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FISH SILAGES PREPARED FROM RAW MATERIAL OF VARYING QUALITY; CHEMICAL ANALYSIS RELATED TO BALANCE EXPERIMENTS IN RATS

By

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ABSTRACT

Capelin *(Mallotus villosus)* and Norway pout (Boregadus esmarkii) raw materials and formic acid silages prepared from them were analysed chemically and the biological values were evaluated in nitrogen balance experiments with rats. The raw materials of capelin and of pout provided similar weight gains, utilizations and digestibilities in the rat experiments. 7 days old silages from fresh capelin were similar to the raw material. Pout raw material showed better weight gain and protein utilization than the corresponding silages. 7 days old silages prepared from capelin raw material stored for 5 days showed similar weight gains and digestibilities but were less well utilized than silages from the fresher raw materials. All the silages stored for 3 months were less well utilized than the 7 days old silages.

The poor utilization of the capelin silages raw material stored for 5 days was reflected in high levels of volatile nitrogen compounds, reduction in essential amino acids and corresponding content of biogenic amines.

INTRODUCTION

The nitrogen balance experiments on rats are part of a project with the aim to gather information on factors influencing the quality of fish silage. Fish silage is used in moist pellets in fish feed, in amounts corresponding to 10–20% of the protein. Ensiling is convenient utilization of fish offal and other resources, but quality criteria are needed to allow for a declaration in buying and selling (Espe et al., 1989). Silage quality depends on the quality of the raw material, on proper ensiling and on reactions in the silage during storage. The type of raw material may also be important.

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The silages used in the present experiments were prepared from fresh frozen whole capelin and Norway pout. These are important potential silage raw materials. Espe et al. (1989 and 1990) have reported experiments with rats using silages prepared from herring offal and from saithe offal.

MATERIALS AND METHODS

Raw materials and silages

Capelin *(Mallotus villosus)* and Norway pout *(Boregadus esmarkii)*, frozen when fresh, were obtained. Capelin contained 125 g protein, 190 g fat and 340 g dry matter per kg, pout contained 160 g protein, 11 g fat and 215 g dry matter per kg. After thawing capelin was stored at ambient temperature (about 15° C) for 1,3 and 5 days, Norway pout was stored for 1 day (Table 1). After the storage time the raw materials were minced and two portions of each of the four samples were ensiled with either 16 or 22 g formic acid (85% w/v). To each portion was added 2 g K-sorbate and 150 mg ethoxyquin (Raluquin, 66%). To obtain stable pH values below pH 4.5 more formic acid was added to four of the silages after one day (Table 1), and after 16 days a new portion of 150 mg ethoxyquin per kg silage was added to all the silages which were stored at room temperature (20° C). One portion of the thawn capelin and Norway pout were freeze-dried.

After 7 days storage half of each of the silages was freeze-dried and minced to be used in feeding experiments, the rest of the silages were stored for 3 months before they were freeze-dried. The freeze-dried silages were very hygroscopic and they were therefore immediately after drying mixed with two thirds (w/w) dry, partially dextrinized potato starch before they were mixed into the experimental diets. The diets were stored at 4° C during the experiments.

Nitrogen balance experiments

Male Wistar Møll rats weighing respectively 58.4 ± 3.1 g (Expt 1) and 63.6 ± 4.1 g (Expt 2) were used in two nitrogen balance experiments by a modified Thomas-Mitchell method (Njaa, 1963; Eggum, 1973; Espe et al., 1989). Ten groups of five rats were used in each experiment. The rats were accustomed to the diet during a 4-day preliminary period. During the following 5 days urine and faeces were collected and analysed for nitrogen and faeces also for chromium.

The rats were offered 10 g dry feed per day in Expt 1 and 8 g per day in Expt 2. In both experiments the diets contained 80 g protein per kg dry diet. The protein sources were the dried raw materials (capelin and Norway pout) and in Expt 1 the 8 silages dried after 7 days and in Expt 2 the 8 silages

dried after 3 months storage. The other constituents of the diets were (g per kg dry weight): Vitamin mixture 10, mineral mixture 40, cellulose powder 10, sugar 50, Cr_2O_3 2. Capelin oil was added to the Norway pout diets to equalize the fat content between diets. Ethoxyquin was added to the raw material diets. Partially dextrinized potato starch was added up to 1000 g. Protein digestibility (AD and TD) and utilization (Bal and NPU) were calculated according to Njaa (1963). Nitrogen intake and faecal output were related to the chromium indicator.

Chemical analysis

Protein (N · 6.25), fat, dry matter and amino acids were analysed as described by Haaland et al. (1988), total volatile nitrogen (TVN), NH_3, amide-N as described by (Haaland and Njaa, 1989), and biogenic amines (cadaverine, tyramine, putrescine, phenethylamine, histamine) according to Haaland et al. (1990). Nitrogen in feed, urine and faeces was determined colorimetrically as for NH_3-N. Chromium was determined as described by Rosenlund and Njaa (1982). All analysis were run in duplicate.

RESULTS

The silages liquified within a couple of days, and pH was stable during storage. The pout silage preserved with 1.85% acid attained pH 4.5. This is assumed to be at the upper limit for proper preservation. When too little acid is added, a microflora will establish and pH will gradually increase. In this silage, though, pH 4.5 was stable for at least 8 months. The different additions of formic acid giving different pH values in the silages (Table 1)

Table 1. Raw materials, total amount of formic acid used, and pH values in the experimental silages.

Raw material	Stored before ensiling	Silage	Formic acid (g/kg)	pH after storage for 1 day	7 days	3 months
Capelin I	1 day	IA	16	4.1	4.2	4.2
»	»	IB	22	3.8	3.9	3.9
Capelin II	3 days	IIA	16	4.1	4.2	4.3
»	»	IIB	22	3.8	3.9	4.0
Capelin III	5 days	IIIA	20	4.4	4.5	4.6
»	»	IIIB	25	4.1	4.2	4.3
Norway pout	1 day	IVA	18.5	4.5	4.5	4.5
»	»	IVB	27	4.2	4.1	4.2

Table 2. Total amino acid content (mg/g protein) and protein (total N · 6.25) (mg/g wet sample) in the raw materials used for the experimental silages.

	Norway pout	Capelin I	Capelin II	Capelin III
Aspartic acid	98	95	89	71
Threonine	44	47	46	32
Serine	45	44	42	29
Glutamic acid	140	137	137	129
Glycine	60	55	56	46
Alanine	61	59	64	57
Valine	42	45	46	47
Methionine	28	27	28	23
Isoleucine	36	35	36	36
Leucine	72	72	77	69
Tyrosine	33	35	37	24
Phenylalanine	38	36	36	35
Lysine	84	83	84	75
Histidine	19	21	21	19
Arginine	57	54	50	29
Protein	168	129	165	122

were neither reflected in the chemical analysis nor in the biological experiments.

Amino acids in the three capelin raw materials and in the Norway pout raw material are given in Table 2. The analyses showed some unsystematic variation, but there was a clear reduction in serine, tyrosine and arginine in the capelin stored for 5 days before ensiling compared to the portions stored for 1 and 3 days.

Table 3. Amines (mg/g protein) in capelin raw material (III) stored for 5 days at 12° C, and silages from the same raw material (IIIA and IIIB) stored for 1 and 7 days and for 1, 2 and 3 months.

	Raw material	Silages 1 day	7 days	1 month	2 months	3 months
Cadaverine[1]	7.7	7.6–9.3[2]	5.7–5.6	6.3–4.6	6.5–5.2	8.8–6.0
Putrescine	3.4	2.9–5.8	2.6–2.8	2.7–2.3	2.9–2.5	3.0–2.8
Histamine	0.3	2.1–1.5	1.8–1.4	1.8–1.3	2.0–1.3	1.9–1.5
Tyramine	8.5	9.2–7.7	8.4–7.6	8.7–7.1	10.0–7.6	8.1–8.4
Phenethylamine	0.6	0.5–0.5	0.5–0.4	0.6–0.5	0.6–0.4	0.4–0.5

[1] the factors for converting the amine values to their corresponding amino acids:
cadavering – lysine: 1.43 putrescine – arginine: 1.98
histamine – histidine: 1.39 tyramine – tyrosine: 1.32
phenethylamine – phenylalanine: 1.36

[2] the first value refers to the low acid level (A), the second to the high level (B)

Table 4. Total volatile nitrogen (TVN), ammonia (NH_3–N) and amide-N (*mg/g total* N) in the raw materials, and in the silages prepared from them after storage for 7 days and for 3 months. The mean of the two silages produced from each raw material (A and B) is given.

			TVN	NH_3–N	amide–N
Capelin raw material	I		11	13	44
Silage	I	7 days	20	16[1]	40 ± 2
Silage	I	3 months	31 ± 1	28 ± 1	29 ± 2
Capelin raw material	II		26	22	37
Silage	II	7 days	24 ± 1	23 ± 1	35 ± 3
Silage	II	3 months	38 ± 1	33 ± 1	28 ± 3
Capelin raw material	III		132	95	41
Silage	III	7 days	127 ± 2	95 ± 1	15 ± 2
Silage	III	3 months	150 ± 6	106 ± 5	8 ± 7
Pout raw material	IV		25	12	49
Silage	IV	7 days	30[1]	16[1]	35 ± 5
Silage	IV	3 months	45 ± 2	35 ± 5	22 ± 7

[1] *only analysis from one of the silages*

Amines were detected only in the portion of capelin raw material stored for 5 days and in the silages from this raw material. The amine concentrations did not change appreciably during the storage of the silages for 3 months (Table 3). The content of tyramine, cadaverine and histamine was reflected in the reduction of the corresponding amino acids. The Norway pout contained no amines in the raw material or in the silages.

In Table 4 are given the results for TVN, NH_3-N and amide-N for the capelin raw material stored for 1, 3 and 5 days before ensiling and for the Norway pout raw material, and for the corresponding silages. Capelin raw material showed low levels of TVN and of NH_3-N 1 and 3 days after thawing and clear increases 5 days after thawing. The slight increase from day 1 to day 3 can partially be explained by the concomitant decrease in amide-N. The 4 to 5 times increases from day 3 to day 5 indicate that α-amino groups from the amino acids were broken down to NH_3 (Haaland and Njaa, 1989). The 7 days old silages reflected to a great extent the contents of TVN and NH_3-N of the raw materials from which they were prepared, and the reduction in amide-N wholly or partially explains the slight increases in TVN and NH_3-N in the silages during storage (from 7 days to 3 months). The Norway pout raw material showed higher TVN and NH_3-N values than the capelin raw material I, and these differences were also reflected in the corresponding silages.

The results from the nitrogen balance experiments are summarized in Tables 5 and 6. Within and between experiments feed intake varied much and the results are therefore somewhat difficult to interprete.

The groups given the two raw materials were offered 10 and 8 g feed per rat per day in Expt 1 and Expt 2, respectively, and these amounts were almost completely eaten (Table 5). Analyses of variance showed there to be no significant differences in growth rate, nitrogen balance and apparent protein digestibility neither between raw materials nor between experiments. The silages stored for 1 week differed insofar that the rats given the Norway pout refused more feed than the rats given the capelin silages, within silages the feed intakes were about equal at the two formic acid levels. The differences in feed intake were reflected in significant differences in growth rates. The differences in apparent digestibility were not significant, but the utilization (nitrogen balance) were significantly better of 7 days old silages that of 3 months old silages, and of the capelin silages compared with the pout silages. Compared with the raw materials, the 7 days old samples of capelin silage were utilized by the rats equally well as these, whereas the utilization of the Norway pout silages were less than of the raw materials as judged from the growth rate, apparent protein digestibility and per cent nitrogen balance criteria. The silages stored for 3 months (Expt 2) were less well accepted by the rats than the corresponding raw materials.

Because of the different feed intakes it is difficult to test the differences in growth rate, apparent protein digestibility and per cent nitrogen balance statistically. However, the results indicates that both raw materials were better utilized than the silages, and that capelin silages were better utilized than Norway pout silages.

The results given in Table 6 summarize the data obtained with the silages prepared from the capelin raw material which had been kept at ambient temperature for 1, 3 and 5 days. The 1-day data are the same as those presented in Table 5. The silages stored for 1 week were equally well accepted by the rats. Analyses of variance showed that the silages made from capelin stored for 3 days before ensiling supported slightly better growth rates than the silages kept for 1 and 5 days before ensiling. This effect was significant, but it was not significantly reflected in better apparent protein digestibility or nitrogen balance. The 7 days old silages prepared from the 5 days old raw material were significantly less utilized (nitrogen balance) than the other capelin silages in Expt 1.

When the silages had been stored for 3 months, the 1-day, the 3-days and the 5-days silages were less well accepted in that order. They are therefore difficult to compare statistically. The growth rates, apparent protein digestibilities and the per cent nitrogen balances were not clearly different between

Table 5. Mean feed intake (g/day), weight gain (g/day), apparent and true protein digestibility (AD and TD, %), nitrogen balance and net protein utilization (Bal and NPU, %) of rats given capelin and Norway pout raw materials (I and IV) and silages prepared from them and stored for 7 days and for 3 months (IA, IB, IVA, IVB).

	Raw material				Silage							
	Capelin		Norway pout		Capelin				Norway pout			
					7 days		3 months		7 days		3 months	
Experiment nr.	1	2	1	2	1		2		1		2	
	I		IV		IA	IB	IA	IB	IVA	IVB	IVA	IVB
Feed intake	9.7	8.0	9.8	8.0	9.7	9.3	6.7	7.9	8.3	8.3	4.5	4.6
Weight gain	2.1	1.9	2.3	2.1	2.5	2.2	0.9	0.5	1.0	0.9	–0.5	–0.3
AD	81.5	88.4	80.4	85.5	79.7	82.5	82.1	82.6	74.8	75.4	78.5	78.7
TD	97.3	104.0	96.2	101.0	95.5	96.6	97.9	98.4	90.6	91.2	94.3	94.5
Bal	66.6	72.6	66.6	67.6	61.9	64.4	49.5	44.5	53.3	52.2	24.1	23.6
NPU	95.2	102.9	95.4	98.3	91.3	92.3	81.3	76.9	83.1	82.0	61.9	61.1

Table 6. Mean feed intake (g/day), weight gain (g/day), apparent and true protein digestibility (AD and TD, %), nitrogen balance and net protein utilization (Bal and NPU, %) of rats given capelin silages (IA, IB, IIA, IIB, IIIA and IIIB) stored for 7 days (Expt 1) and for 3 months (Expt 2).

Experiment nr.	1		2		1		2		1		2	
Silage stored for	7 days		3 months		7 days		3 months		7 days		3 months	
	IA	IB	IA	IB	IIA	IIB	IIA	IIB	IIIA	IIIB	IIIA	IIIB
Feed intake	9.7	9.3	6.7	7.9	9.3	9.7	6.3	6.2	9.0	9.3	5.8	5.4
Weight gain	2.5	2.2	0.9	0.5	2.9	3.1	0.7	0.4	2.0	2.3	0.4	0.0
AD	79.7	82.5	82.1	82.6	80.5	79.2	82.2	79.1	77.3	77.7	82.2	79.8
TD	95.5	96.6	97.9	98.4	96.3	95.0	98.0	94.9	93.1	93.5	98.0	95.6
Bal	61.9	64.4	49.5	44.5	62.7	62.9	38.5	46.7	49.0	48.3	46.8	43.5
NPU	91.3	92.3	81.3	76.9	92.6	92.4	71.6	79.7	79.0	76.8	80.9	78.6

them, but they were clearly lower than the values seen with the raw material (Table 5).

DISCUSSION AND CONCLUSIONS

Good digestibility and utilization of ensiled fish compared to the unpreserved raw material have been shown for silages prepared from herring offal (Espe et al., 1989) and from saithe offal (Espe et al., 1990 – this issue) and of cod and saithe viscera silage (Strøm and Eggum, 1981). This was found for capelin, while ensiled pout was less well accepted by the rat than the raw material. The capelin silages became souplike within few days while the pout silage became even more watery and the smell was somewhat acrid, chemical analysis did not reveal any obvious differences between the raw material and the silages.

Espe et al. (1989) found no change in digestibility of herring offal silage stored for up to 6 months at 20° C. Reduced utilization was found for these silages after storage for more than 90 days and also for saithe offal stored for 2 and 5 months (Espe et al 1990). This might be due to less utilization of partly pre-digested protein, but rancidity may also have been involved (Espe et al., 1989).

In silages of uncooked raw material autolysis is rapid and continuous, though temperature dependent (Backhoff, 1976; Espe et al., 1989; Haaland and Njaa, 1989b). Severe autolysis seems to have adverse effect on the protein value of silages when used as the sole protein source. Slightly autolysed silage, or at low inclusion levels which seem to be well accepted.

Amines may reflect the quality both of the raw material and of the silage. Work is in progress to establish amine levels for fish meal raw materials, and similar levels will probably be convenient in silage production. Further, it seems necessary to obtain chemical criteria, preferably only one, on the degree of autolysis of a silage.

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REFERENCES

Backhoff, H.P., 1976. Some chemical changes in fish silage. J. Food Tech., *11*, 353–363.

Eggum, B.O., 1973. A study of certain factors influencing protein utilization in rats and pigs. Beretning fra forsøkslaboratoriet. Copenhagen.

Espe, M., Raa, J. and Njaa, L.R., 1989. Nutritional value of stored fish silage as a protein source for young rats. J.Food Sci.Agric., *49*, 259–270.

ESPE, M., HAALAND, H. and NJAA, L.R., 1990. Digestibility and utilization in young growing rats given saithe offal silage stored for different length of time as the sole protein source. Fisk.Dir.Skr.Ser. Ernæring – this issue.

HAALAND, H., ARNESEN, E. and NJAA, L.R., 1988. Amino acid composition of whole herring over the spawning season and during anaerobic storage at 2° C. Int.J.Food Sci.Tech. *23*, 521–524.

HAALAND, H. and NJAA, L.R., 1989. Total volatile nitrogen – a quality criterion for fish silage? Aquaculture, *79*, 311–316.

HAALAND, H. and NJAA, L.R., 1986. Effect of temperature on the autolysis of capelin silage stored for one year. Fisk.Dir.Skr.Ser.Ernæring, Vol. II, No. 7, 219–226.

HAALAND, H., ARNESEN, E. and NJAA, L.R., 1990. Amino acid composition of whole mackerel *(Scomber scombrus)* stored anaerobically at 20° C And at 2° C. Int.J.Food Sci.Tech., *25*, 82–87.

NJAA, L.R., 1963. A study of the Mitchell method for determination of the biological value of protein. Fisk.Dir.Skr.Ser.Tekn.Unders., Vol. IV, No. 5, pp 118.

ROSENLUND, G. and NJAA, L.R., 1982. Protein digestibility in rats determined with TiO_2 and Cr_2O_3 and comparisons with a rapid enzyme test. Acta Agr. Scand. *32*, 115–121.

STRØM, T and EGGUM, B.O., 1981. Nutritional value of fish viscera silage. J.Sci. Food Agric., *32*, 115–120.