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PROTEIN VALUE OF COD AND COALFISH AND SOME PRODUCTS FOR THE YOUNG RAT

By

LEIF REIN NJAA, FINN UTNE and OLAF R. BRÆKKAN

Goverment Vitamin Laboratory
Norwegian Fisheries Research Institute
Bergen—Norway

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INTRODUCTION

A knowledge of the nutritional value of fish protein is of importance considering the established use of fish in various diets and the potential use of fish and fish flours to meet the world's increasing demand for high quality protein foods.

In a previous communication results obtained with acetone dried herring fillets and herring organs were reported (NJAA, UTNE and BRÆKKAN, 1966). In the present paper some results obtained with products of the gadus species cod (*Gadus morrhua*) and coalfish (*Gadus virens*), are reported. The amino acid composition of teleost fish show little variation. (BRÆKKAN and BOGE, 1962) and it is therefore assumed that the results obtained may be valid also for other species than those tested.

MATERIALS

In Expts 1—5 the samples were prepared from live cod bought at the Bergen Fishmarket. The fish was filleted at purchase. The fillets were used raw or acetone dried as previously described (NJAA et al. 1966). Acetone dried samples were prepared from raw and from boiled minced fish, from raw minced fish kept for one day at 4° with or without addition of ascorbic acid and from raw minced fish kept for one day at room temperature.

In Expt. 6 two flours produced in a pilot plant from cod fillet cuttings (BAKKEN, 1962) and freeze dried commercial frozen cod fillets were tested.

In Expt 7 four samples of freeze dried coalfish fillet paste containing varying amounts of fillet bones were compared.

In Expt 8 were tested three flours prepared from three batches of deskinned stockfish. The stockfish had been produced from coalfish.

In Expt 9 were compared a flour prepared from deskinned stockfish from cod, and three pulps prepared from the same stockfish by soaking in water, in sodium hydroxide or in sodium carbonate. The two latter pulps were prepared from the raw material used when the Norwegian dish «lutefisk» is made.

In Expt 10 were tested acetone dried fillets of fresh raw cod and herring and acetone dried «meats» from basking shark (*Cetorhinus maximus*) and porbeagle (*Lamna nasus*).

Further details of the samples are given in connection with the description of the various experiments. The acetone powders usually contained 85—95 % protein (N × 6.25). The acetone dried cod liver contained about 68 %.

METHODS

Nitrogen and dry matter was determined as previously described (NJAA et al. 1966). Calcium was determined by a flame spectrographic technique after isolation of the oxalate. Tryptophan was determined colorimetrically as described by GRAHAM, SMITH, HIER and KLEIN (1947).

The nitrogen balance experiments were performed at 21—22° with 5-day collection periods following a 4-day preliminary period. In Expts 1 and 2 groups of 12 and 8 littermate rats of the same sex within pairs were used, in the other experiments four groups were set up from five or six quartets of littermate rats of the same sex within each quartet. The amount of food offered daily to each rat was equivalent to 10 g air dry diet, usually this amount was eaten completely. The protein content of the diet was about 8 % on an air dry basis. The net protein utilizations (NPU_C) given in the tables were obtained from the nitrogen balance data by assuming that the maintenance protein requirement is equivalent to 190 mg N per kg body weight taken to the power of 0.73 (NJAA, 1965). Methods and diets are described in more detail by NJAA (1959, 1963) and NJAA et al. (1966).

EXPERIMENTS AND RESULTS

Expts 1 and 2 Comparison of fresh raw cod fillets and egg albumin.

Fresh raw cod fillets were mixed with a basal protein free diet at the rate of 32 parts minced fish and 68 parts basal diet. The percentage composition of the basal diet was: Partly dextrinized potato starch 68, succrose 22.1, arachis oil 5.5 and salts 4.4. The daily ration of 13.3 g/rat was calculated to contain 128 mg N on the assumption that the cod fillets contained 19 % protein.

In *Expt 1* the fish was bought daily and mixed with the basal diet shortly after purchase. Daily portions of the mixed diet were taken for nitrogen analyses.

In *Expt 2* the whole portion of the experimental diet was mixed before the experiment was started, daily portions were weighed into feeding cups and these were kept below —20° until the day of use.

The results of these experiments are given in Table 1.

Table 1. Apparent digestibility (D_a), percentage nitrogen balance (Bal %) and calculated net protein utilization (NPU_c) of raw cod fillets and egg albumin.

Expt no	Description of sample	Number of rats per group	Nitrogen intake mg/day	D_a	Bal %	NPU_c	Body weight (g)	Weight gain (g/5days
1	Raw minced cod fillets .	12	131.5	86.3	47.3	70.4	81.5	8.3
	Egg albumin	12	122.8	83.6	60.8	85.7	82.4	9.8
2	Raw minced cod fillets .	8	126.4	85.6	48.4	72.0	79.4	8.0
	Egg albumin	8	122.6	83.8	58.2	83.3	82.8	8.3

Expts 3—5 Comparison of acetone dried cod fillets and egg albumin.

Expt 3 The cod fillets were minced and divided in three 1-kg portions. One portion was acetone dried immediately, the two other portions were spread in thin layers and kept for one day at 4°. To one of these portions 0.1 % ascorbic acid was added. After the one-day storage the samples were acetone dried as usual.

Expts 4 and 5 The cod fillets were minced, a 1-kg portion was acetone dried immediately and another 1-kg portion after one-day storage in a thin layer at room temperature. Two portions of 0.5 kg each were brought into 0.5 l boiling 3 % NaCl and boiled for half an hour. They were then filtered through cotton wool and acetone dried. Two further 0.5-kg portions were brought into 3 % NaCl at room temperature, heated to boiling and boiled for half an hour. The samples were filtered off and acetone dried as above. The recovery was about 160 g per kg raw minced fish for the two boiled samples, whereas it was about 180 g for the other samples. In Expt 5 was also included an acetone dried sample of cod liver. The livers were first cooked and pressed for oil, and the press cake was acetone dried. In these experiments the rats did not always eat the total amount of food offered. However, all results obtained with rats eating between 8 and 10 g food per day were included in the calculations.

The results of these experiments are given in Table 2.

Table 2. Apparent digestibility (D_a), percentage nitrogen balance (Bal%) and calculated net protein utilization (NPU_c) of acetone dried cod fillets and cod livers and of egg albumin.

Expt no	Description of sample	Number of rats per group	Nitrogen intake mg/day	D_a	Bal%	NPU_c	Body weight (g)	Weight gain (g/5days
3	Fresh raw cod	5	120.1	86.3	52.5	78.5	84.4	4.4
	Fresh raw cod, 1 day 4°	5	120.0	86.7	52.8	78.2	84.5	8.6
	Fresh raw cod, 1 day 4°, 0.1% asc.ac.....	5	122.0	87.0	52.4	77.6	83.5	5.4
	Egg albumin	5	122.1	84.2	62.9	89.2	88.1	7.4
4	Fresh raw cod	5	126.6	85.9	50.1	71.6	69.2	4.7
	Fresh raw cod, boiling NaCl, boiled 1/2 hr. ...	5	125.0	84.1	51.8	73.6	69.3	4.8
	Fresh raw cod, cold NaCl, boiled 1/2 hr.	5	123.7	84.7	50.2	72.5	70.8	5.3
	Egg albumin	5	126.7	83.7	56.3	78.0	71.4	6.5
5	Fresh raw cod	6	111.5	87.3	44.6	71.1	78.1	3.3
	Fresh raw cod, 1 day room temp.....	6	108.9	86.6	43.6	70.6	77.3	4.9
	Cod livers, cooked, pressed	6	124.2	83.0	42.4	65.6	76.2	6.5
	Egg albumin	6	128.1	85.6	60.5	84.5	82.8	7.9

Expt 6 Comparison of two pilot plant fish flours, freeze dried frozen cod fillets and egg albumin.

The two fish flours were produced in a pilot plant by three-stage alcohol extraction. The raw material was cuttings from cod fillets. The pilot plant together with preliminary results have been described previously (BAKKEN, 1962). The freeze dried frozen cod fillets were prepared in the laboratory using commercial frozen fillets as raw material.

The results are given in Table 3.

Expt 7 Comparison of four freeze dried coalfish fillet pastes containing different amounts of fillet bone residues.

The fillet pastes were prepared in a commercial fillet plant and arrived in the laboratory in frozen condition. One sample (A) was of ordinary fillets which had been cut to contain minimum bone residues. The other samples (B, C and D) contained more residual bones. Sample B contained the main fillet bones, sample C contained the main fillet bones

Table 3. Expt. 6. Apparent digestibility (D_a), percentage nitrogen balance (Bal%) and calculated net protein utilization (NPU_c) of two pilot plant fish flours, freeze dried frozen cod fillets and egg albumin.

Description of sample	Nitrogen intake (mg/day)	D_a	Bal%	NPU_c	Body weight (g)	Weight gain (g/5days)
Fish flour 1, Pilot plant	118.3	85.3	60.6	84.4	73.3	8.0
Fish flour 2, Pilot plant	127.2	84.0	61.1	84.0	76.6	10.2
Freeze dried frozen cod fillets..	125.5	84.3	56.2	78.6	73.0	9.0
Egg albumin	123.6	83.1	68.0	91.5	76.7	11.7

and belly sides with bones, and sample D contained in addition to the bones included in sample C spike bones from the backbone. The spike bones included were those following the fillets when these were intentionally carelessly cut.

The pastes were freeze dried and equilibrated toward air after the drying. The dry samples contained from 90 to 85 % dry matter, falling gradually from sample A to D.

The pastes were analysed for dry matter, protein, ash and calcium. The dried samples were analysed for dry matter, protein, calcium and tryptophan. The analytical data are given in Table 4 and the results of the nitrogen balance experiment in Table 5. It is noted that the sum of protein and ash is higher than 100 %. This indicates that the factor 6.25 used for the conversion of nitrogen to protein is to high (BRÆKKAN and BOGE, 1962).

Table 4. Expt 7. Analyses of four coalfish fillet pastes before and after freeze drying.

Sample*	Paste				Freeze dried paste			
	Dry matter	Protein (% of dry matter)	Ash (% of dry matter)	Ca (% of dry matter)	Dry matter	Protein (% of dry matter)	Ca (% of dry matter)	Tryptophan (% of protein)
A	18.5	98.4	6.47	0.06	90.2	96.5	0.06	1.16
B	18.3	97.8	6.65	0.12	89.6	94.9	0.12	1.15
C	18.2	95.6	7.17	0.25	88.7	93.7	0.24	1.14
D	18.7	95.2	7.13	0.28	85.6	92.9	0.32	1.17

* Sample A contained minimum bone residues, sample B contained the main fillet bones, sample C contained the main fillet bones and belly sides with bones, sample D contained the main fillet bones, belly sides with bones and spike bones from the backbone.

Table 5. Expt 7. Apparent digestibility (D_a), percentage nitrogen balance (Bal%) and calculated net protein utilization (NPU_c) of four freeze dried coalfish fillet pastes.

Sample*	Nitrogen intake (mg/day)	D_a	Bal%	NPU_c	Body weight (g)	Weight gain (g/5days)
A	127.2	87.1	59.8	81.6	71.4	10.1
B	127.9	87.2	58.2	80.0	71.8	9.7
C	126.5	87.2	61.7	83.6	71.5	10.2
D	124.0	85.4	58.9	81.2	71.4	9.7

* See note to Table 4.

Expt 8 Comparison of stockfish flour and egg albumin.

Three samples from different batches of stockfish made from split coalfish were deskinned and milled to flours. The samples were provided by the department for salted and dried products. The protein contents of the flours ranged from 82 to 85 %.

The results of the balance experiment are given in Table 6.

Table 6. Expt 8. Apparent digestibility (D_a), percentage nitrogen balance (Bal%), and calculated net protein utilization (NPU_c) of three flours from deskinned stockfish from coalfish and of egg albumin.

Sample	Nitrogen intake (mg/day)	D_a	Bal%	NPU_c	Body weight (g)	Weight gain (g/5days)
1	128.3	85.4	43.0	68.0	88.0	7.4
2	128.6	85.7	43.1	67.9	87.0	7.5
3	126.7	84.4	42.6	67.9	88.2	8.1
Eggalb.	113.9	81.6	54.3	83.2	90.2	9.3

Expt 9 Comparison of stockfish flour, and stockfish soaked in water, sodium hydroxide and sodium carbonate.

The four protein sources used in this experiment were prepared from a batch of stockfish made from split cod. The samples were prepared by

the department for salted and dried products. Sample E was deskinned and milled to a flour. Sample F was soaked in running water for 9 days, deskinned and made into a pulp. Sample G was soaked in running water for three days, deskinned, the deskinned product was soaked in 0.3 % NaOH for one day and then in running water for 18 hours, and made into a pulp. Sample H was soaked in running water for 3 days, deskinned, the deskinned product was soaked in 3 % Na_2CO_3 for three days and then in running water for one day, and made into a pulp. The temperature during soaking was 8°. The protein content of the four samples were 83.2 %, 17.4 %, 9.7 % and 12.1 %. The wet products were mixed with the basal diet described in Expts 1 and 2, and amounts containing by calculation 56 g protein were mixed with 633 g basal diet. The daily allowances of the diets were calculated to provide 128 mg N/rat. The daily portions were weighed into feeding cups and kept frozen as in Expt 2. Each day during the experimental period one portion of each diet was analyzed for nitrogen and nitrogen intakes were calculated from the analyses. The low nitrogen intakes for the group given the sodium hydroxide-treated sample assumedly must be due to an error during the mixing of the diets resulting in that 100 g less of sample G was mixed into the diet than was intended. Thus, the rats given this diet received the protein at a level about 6.7 % instead of 8 % of the air dry diet intended.

The results of the balance experiment are given in Table 7.

Table 7. Expt 9. Apparent digestibility (D_a), percentage nitrogen balance (Bal %) and calculated net protein utilization (NPU_c) of deskinned stockfish flour and of pastes prepared from stockfish soaked in water, sodium hydroxide or sodium carbonate.

Sample*	Nitrogen intake (mg/day)	D_a	Bal %	NPU_c	Body weight (g)	Weight gain (g/5days)
E	129.4	87.1	55.9	79.1	79.8	9.5
F	140.6	86.4	57.3	78.9	81.4	9.3
G	110.0	83.8	57.3	85.1	82.8	9.8
H	126.9	85.4	57.5	81.5	81.4	9.9

* Sample E was a flour from deskinned stockfish from cod; sample F was a pulp from deskinned stockfish soaked in water; sample G was a pulp from deskinned stockfish soaked first in water and then in 0.3 % sodium hydroxide; sample H was a pulp from deskinned stockfish soaked first in water and then in 3 % sodium carbonate. Samples G and H were washed in water after the last treatment.

Expt 10 Comparison of acetone dried samples of raw cod and herring fillets, and raw «meats» from frozen basking shark and porbeagle.

The results obtained in Expts 3—5 with cod fillets gave the impression that cod protein might be slightly better utilized than herring protein as reported by NJAA et al. (1966). Therefore samples of raw cod and herring fillets were acetone dried parallelly in the laboratory. Included in the experiment were also two samples prepared by acetone drying at about the same time from frozen meats of basking shark and porbeagle.

The results of this experiment is given in Table 8.

Table 8. Expt 10. Apparent digestibility (D_a), percentage nitrogen balance (Bal%) and calculated net protein utilization (NPU_c) of acetone dried fillets of cod and herring, and meats of basking shark and porbeagle.

Sample	Nitrogen intake (mg/day)	D_a	Bal%	NPU_c	Body weight (g)	Weight gain (g/5days)
Cod	127.7	88.3	60.8	83.9	77.7	9.9
Herring	129.6	88.0	59.2	82.0	78.4	9.6
Basking shark	118.8	87.0	43.0	66.8	73.8	7.1
Porbeagle	125.7	87.3	47.3	70.5	76.7	6.9

DISCUSSION

The value of fish protein measured in biological utilization studies or calculated from the amino acid composition is generally accepted to be high. FAO (1957) data indicate that the quality of fish protein is about equal to that of meat, casein and soya, but that it is slightly inferior to milk, egg and egg albumin. Many studies, however, show that the protein value of fish meals and flours may vary considerably (MILLER, 1956; BENDER & HAIZELDEN, 1957; MORRISON & MCLAUGHLAN, 1961).

In the present experiments egg albumin was usually the standard of reference, and in accordance with the FAO (1957) data it was always found to be significantly better utilized than the fish protein preparations. In this respect the present findings differ from the results of BEVERIDGE (1947) who found higher protein values for four species of fish than of egg albumin and beaf. A possible explanation of this discrepancy is that the egg albumin used in his experiments may have been of inferior quality. It was a pan dried product whereas the egg albumin used in the present study was spray dried.

The fish products used in our experiments were either completely unprocessed (Expts 1 and 2) or they were treated under very mild conditions. A possible exception to this may be the alkali treatments of stockfish in Expt 9. The treatments used included acetone drying of either fresh or boiled cod, freeze drying, commercial air drying (stockfish), alcohol extraction, and alkali treatment of stockfish. The apparent digestibility ranged in all cases from 84 to 88 per cent, and there was no indication that the treatments affected the digestibility of the protein. The values obtained were either equal to or slightly higher than simultaneously determined values for egg albumin. The nitrogen balance values were not so uniform, but in no case was there a significant difference between fish protein sources tested in the same experiment. Comparisons between experiments are difficult even when the results are expressed relative to egg albumin. Thus, in Expts 4 and 5 acetone dried fresh cod muscle showed nitrogen balance values of about 50 and 45 per cent, respectively. Relative to egg albumin the values were about 90 and 75 per cent. It is suggested, therefore, that the experimental results do not indicate any adverse effect of the treatments of the fish. Thus, the nutritional value of the treated product must be considered to be equal to that of raw unprocessed fish. It is of special importance that the results indicate that the protein value of stockfish is about equal to that of raw fish and of carefully dried laboratory samples of cod muscle. Also, the special Norwegian delicacy, «lutefisk», which is made by alkali treatment of stockfish, had about the same nutritional value as the stockfish from which it was prepared. Much of the original protein is extracted by the alkali, but the remaining protein show the same high quality as the raw material. In this connection it may be noted that MILLER (1956) found alkali soluble fish protein to be of high nutritional value.

In our experience the protein digestibility is usually reduced when a treatment of the raw fish leads to impairment of the protein quality (NJAA et al. 1966). The present results also show that the high nutritional values are associated with high digestibility values. This is in agreement with the findings of PUJOL (1958). He tested eleven samples of fresh, frozen and salted marine products of which six derived from teleost fishes. The samples were dried at 60°. He found apparent digestibility values for the fish samples fed at about the 11 per cent protein level to range from 82 og 89 per cent. The nitrogen balance values calculated from his data range from 57 to 76 per cent. The former range is in accordance with that found by us, but the balance values are considerably higher. However, this does not necessarily indicate better protein utilization in PUJOL's experiments. The values reported by him for endogenous urinary nitrogen showed considerable variation. Thus, rats

1—20 excreted 7—17 mg/day whereas rats 21—44 excreted 2.5 to 4.6 mg/day. These abnormal low excretions indicate that not all factors affecting the amount of nitrogen found in the urine were completely under control.

The apparent correlation between digestibility and utilization values form the basis for the renewed interest in pepsin digestibility tests for protein quality evaluation (OLLEY and PIRIE, 1966). It should, however, be noted that extraction of fish with ethylene dichloride seems to impair the protein utilization without affecting the apparent digestibility as determined with young rats (MORRISON and MCLAUGHLAN, 1961). The impairment of protein quality by ethylene dichloride was also observed with extracted meat (CLARK, HOOPER and MCCORD, 1955). Thus, even though poor quality of fish protein can be detected by observing digestibility only, this can in certain cases lead to erroneous conclusions.

The results with coalfish fillet protein containing varying amounts of fillet bones showed that the content of bone protein was too small to influence upon the protein value. The higher bone content was only reflected by the higher calcium contents.

The impression from the first experiments in this investigation that cod fillet protein had a higher nutritional value than herring fillet protein (NJAA et al. 1966) was not substantiated. However, the two acetone powders of shark meat showed a lower protein utilization. This was not accompained by a reduced digestibility. In contrast MASHELKAR and SOHONIE (1958) found not only lower biological value but also lower digestibility for shark and skate protein than for casein. The low utilization of elasmobranch «protein» may be due to high urea contents being included as protein. As free urea is soluble in an acetone-water mixture, some extraction would most likely take place during the first acetone treatment.

The high quality found for the alcohol extracted fish flours indicate that this process gives a nutritionally satisfactory product. So far, however, no commercial production of this type of flour has been started in this country.

SUMMARY

The protein quality of cod muscle protein and of some products of cod and coalfish was determined in nitrogen balance experiments with young rats. In most of the experiments spray dried egg albumin was used as a standard of reference. All the fish proteins showed as expected a lower utilization than egg albumin, but the results indicated that the preparations tested had high biological values.

There was no appreciable difference between raw cod fillets, acetone dried cod fillets, or acetone dried fillets which had been boiled before drying. Two pilot plant fish flours from cod fillet waste showed high biological values.

There was no difference in the utilization values between freeze dried coalfish fillet pastes containing different amounts of residual bones. Stockfish flours showed utilization values of about the same magnitude as the acetone dried fillets.

There was no difference in the utilization values for stockfish and paste made from stockfish soaked in NaOH or $NaCO_3$ («lutefisk»). A comparison between acetone dried cod and herring fillets and meat from porbeagle and basking shark showed the elasmobranchs to be less well utilized than the teleosts.

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