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DIGESTIBILITY AND UTILISATION IN YOUNG GROWING RATS GIVEN SAITHE OFFAL SILAGE STORED FOR DIFFERENT LENGTH OF TIME AS THE SOLE PROTEIN SOURCE

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ABSTRACT

Rats were given saithe offal silage as the sole protein source in a nitrogen balance experiment. Four formic acid silages were prepared from the same batch of frozen saithe offal 2 weeks, 2 months and 5 months before the start of the experiment. The raw material was minced before addition of acid. Cooked raw material was also ensiled five months before start. Thus in three of the silages the proteolytic enzymes were intact and in one the enzymes were denatured. Minced raw saithe offal was used as a control.

Chemical analyses showed varying degrees of protein solubilisation in the four silages. The digestibility showed only small differences whereas the two silages stored for 2 and 5 months showed significantly poorer protein utilisation values than the silages stored for 2 weeks, the one of cooked raw material stored for 5 months and the raw material itself. It is suggested that the high degree of solubilisation in the two stored silages resulted in a rapid amino acid absorption overwhelming the synthetic capacity of the liver.

INTRODUCTION

Fish silage as used in Norway, is fish and fish offal preserved by addition of formic acid to lower pH below 4.5. It has been used as a feed ingredient for farmed fish and for fur animals with good results (Lie et al. 1986). However, poor performance in animals given fish silage in the diet has also been reported (Hardy et al. 1984; Stone and Hardy 1986).

During storage the fish silage change consistence from porridge-like to soup-like. This is due mainly to proteolytic enzymes present in the fish, and

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peptides of varying length and free amino acids are produced. The liquification is not due to bacterial degradation as silage from heated fish does not liquify during storage (Backhoff 1976; Wood et al. 1985; Stone et al. 1989).

Reduced weight gain in farmed fish given silage is often explained by the solubilisation of the protein in the silage. As reduced digestibility of silage protein has not been reported in rats (Strøm and Eggum 1981; Espe et al. 1989) it is likely that it is the utilisation of the absorbed protein which may be impaired. To study this a nitrogen balance experiment was run with rats given fish silages stored for different length of time as the sole protein sources. As controls were used a silage prepared from cooked fish offal and fish offal stored frozen.

MATERIAL AND METHODS

Raw material

Fish offal from saithe (*Pollachius virens L.*) was obtained in april 1989 frozen in 20 kg blocks and stored at–20° C for future use in experiments with rats and salmon.

Fish silage production

Fish silages were produced by adding 2.0% (w/w) formic acid (85%) as a preservative. 0.2% K-sorbate and 0.02% ethoxyquine (EMQ-RALUQUIN, Roche 66%) were added as fungicide and antioxydant, respectively.

One silage was made 2 weeks prior to use (RS). A second silage was made 2 months (2M) prior to use and a third silage was made 5 months (5M) prior to use. A fourth silage was made from cooked saithe offal, cooked for 10 minutes at 90° C, to denature the endogenous enzymes (CS) and stored for 5 months prior to use. As a standard of comparison, minced raw, saithe offal was used (RM).

Chemical methods

Dry matter, crude protein (N*6.25), fat, ash, non protein nitrogen (NPN) and chromium were analysed as described by Espe et al. (1989). Total volatile nitrogen (TVN) and ammonium nitrogen (NH_3–N) in the silages were analysed according to Haaland and Njaa (1988).

Biological methods

A nitrogen balance experiment using the Thomas Mitchell method as modified by Eggum (1973) was performed. 5 male albino rats (Wistar Møll, Køge Denmark) weighing approximately 80 g were used for each dietary treat-

ment. Each of the 4 fish silages and the minced, raw saithe offal were used as the sole protein sources in the diets. The fish silages and the raw, minced saithe were added to a N-free basal mixture consisting of (g kg^{-1}): pre-cooked potato starch 655, sucrose 100, cellulose 20, mineral mixture 40, vitamin mixture 10 and soy bean oil 30. The mineral and vitamin mixtures were the same as used by Espe et al. (1989). 0.1% Cr_2O_3 was added to the feed to allow for calculation of the apparant digestibility (AD) as described by Rosenlund and Njaa (1982). The preliminary period lasted for 4 days and faeces and urine were collected for an experimental period of 5 days. Each animal received 1.6% N (10% protein) in the feed and 10 g dry feed throughout the preliminary and experimental periods. True digestibility (TD) was calculated assuming a metabolic faecal N-excretion of 2.02 mg g^{-1} food eaten (Njaa 1963). Net protein utilisation (NPU) was calculated by taking into account the urinary N-excretion and assuming and endogenus urinary N-excretion of 0.645 mg g body $weight^{0.75}$ (Njaa 1963). Biological value (BV) was calculated from NPU/TD.

Statistical analysis

Differences in digestibility and utilisation with different dietary treatments were evaluated by analysis of variance. The significance of the differences observed was evaluated by Duncans (1955) multiple range test.

RESULTS AND DISCUSSION

The silages kept well throughout the storage periods. The pH was constant at about 4.2, the minced saithe offal had a pH of 7.0. Fish silage from uncooked material liquified during storage, the silage from cooked saithe offal had a porridge like consistentce during the storage period of 5 months. Dry matter, crude protein, fat and ash were about 218 mg g^{-1}, 155 mg g^{-1}, 32 mg g^{-1} and 33 mg g^{-1}, respectively and no differences between the silages could be noted (Table 1), except for a slight increase in fat by storage. This increase may be related to fat floating up by storage of silage making it difficult to make homogeneous samples. Non protein nitrogen (NPN), determined as nitrogen soluble in 10% trichloro-acetic acid, showed great differences between silages (Table 1).

The liquification of silages during storage is a well known phenomenon (Hardy et al. 1984; Stone and Hardy 1986; Espe et al. 1989; Haaland and Njaa 1989) as is prevention of liquification when the fish material is heated before adding the acid (Backhoff 1976; Wood et al. 1985).

As liquification of the silages increased, total volatile nitrogen (TVN) also

Table 1. The composition of the different saithe offal silages and the raw, minced saithe offal. pH, non protein nitrogen (NPN) in % of total N, crude protein, fat and ash in mg g^{-1} dry matter. Total volatile nitrogen (TVN) and ammonium-nitrogen (NH_3-N) are given in mg g^{-1} total protein.

	5M	2M	RS	RM	CS
pH	4.2	4.4	3.8	7.0	4.0
NPN	80.5	74.1	50.0	18.1	19.9
Protein	702	668	686	691	706
Fat	171	152	143	115	110
Ash	132	166	133	129	162
TVN	15.3	12.1	6.3	7.3	7.1
NH_3-N	14.1	11.2	5.4	6.3	6.4

5M: silage stored for 5 months.
2M: silage stored for 2 months.
RS: silage stored for 2 weeks.
RM: raw, minced saithe offal.
CS: silage made from cooked saithe offal and stored for 5 months.

increased (Table 1). This increase in TVN was mainly due to an increase in NH_3-N. This has been reported earlier (Haaland and Njaa 1988, Espe et al. 1989), the former reported this increase in ammonium to be due to amide-N from glutamine and probably also from asparagine.

The results of the nitrogen balance experiment are given in Table 2. The rats given the stored fish silages (5M, 2M) ate significantly less ($p < 0.05$) than the rats in the other groups. Weight gain was also significantly lower ($p < 0.05$) for the rats given the autolysed fish silages as shown in Table 2. Rats given silage stored for 5 and 2 months did not grow at all during the experimental period whereas the rats given RS, CS and the raw, minced saithe offal showed good growth. The better growth of the rats given intact protein compared to those given autolysed proteins is in accordance with results reported by Itoh et al. (1973; 1974) who fed rats a casein diet and a diet of free amino acids simulating casein.

The differences in apparent and true digestibility or differences in the food intake in the five groups of rats were too small to explain the low growth rates seen in the two groups given the silages stored for 2 and 5 months. This indicates that with increased degree of protein solubilisation the absorbed amino acids were less well utilised for growth. The values obtained for nitrogen balance (Bal) and those calculated for net protein utilisation (NPU) and biological value (BV) showed greater differences between groups than can be accounted for by the small differences in the digestibility values.

Thus the results show that when the silages were stored for a long time with the proteolytic enzymes intact, the protein (N*6.25) solubilised to such

Table 2. Feed intake (FI, g day^{-1}), weight gain (ΔW, g day^{-1}), apparent digestibility (AD, %), true digestibility (TD, %), nitrogen balance (Bal, %), net protein utilisation (NPU) and biological value (BV) during the experimental period of 5 days. Each value is the mean of 5 with standard error of mean given in parantheses.

Diet	FI	ΔW	AD	TD	Bal	NPU	BV
5M	$7.8(0.4)^{c}$	$-0.9(0.9)^{c}$	$68.7(1.2)^{c}$	$87.2(2.4)^{b}$	$2.5(2.0)^{c}$	$33.9(2.2)^{b}$	$38.9(2.6)^{c}$
2M	$9.0(0.4)^{b}$	$0.4(0.3)^{b}$	$71.7(1.3)^{bc}$	$87.2(1.6)^{b}$	$21.0(4.5)^{b}$	$48.3(4.4)^{b}$	$55.2(4.2)^{b}$
RS	$9.7(0.2)^{ab}$	$1.4(0.3)^{ab}$	$73.2(1.4)^{b}$	$90.8(1.2)^{ab}$	$40.9(1.7)^{a}$	$69.5(1.4)^{a}$	$76.5(1.0)^{a}$
CS	$10.2(0.2)^{a}$	$2.0(0.2)^{a}$	$77.7(0.6)^{a}$	$93.9(0.5)^{a}$	$45.3(1.6)^{a}$	$73.3(1.7)^{a}$	$78.0(1.8)^{a}$
RM	$10.4(0.1)^{a}$	$1.5(0.2)^{ab}$	$74.2(1.3)^{ab}$	$91.1(1.2)^{ab}$	$39.8(2.0)^{a}$	$68.3(1.4)^{a}$	$74.9(1.3)^{a}$

5M, 2M, RS, RM and VB is short for the different silages, see Table 1.
Vertical means with different superscript differ significantly ($p < 0.05$).

an extent that the protein utilisation was impaired. Also Espe et al. (1989) reported lower protein utilisation when rats were fed stored herring silage compared to freshly prepared silage, although the differences were not so great as found in this experiment.

The silages were used as the sole protein sources and it is suggested that the presence in the digestive tract of high amounts of predigested protein may have accelarated the amino acid absorption rate to such an extent that the anabolic synthetic capacity of the liver was overwhelmed. Solubilisation up to 50% of the total nitrogen in diets seems to have litle effect on the protein utilisation.

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