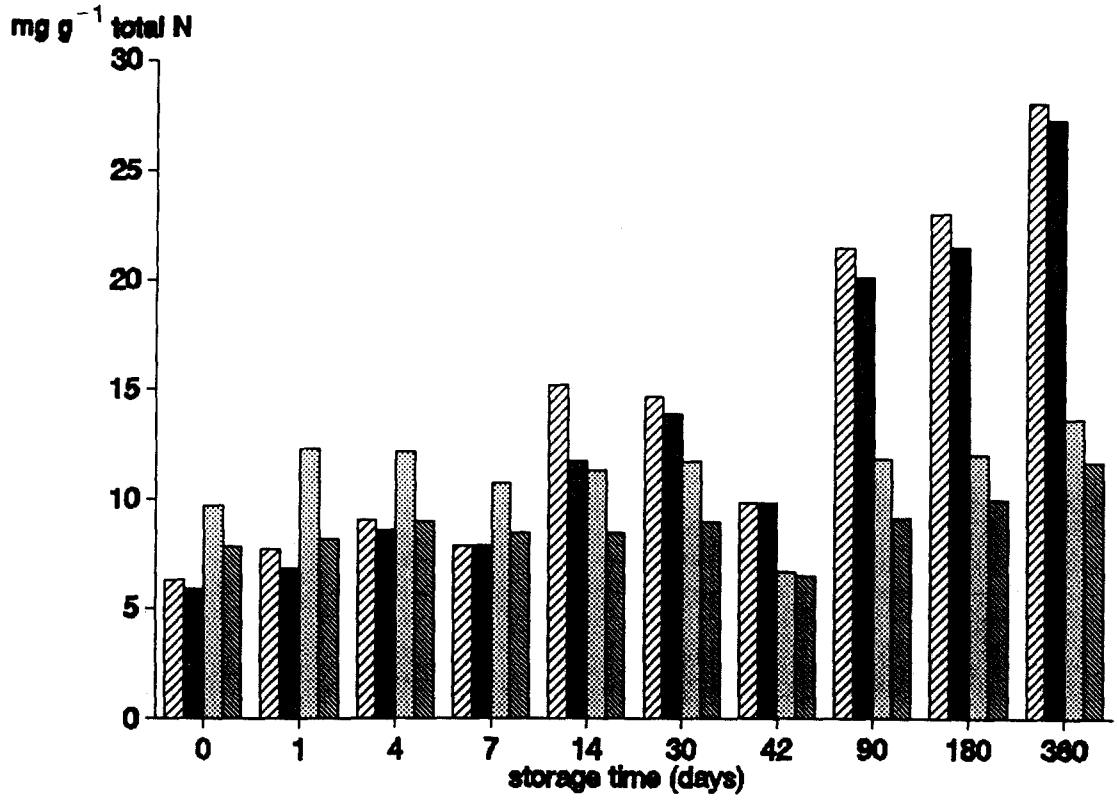

Fig. 2. Total volatile nitrogen (TVN, mg g^{-1} total N) in raw silage (▨) and in cooked silage (▦) and ammonium nitrogen (NH_3-N, mg g^{-1} total N) in raw silage (▩) and in cooked silage (▩) stored for 360 days at 12 ± 2°C.

hypothesis is supported by the fact that there was no increase in dry matter in the cooked silage.

The silage from non-cooked capelin liquefied after 2–3 days, while, as expected, the cooked silage was porridge-like during the entire storage period in accordance with earlier reports (Backhoff, 1976; Wood *et al.*, 1985). In the former the NPN measured as soluble N in 10% (w/v) trichloroacetic acid (TCA) increased from 180 g kg^{-1} at start to 920 g kg^{-1} after 360 days. In the cooked silage the soluble N increased to only 350 g kg^{-1} (Fig. 1). Similarly, the TVN increased in the uncooked silage from 9·7 to 28·5 mg g^{-1}N whereas in the cooked silage an increase could hardly be observed (Fig. 2). Since the increase of TVN was mainly due to NH_3-N, and earlier experiments have shown no differences in the total amino acids during storage of fish silages (Espe *et al.*, 1989), it is likely that ammonia is formed by hydrolysis of the amide N of glutamine and asparagine, as suggested earlier (Espe *et al.*, 1989; Haaland & Njaa, 1989).

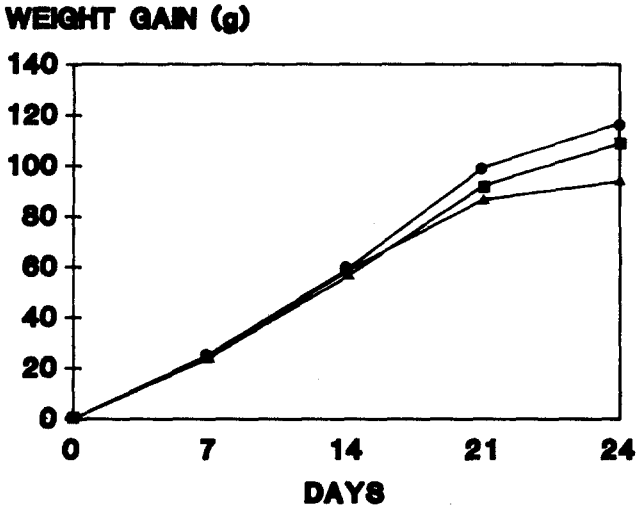

Fig. 3. Mean total weight gains (g) in Expts 1–4 for rats fed diets of raw silage (■), cooked silage (●) and raw capelin not ensiled (▲) as the sole protein source for 24 days.

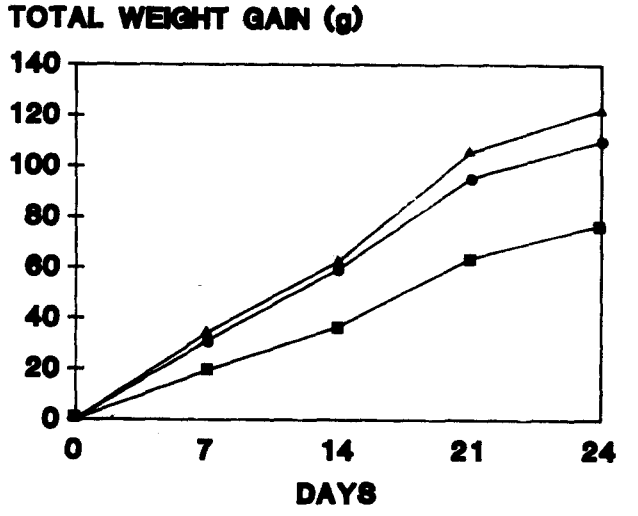

Fig. 4. Total weight gain in Expt 5 for rats fed diets in which the sole protein source was raw silage (■), cooked silage (●) and raw capelin not ensiled (▲) for 24 days.

Table 1. Total weight gain, feed intake, hepatosomatic index and apparent digestibility in five growth experiments on young rats given the two silages and frozen capelin for 24 days

Expt	Weight gain (g day^{-1})	Eaten feed (g day^{-1})	PER	HI	AD (%)
1: 7 days					
RS	4·6 ± 0·3	15·8 ± 0·6	2·9 ± 0·1	4·4 ± 0·1	83·1 ± 1·2
RM	3·6 ± 0·3	13·8 ± 0·5	2·6 ± 0·1	4·5 ± 0·1	78·9 ± 1·3
CS	5·0 ± 0·3	15·8 ± 0·6	3·1 ± 0·1	4·5 ± 0·1	80·9 ± 1·1
X	4·39 ± 0·21	15·11 ± 0·40	2·88 ± 0·08	4·47 ± 0·06	80·96 ± 0·73
2: 42 days					
RS	4·4 ± 0·2	14·8 ± 0·4	3·0 ± 0·1	4·2 ± 0·1	88·1 ± 0·5
RM	4·3 ± 0·4	15·3 ± 0·3	2·8 ± 0·2	4·1 ± 0·1	85·4 ± 1·0
CS	5·3 ± 0·3	16·0 ± 0·5	3·3 ± 0·1	4·7 ± 0·1	85·1 ± 0·8
X	4·71 ± 0·20	15·34 ± 0·22	3·05 ± 0·09	4·34 ± 0·08	86·20 ± 0·55
3: 90 days					
RS	4·6 ± 0·2	15·7 ± 0·7	2·9 ± 0·1	4·5 ± 0·1	79·7 ± 1·8
RM	3·9 ± 0·3	15·0 ± 0·5	2·6 ± 0·1	4·5 ± 0·1	79·4 ± 3·1
CS	4·6 ± 0·3	15·6 ± 0·6	2·9 ± 0·1	4·8 ± 0·1	75·9 ± 1·6
X	4·38 ± 0·18	15·42 ± 0·34	2·83 ± 0·07	4·58 ± 0·05	78·32 ± 1·33
4: 180 days					
RS	4·5 ± 0·1	17·1 ± 0·3	2·6 ± 0·1	4·7 ± 0·1	79·9 ± 5·1
RM	3·8 ± 0·6	15·7 ± 1·0	2·4 ± 0·3	4·7 ± 0·3	78·6 ± 2·8
CS	4·6 ± 0·2	16·0 ± 0·5	2·9 ± 0·1	4·8 ± 0·1	78·5 ± 2·0
X	4·29 ± 0·23	16·25 ± 0·41	2·62 ± 0·10	4·72 ± 0·09	79·01 ± 1·91
5: 360 days					
RS	3·2 ± 0·2	14·2 ± 0·4	2·3 ± 0·1	4·4 ± 0·1	78·0 ± 0·6
RM	5·1 ± 0·1	17·0 ± 0·3	3·0 ± 0·1	5·2 ± 0·1	78·3 ± 0·7
CS	4·6 ± 0·2	15·1 ± 0·3	3·0 ± 0·1	4·4 ± 7·6	75·3 ± 0·5
X	4·31 ± 0·23	15·44 ± 0·35	2·76 ± 0·10	4·68 ± 0·11	77·21 ± 0·48
Diet	**	NS	***	*	NS
Expt	NS	NS	***	***	***
Diet × Expt	***	**	***	***	NS

HI, Hepatosomatic index.
AD, Apparent digestibility.
The silages had been stored for 7, 42, 90, 180 and 360 days.
Each value is the mean of 5 ± SEM.
X is the mean in each experiment ± SEM.
Results of the statistical analysis are indicated:
NS, Non-significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Experiments with rats

The results of the feeding experiments with rats are given in Tables 1 and 2 and in Figs 3 & 4. The feed given was the same in all groups and the intakes were not significantly different except in Expt 5 where silages stored for 1 year were used. In this experiment rats fed the raw material ate significantly more feed than the rats fed the silage diets. The rats fed the non-autolysed protein (CS) ate significantly more feed than those fed the autolysed silage (RS). Equal feed intakes have been reported in rats fed a casein and an amino acid mixture simulating casein at the 10% protein level, but at higher protein levels the feed intake of the amino acid mixture was depressed (Itoh *et al.*, 1973, 1974; Kishi *et al.*, 1978).

Weight gain was lower in rats given capelin RM than in rats given the silages in the 4 first experiments (Fig. 3); in Expt. 5 the opposite was true (Fig. 4). Capelin is known to contain the enzyme thiaminase which destroys the thiamin, and a greater thiamin degradation may have taken place when the feed was prepared every 3rd and 4th day (Expts 1–4) than when it was prepared every 2nd day (Expt 5). This may have led to deficiency in thiamin in the last part of the experimental period in Expts 1–4, resulting in lower growth (Fig. 3). After ensiling, thiaminase activity is apparently absent (Anglesea & Jackson, 1985). The raw material could therefore not be used as a reference feed in Expts 1–4. Total weight gains in rats fed the RS diet and the CS diet showed no significant differences up to 180 days of storage. In rats fed the RS diet in Expt 5, significantly lower weight gain was found. This was probably due to the longer storage and consequently a higher degree of protein solubilization in this silage.

The PER which relates the weight gain to the total protein intake, ranged from 2·3 to 3·1. Rats given the diet with cooked silage showed higher PER values than

Table 2. Liver fat, liver protein, liver glycogen and liver ash in young rats given the two silages and frozen capelin for 24 days

Expt	LF (% dry wt)	LP (% dry wt)	LG (% dry wt)	LA (% dry wt)
1: 7 days				
RS	17·5 ± 3·5	51·6 ± 1·6	16·1 ± 3·6	3·5 ± 0·1
RM	19·2 ± 2·4	50·7 ± 1·8	13·1 ± 1·0	3·3 ± 0·1
CS	20·6 ± 3·2	50·7 ± 2·2	13·2 ± 2·2	3·4 ± 0·2
X	19·10 ± 1·67	51·00 ± 1·00	14·14 ± 1·30	3·42 ± 0·08
2: 42 days				
RS	13·0 ± 1·5	56·3 ± 0·9	20·7 ± 2·1	3·9 ± 0·1
RM	12·6 ± 1·1	62·1 ± 1·3	16·3 ± 2·7	4·1 ± 0·1
CS	17·7 ± 2·4	57·0 ± 1·0	14·1 ± 0·9	3·8 ± 0·1
X	14·45 ± 1·12	58·48 ± 0·91	17·05 ± 1·32	3·94 ± 0·05
3: 90 days				
RS	12·8 ± 1·3	57·8 ± 1·1	16·5 ± 1·6	4·0 ± 0·1
RM	12·5 ± 1·8	60·2 ± 0·4	14·4 ± 0·5	3·9 ± 0·1
CS	14·4 ± 1·0	57·5 ± 1·1	17·6 ± 1·9	3·9 ± 0·1
X	13·24 ± 0·77	58·48 ± 0·60	16·16 ± 0·85	3·93 ± 0·05
4: 180 days				
RS	15·6 ± 1·7	54·1 ± 1·7	17·1 ± 0·5	3·8 ± 0·1
RM	17·4 ± 3·5	56·4 ± 3·4	14·1 ± 2·2	3·6 ± 0·2
CS	20·7 ± 1·0	53·1 ± 1·3	14·3 ± 0·9	3·5 ± 0·1
X	17·87 ± 1·35	54·51 ± 1·27	15·18 ± 0·83	3·59 ± 0·08
5: 360 days				
RS	10·8 ± 1·5	59·3 ± 2·2	12·6 ± 0·7	3·8 ± 0·2
RM	20·1 ± 2·1	52·8 ± 1·4	9·5 ± 1·4	3·8 ± 0·1
CS	13·4 ± 1·5	59·9 ± 0·5	7·4 ± 1·2	4·1 ± 0·1
X	14·77 ± 1·40	57·33 ± 1·20	9·83 ± 0·73	3·74 ± 0·11
Diet	*	NS	**	NS
Expt	**	***	***	***
Diet × Expt	NS	***	NS	*

See Table 1 footnotes.
LF, Liver fat.
LP, Liver protein.
LG, Liver glycogen.
LA, Liver ash.

the rats fed the autolysed raw silage diet. The difference in PER was not due to different digestibilities, as digestibility did not change when the solubility of the feed protein increased. This is in accordance with results reported by Strøm and Eggum (1981) and Espe *et al.* (1989). The better growth and higher PER value for intact feed protein indicate that this is better utilized than shorter peptides and free amino acids. This has been suggested previously on the basis of experiments with rats (Ahrens *et al.*, 1966; Espe *et al.*, 1989), fish (Hardy *et al.*, 1983, 1984) and poultry (Kompiang *et al.*, 1980). As amino acids are absorbed in the free form or as di- and tri-peptides (Holdsworth, 1972; Adibi & Young, 1981; Silk *et al.*, 1985), they may be absorbed faster from a diet containing a high level of predigested protein as in the RS diet than from a diet containing intact proteins as in the CS diet. This may result in an 'overflow' of the anabolic pathways in the liver and more amino acids may enter the catabolic pathways. Reduced utilization of the absorbed amino acids for protein synthesis would then be expected. If this is so, it would be expected that more of the carbon skeleton of the short peptides and free amino acids from the hydrolysed feed would be stored in the liver and body as fat and/or glycogen.

Slightly more glycogen was found in the livers of rats given the uncooked silage diet in Expts 1–4 than in those given the cooked silage diet, but in Expt 5 significantly more glycogen ($p < 0·010$) was found in liver in rats fed the diet of the autolysed silage. The hepatic fat content and consequently the hepatosomatic index were higher in rats given the cooked silage diet than observed for rats fed the autolysed silage diet. The hepatic ash and protein contents did not show any differences between the three diets.

In Expt 5, where thiamin probably had not been destroyed to the same extent as in Expts 1–4, the weight gain and PER in rats fed the raw material were approximately the same as observed for rats fed the diet composed of cooked silage. Rats fed both the RM and the CS diets showed significantly better ($p < 0·010$) weight gains and PER than was observed for the rats

fed the RS diet. This supports the assumption that intact protein is better utilized for growth than is hydrolysed protein.

CONCLUSIONS

Rats given freshly prepared fish silage as the sole protein source, showed the same weight gain as those fed the non-autolysed protein. When the silage was allowed to liquefy for 360 days, both the total weight gain and PER decreased. The explanation may be that the absorption of amino acids is faster when a hydrolysed protein is ingested and that the influx of amino acids exceeds the capacity of the liver to synthesize proteins. In such a situation, excess amino acids may be degraded or transformed to glycogen and lipid. An increase in liver glycogen was detected with increased solubilization of the feed protein, but the liver fat content was not increased. The reason for this is not known and unfortunately whole body fat was not analysed in this experiment. More work is needed to discover whether fat is stored in muscle or not when the feed protein is autolysed.

ACKNOWLEDGEMENT

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