CHEMICAL STUDIES ON HERRING MEAT (2)*

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VIII. CHEMICAL COMPOSITION OF THE PROTEIN OF HERRING MEAT

1. Amounts of nitrogen in the meat muscle of herrings soluble by various kinds of solvents

To determine the solubilities of the nitrogenous compounds in the meat muscle of herring, the amounts of nitrogen dissolved in various kinds of solvents were estimated. As salmples, "Spring herring" ("Fore-coming herring") and "Small herring" were employed. Each 20 fishes of "Spring herring" were divided into two groups, and were used, and also 20 fishes of "Small herring" were employed for the determination. Each solvent, water, 0.2 % KOH, 70 % alcohol and 10 % NaCl solution, was employed in the amount of 10-fold the weight of the muscle meat of the herring. By those solvents, the nitrogenous compounds were extracted at room temperature.

The extracted solutions were estimated for the determinations of the amounts of the total nitrogen and protein nitrogen. Results obtained are shown in Tables 24 and 25. Numerals in Tables, show the content (%) in the meat muscle as dried matter.

Table 24. The solubilities of nitrogenous compounds in "Spring herring" meat

Solvents	Group No.	Dissolved total-N (A)		Dissolved protein-N (B)		$B/A \times 100$ (%)
			average		average	average on I and II
Water	I	4.00	4.03%	2.76	2.80%	69.4%
	II	4.06		2.84		
0.2% KOH	I	9.10	9.03	8.04	7.98	83.3
	II	8.96		7.92		
70% alcohol	I	2.08	2.24	1.22	1.27	56.6
	II	2.40		1.32		
10% NaCl	I	8.05	8.19	5.86	5.93	72.4
	II	8.33		6.00		

*[Contiuned from the previous reports on "Chemical Studies on Herring Meat (I～VII)", described in this bulletin, Vol. 8, No. 4, pp. 319—345, 1958.

This manuscript left by Late Sasa is published in the form of this paper after arrangement, revision and the addition of new data by his follows, Dr. Eiichi Tanikawa, Prof. of Faculty of Fisheries, Hokkaido University, and Mr. Minoru Akiba, Assist. Prof. of the same Faculty.

Table 25. The solubilities of nitrogenous compounds in "Small herring" meat

Solvents	Dissolved total-N (A)	Dissolved protein-N (B)	$B/A \times 100$ (%)
Water	5.47%	2.82%	51.6%
0.2% KOH	9.98	7.07	70.9
70% alcohol	2.50	1.03	41.2
10% NaCl	7.97	6.09	76.3
Remarks	Percentage of total-N in the dried matter of sample was 10.7%		

As seen in Tables 24 and 25, the nitrogenous compounds in the meat muscle are easily dissolved by 0.2 % KOH and 10 % NaCl solutions. The solubilities by water and 70 % alcohol are next in order.

2. Distribution of the nitrogens in the herring meat muscle protein

The amount of myogen-form nitrogen and the nitrogens in 5 % NaCl soluble extract and in non-protein substance in herring meat muscle were estimated. Results obtained are shown in Table 26.

Table 26. Various forms of nitrogenous compounds in herring meat

Nitrogens	% in dried matter	% in 5% NaCl soluble-N
5% NaCl soluble-N	8.04	100
Myosin-N	3.83	47.6
Myogen-N	2.30	28.6
Non-protein-N	1.91	23.8

As seen in Table 26, in 5 % NaCl-soluble protein, a half part was occupied by myosins. The rest was occupied by almost equal amounts of myogens and non-protein substance.

3. Nitrogenous compounds from the hydrolyzate of herring meat protein

The properties and nutritive value of the proteins are different according to their constituents and construction. Herring meat protein was hydrolyzed by the usual method and the amounts of nitrogenous components were estimated. Fresh herring meat ("Fore-coming herring"), from which the reddish meat ("Chiai" meat) and small bones were removed, was taken and crushed. To the crushed meat toluene was added and the preparate was extracted by water three times in a cold place. The remainder was extracted by 70 % alcohol twice, by absolute alcohol three times, and then by ether three times. After the extraction, the remainder was dried at low temperature and crushed. The crude meat protein thus made was stored in an air-tight vessel.

(1) *Nitrogen distribution*

The herring meat protein was hydrolyzed with sulfuric acid and the amounts of the various forms of nitrogen were estimated by the usual method. The results obtained are shown in Table 27.

Table 27. Distributions of nitrogens in herring meat protein

Nitrogens	% in dried matter	% in ash-free dried matter	% in total-N
Total-N	12.52	15.13	100
Amino-N	7.56	9.13	60.34
Basic-N	3.92	4.74	31.33
Ammonia-N	0.41	0.50	3.30
Melanin-N	0.63	0.76	5.03

As seen in Table 27, about 60 % of the total amount of nitrogen was amino nitrogen and 30 % was basic-form nitrogen. The rest was considered to consist of small amounts of ammonia and melanin-form nitrogen.

(2) *Amino nitrogen*

(i) **Monoamino acids**

One hundred *g* of the meat protein above described was hydrolyzed at first in a water bath with three-fold volume of 33 % H_2SO_4 solution, and then in an oil bath until the Biurett's reaction became negative. After the hydrolysis, the hydrolyzate was filtered and was treated with blood charcoal; then it was neutralized and evaporated in vacuum. After cooling, fractional crystallization was carried out. By this crystallization, 0.507 *g* of cystine, 2.1667 *g* of tyrosine were isolated. The solution from which cystine and tyrosine had been removed was concentrated by evaporation, then saturated with gaseous hydrochloric acid and was kept at $-5°\sim-6°C$ for several days. Here 10.2048 *g* of hydrochloride of glutamic acid (8.2334 *g* as glutamic acid) was obtained.

(a) Detection of cystine: From 0.3637 *g* of the sample obtained as cystine, 0.5920 *g* of barium sulfate was obtained, that is to say, 22.36 % of sulfur was experimentally obtained against the calculated amount, 22.53 %, of sulfur in cystine ($C_6H_{12}O_4N_2S_2$).

(b) Detection of tyrosine: From 0.3126 *g* of the sample obtained as tyrosine, 0.0259 *g* of nitrogen was obtained, that is 8.30 % of N, against the calculated amount of N in tyrosine ($C_9H_{11}O_3N$) being 7.74 %.

(c) Detection of hydrochloride of glutamic acid: From 0.2023 *g* of the sample obtained as hydrochloride of glutamic acid, 0.0159 g of N was estimated, that is 7.83 %

of N, against the calculated amount, 7.63 %, of N in hydrochloride of glutamic acid ($C_5H_{10}O_4NCl$).

The mother liquid from which the crystals had been isolated as above described and the washing solution were together concentrated by evaporation, and the concentrated solution was treated with absolute alcohol and gaseous hydrochloric acid. The crystal of hydrochloride of glycocoll ester was not yielded. Here, after the washing of free ester, the ester was factionally distilled as shown in Table 28.

The amount of yield are shown also in the Table.

Table 28. Conditions of the fractional distillation

Distillate No.	Temperature (°C)	Pressure (*mm* Hg)	Yields (*g*)
Part 1	40	10	5
Part 2	60	10	8.4
Part 3	90	10	7.2
Part 4	100	2	20
Part 5	130	1	13
Part 6	160	1	15.2

To the first part of the distillate, water was added and boiled until the alkali reaction disappeared, and evaporated in vacuum. Here 2.210 *g* of alanine was obtained. Also, 0.319 *g* of alanine was separately obtained by the evaporation of ether from ether solution of the total ester. The total amount of alanine was 2.529 *g*.

(d) Detection of alanine: From 0.3463 *g* of the sample obtained as alanine, 0.0524 *g* of N was estimated, that is 15.14 % of N against the calculated amount, 15.73 %, of N in alanine ($C_3H_7O_2N$).

The second and the third parts of distillate described in Table 28 were boiled with 10-fold volume of water, then concentrated by evaporation in vacuum, and kept for several days. Then leucine was isolated. After the isolation of the crystal of leucine, the mother liquid was extracted by hot alcohol, and 2.17 *g* of proline was obtained. Proline was changed to copper salt, and 0.437 *g* of racemic copper salt of proline was obtained. This salt was dried at 120°C and analyzed. The remainder from which proline has been removed, was dissolved by hot water, and the crystal of leucine was again isolated. The total sum of leucine which was isolated was 1.1745 *g*.

(e) Detection of leucine: (I) 10.25 % of N was estimated from 0.3621 *g* of the sample obtained as leucine. (II) 10.10 % of N was estimated from 0.3947 *g* of the sample. As compared with the experimental amount of N, the calculated amount of nitrogen is 10.69 % in leucine ($C_6H_{13}O_2N$).

(f) Detection of copper salt of proline: From 0.255 *g* of the anhydrous sample, 0.0685 *g* of copper oxide (corresponding to 0.0547 *g* of copper) was estimated. Therefore, the experimental amount of Cu was 21.38 % against 21.79 % calculated amount of Cu in copper salt of proline ($C_{10}H_{16}O_4N_2Cu$).

To the 4th part of the distillate in Table 28, water was added and saponified. This soap was extracted with absolute alcohol and proline was again isolated. The total sum of proline was 2.35 *g*. The rest was treated with baryta and 10.0176 *g* of racemic leucine was obtained. This leucine was also confirmed.

To the distillate of the 5th part in Table 28, water of 5-fold volume and ether of equal volume were added and shaken. After the separation of ether layer, the ether layer was evaporated and heated with conc. hydrochloric acid, and 3.6818 *g* of hydrochloride of phenylalanine was obtained (This is 3.0158 *g* as phenylalanine). The water layer which was separated from ether layer was treated with petroleum ether several times, and was saponified, and thus 1.324 *g* of serine was obtained. The solution from which serine was removed was treated with baryta, and thus barium salt of asparatic acid was obtained. This barium salt was neutralized with sulfuric acid and 2.322 *g* of asparatic acid was obtained.

(g) Detection of hydrochloride of phenylalanine: From 0.3260 *g* of the sample as hydrochloride of phenylalanine, 6.84 % of nitrogen was obtained against 6.95 % calculated amount of N in hydrochloride of phenylalanine ($C_9H_{11}O_2N \cdot HCl$).

(h) Detection of serine: From 0.3013 *g* of the sample as serine, 13.54 % of nitrogen was obtained against 13.34 % calculated amount of N in serine ($C_3H_7O_3N$).

(i) Detection of asparatic acid: From 0.3158 *g* of the sample as asparatic acid, 11.21 % of nitrogen was obtained against 10.53 % calculated amount of N in asparatic acid ($C_4H_7NO_4$).

The distillate of the 6th part in Table 28 was treated with petroleum ether. The remainder was again treated with hydrochloric acid and gaseous hydrochloric acid, and was kept in a cold place for several days. Thus 0.7232 *g* of hydrochloride of glutamic acid was obtained (This corresponds to 0.5835 *g* of glutamic acid). The mother liquid was concentrated by evaporation in vacuum; the concentrate was dissolved in water; hydrochloric acid in the solution was removed by lead oxide. The excess amount of the lead was removed by sulfuretted hydrogen. Thus 0.81 *g* of asparatic acid was obtained. The hydrochloride of glutamic acid and the asparatic acid thus obtained were confirmed.

(j) Determination of tryptophane: As above mentioned, the isolation of monoamino acids was tried. Here tryptophane was detected separatedly. To 70 *g* of the completely crushed meat protein, water, sodium carbonate and pancreatine were added. To this solution, chloroform and toluene were added in order to prevent putrefaction; preparate was kept at 40°C for 14 days. The reaction of tryptophane was observed to be positive.

The amount of tryptophane was estimated colorimetrically by May and Rose's method[38], and obtained 1.02 % of tryptophane. This amount was 1.13 % by calculation in ash-free sample of anhydrous.

(k) Estimation of the amount of valine: As no estimation of the amount of valine has previously been carried out, here the estimation was performed as follows: 100 *g* of herring meat protein was boiled with hydrochloric acid of 3-fold volume; the ester was thus made, and then it was fractionally distilled. The conditions of the fractional distillation are described in Table 29. The yielded amounts are also shown in the Table.

Table 29. Conditions of the fractional distillation

Distillate No.	Temperature (°C)	Pressure (*mm* Hg)	Yields (*g*)
Part 1	65	10	20.8
Part 2	100	10	25.5
Part 3	110	3	10.0
Part 4	190	3	30.7

From alcohol-undissolved parts of the distillates of the lst, 2nd and 3rd parts in Table 29, valine was obtained by fractional crystallization. From the lst part 0.2124 *g*, from the 2nd part 0.7323 *g*, from the 3rd part 0.5925 *g* of valine were obtained. The sum was 1.5372 *g*. The samples of valine from the lst, 2nd and 3rd parts were analyzed. The experimental amount of nitrogen was 11.99 % for the lst part, 11.77 % for the 2nd part, and 12.08 % for the 3rd part. The calculated amount of nitrogen is 11.96 % in valine ($C_5H_{11}O_2N$). Thus the sample of crystal was identified as valine.

(ii) **Diamino acids**

One hundred *g* of the herring meat protein which was the same as the sample employed for the isolation of monoamino acids, was hydrolyzed with three-fold amount of fuming sulfuric acid for about 15 hours, and then 50 % phosphotungstic acid was added. The precipitate yielded was treated with 5 % H_2SO_4 and decomposed by baryta. Then the determination of diamino acid was carried out according to Kossel's method[39].

The results are as follows.

Hydrochloride of histidine: 2.593 *g* (This is 2.099 *g* as histidine).

Picrate of arginine: 10.7108 *g* (This is 4.626 *g* as arginine).

Picrate of lysine: 26.1200 *g* (This is 10.833 *g* as lysine).

Those isolated amino acids were confirmed by the analysis.

(a) Detection of hydrochloride of histidine: 18.31 % of N was obtained from 0.2021 *g* of the sample as hydrochloride of histidine. The calculated amount of N in hydrochloride of histidine ($C_6H_9N_3O_2 \cdot 2HCl$) is 18.42 %.

(b) Detection of picrate of arginine: 23.78 % of N was obtained from 0.2471 *g* of the sample as picrate of arginine. The calculated amount of N in picrate of arginine ($C_6H_{14}O_2N_4 \cdot C_6H_3O_7N_3$) is 24.3 %.

(c) Detection of picrate of lysine: 18.57 % of N from 0.1014 *g* of the sample and 61.82 % of picric acid from 0.3164 *g* of the sample as picrate of lysine were estimated. The calculated amount of N in picrate of lysine ($C_6H_{14}N_2O_2 \cdot C_6H_3N_3O_7$) is 18.67 %, and that of picric acid is 61.07 %.

(3) *Considerations*

Results obtained are summarized in Table 30. In that table the amino acids of beef crude protein (water content 71.23 %, fat 6.95 %, total N 3.30 %, ash 1.21 %) as obtained by the present author and of bonito (*Euthynnus Pelamys* Linne') and amber jack ("Buri") (*Seriola quinqueradiata*) meat proteins which were obtained respectively by Ogura,[40] Okuda[22] and Oya[41], were shown together.

Table 30. Comparison of amino acids in herring meat protein with other fish meat and beef meat proteins (% in ash-free dried matter)

Amino acids \ Proteins	Herring meat protein	Beef meat protein I	Beef meat protein II[40]	Bonito meat protein[22]	Amber jack meat protein[41]
Glycine	0.00 *g*	···	2.06	0	0
Cystine	0.57	1.20	···	+	+
Tyrosine	2.42	2.01	2.20	2.1	4.81
Glutamic acid	9.86	15.08	15.49	8.1	5.55
Alanine	2.84	4.00	3.72	2.3	2.29
Leucine	12.51	12.13	11.65	10.4	5.58
Proline	2.63	6.32	5.81	3.1	2.75
Phenylalanine	3.37	2.93	3.15	4.1	2.60
Serine	1.48	1.20	?	?	?
Asparatic acid	3.50	3.99	4.51	3.3	2.42
Histidine	2.35	4.35	1.76	3.04	2.27
Arginine	5.17	7.15	2.47	7.80	5.45
Lysine	12.11	9.49	7.59	7.41	5.81
Tryptophane	1.13	+	+	+	+
Valine	1.70	···	0.81	2.8	1.15

As seen in Table 30, the amount of nitrogen of diamino acids in herring meat protein was larger than that of monoamino acids. The amounts of leucine and glutamic acids nitrogens are larger than the other amino acids in monoamino acids. The amount of histidine nitrogen in diamino acids is less than that of arginine or lysine. The fact that there are larger amounts of lysine, arginine and considerably larger amounts of histidine, tyrosine, phenylalanine and tryptophane in the herring meat, indicates the high nutritive value of herring meat.

In comparing herring meat protein with beef meat protein, the amounts of glutamic acid, alanine, proline, histidine and cystine of the former are found to be less than those of the latter, but the amounts of other essential amino acids are almost the same in their quantities. By comparison the herring meat protein with the bonito and amber jack meat proteins, the amounts of glutamic acid, asparatic acid and lysine of the former are to be found larger than those of the latter. The nutritive value of the herring meat, therefore, is considered to be large.

4. Comparison of the chemical composition of herring meat protein in every stage of the maturity of herring

From the experimental results obtained as above described, it can be suggested that the nutritive value of herring meat protein is no less than that of other fish meat protein or beef meat protein. As the amounts of the chemical components of herrings meat vary remarkably according to maturity and sex, experiments were carried out using samples of herrings which were classified by their maturity and sex.

(*1*) *Samples of herrings*

Identification of the maturity was made by the method offered by Hokkaido Fisheries Station. When the finger tips are rubbed along the belly side of the herring bodies from breast fin to tail fin, if milt or roe spring out, then those fishes are of full maturity, but if milt or roe does not spring out, those fishes are considered to be unmatured. The herring which have spawned are called **spent herring**. As unmatured herrings, 5 male fishes and 4 female which were caught off Otaru, Hokkaido on 13th December, 3 years old, were employed. As fully matured herrings, 10 fishes, each male and female, caught near Yoichi and Oshoro, Hokkaido, from 25th to 31st of March, 6～7 years old, were employed.

As spent herrings, 5 male fishes and 4 female which were caught near Zenibako, Hokkaido, on lst of June, 4 years old, were employed. The body length and weight of those herrings are shown in the lower line of Table 31.

In this experiment, from each of the samples, the meat was taken. It was treated with water, alcohol, and ether, and was made to the crude fish protein.

The amounts of the total nitrogen of each crude fish protein were estimated as usual. In this experiment, it is to be regretted that it was not possible employ the fish of the same age as the sample of each stage of the maturity. But in this experiment, the comparison of the properties of meat protein was carried out by the comparison of the ratios of each form of nitrogen to the total amount of nitrogen in the meat, because the difference of the ratios by age of fish was considered to be not very remarkable.

(*2*) ***Results and considerations***

The results obtained are shown in Table 31.

Table 31. Nitrogen distribution of herring meat protein in various stages of maturity

Items / Nitrogen forms	Unmatured herring				Full-matured herring				Spent herring			
	♂		♀		♂		♀		♂		♀	
	% in sample	% in total-N	% in sample	% in total-N	% in sample	% in total-N	% in sample	% in total-N	% in sample	% in total-N	% in sample	% in total-N
Total-N	16.16	100	16.13	100	15.59	100	15.92	100	16.43	100	16.41	100
Monoamino-N	10.34	63.99	10.25	63.55	9.70	62.20	10.26	64.41	10.52	64.03	10.84	66.06
Diamino-N	4.79	29.64	4.82	29.88	4.89	31.34	4.64	29.14	5.04	30.68	4.96	30.23
Amide-N	0.83	5.14	0.29	1.80	0.49	3.13	0.32	2.03	0.71	4.32	0.37	2.25
Humine-N	0.20	1.24	0.77	4.77	0.51	3.28	0.70	4.41	0.16	0.97	0.24	1.46
Basic-amino-N	2.95	18.25	2.99	18.60	3.01	19.26	2.81	17.63	3.49	21.24	3.33	20.29
Non-amino-N	1.84	11.39	1.83	11.35	1.89	12.10	1.83	11.50	1.55	9.43	1.63	9.87
Cystine-N	0.07	0.43	0.09	0.56	0.07	0.45	0.05	0.32	0.07	0.43	0.08	0.49
Arginine-N	2.18	17.39	2.00	12.40	2.11	13.54	1.91	11.98	2.00	12.17	1.94	11.82
Histidine-N	0.31	1.92	0.50	3.10	0.45	2.90	0.61	3.83	0.08	0.49	0.26	1.58
Lysine-N	2.23	13.80	2.23	13.83	2.29	14.72	2.07	13.00	2.89	17.59	2.68	16.33

Herrings used		Unmatured ♂	Unmatured ♀	Full-matured ♂	Full-matured ♀	Spent ♂	Spent ♀
	Average body length	21.8(*cm*)	22.8	31.4	31.6	22	22.6
	Body weight	101.2(*g*)	103.6	176.3	187.2	104.6	107.4
	Ages	3 years old	3	6, 7	6, 7	4	4
	Catching date and place	XII, 13, off Otaru	ditto	III, 25-31; off Yoichi	ditto	VI, 1; off Zenibako	ditto
	Individual No.	5	4	10	10	5	4

As in Table 31, the comparison of the properties of meat protein at various stages of the maturity by sex, may be stated as follows: The total amount of nitrogen of herring meat protein in full-matured female was larger than that in male. But that of unmatured or spent herrings of female was less than that of male.

The amount of monoamino acid nitrogen of female herring in the stages of full-matured or spent, is larger than that of male. But that of female herring in unmatured stage is less than that of male. The amount of diamino nitrogen was the opposite to the amount of monoamino nitrogen. The amount of amide nitrogen of male herrings in each stage of maturity was larger than that of female. The amount of humine-form nitrogen was the contrary. The amount of basic amino nitrogen, *e.g.*, arginine or lysine nitrogen, of female herring in full-matured and spent stages was less than that of male as was also true of the amount of diamino nitrogen. But that of unmatured female herrings was considerably larger than that of male or the values were all the same. The amount of non-amino acid nitrogen of female herrings in full-matured or unmatured stages was less than that of male. But that in spent herring was the contrary. The amount of cystine nitrogen of female herring in full-matured stage was less than that of male. But that in unmatured or spent stage was the opposite. The amount of histidine nitrogen of female in all stages was larger than that of male.

Next, as to the difference of the amount of nitrogen of various forms among the respective stages of maturity: the amounts of the total nitrogen and nitrogens of mono-amino acids, diamino acids, basic amino acids, and lysine of spent herring were larger than those of unmatured and full-matured. The order was first, unmatured and then full-matured herring meat. On the contrary, the amounts of nitrogens of humine-form, non-amino acid and histidine showed the maximum value at full-matured stage. Those amounts in unmatured herring and spent berring followed next to the full-matured herring in order.

The amounts of amide and cystine nitrogens of unmatured herrings meat were larger than those of herring meat of full-matured and spent. The amount of arginine nitrogen of unmatured herring was larger than that of full-matured and spent herrings. In the results above obtained, it was remarkable that the amounts of nitrogens of histidine, arginine and non-amino acids decreased after the spawning; on the contrary, the amount of monoamino acids and diamino acids (basic amino acids), especially lysine nitrogen, increased after the spawning.

Sekine[42] has compared the chemical components of muscle meat of herring and other fish before and after the spawning. According to his findings as well as in the present author's result, the amounts of nitrogens of arginine and histidine decreased after the spawning, and on the contrary, the amount of lysine nitrogen increased. Fukuhara[7] has also compared the chemical components of salmon meat before and after the spending of milt. According to his observations, the amounts of nitrogens of arginine and histidine decreased after the spending of milt, on the contrary, the amounts of lysine and mono-amino acids increased. In general, with the progress toward maturity, the amounts of arginine and histidine decrease by the spending of them in order to synthesize purine base which is necessary to compose the nucleo matter of the cell. According to the results of Sekine,[42] as above described, the amounts of total nitrogen and histidine of female fish were larger than those of male, and on the contrary, the amounts of nitrogens of cystine, arginine and lysine of the male fish were larger than those of female. Those results agreed with the present author's result.

Igarashi[43] has estimated the amounts of nitrogens of various forms, and obtained the following results which were compared with the present author's results as shown in Table 32.

Table 32. Nitrogen distribution of herring meat protein (% in ash-free dried matter)

	Author's (Full matured)		Igarashi's[43] (Sex was unknown)
	I	II	
Total-N	15.59	(15.13)	15.452
Ammonia-N	—	(0.50)	1.423
Melanin-N	—	(0.76)	0.283
Diamino-N	4.89	(4.74)	5.285
Monoamino-N	9.70	(9.13)	8.462
Arginine-N	2.11		2.344
Histidine-N	0.45		0.983
Cystine-N	0.70		0.082
Lysine-N	2.29		1.876

Igarashi's results are, for reference, summarized as follows. According to the data[44] of the determination of nitrogens of the soluble protein by NaCl solution, the amount of diamino acid nitrogen in NaCl soluble protein was less than that of the original meat protein of herring, but the amount of mono-amino acids was greater. As to the differences of the parts of fish body and sex[10], the amounts of total nitrogen and ash (phosphoric acid, calcium and magnesium), nitrogens of amide and melanin in belly meat were larger in amount than in the back meat. The amounts of the nitrogens of diamino acids, especially arginine, cystine and histidine of the back meat were larger than those of the belly meat, but the amount of lysine nitrogen was contrary. The amount of arginine nitrogen of male herring meat was less than that of female, but the amount of lysine nitrogen was the opposite. As to the amount of non-amino acid nitrogen in monoamino acids fraction, the back meat of female contained more than that of male. According to the heat coagulating of muscle plasma of herring meat which was extracted with 0.6 % NaCl solution[45], the coagulating temperatures of two groups of heat cogulable proteins in muscle plasma are different according to sex. The salting conditions of the protein of herring meat by ammonium sulfate somewhat different by sex. Those facts accorded with the specific difference in muscle plasma protein by sex.

As above described, the properties of meat protein of herring were different more or less in accord with age, fishing season and the spawning. But there was no difference in chemical composition between the herring meat protein and that of other fish, bonito or amber jack, or beef meat protein. From those data, it can be said that the herring meat is as good as other meat from the nutritive point of view.

IX. DIGESTIBILITY OF HERRINGS MEAT

The digestibility of fresh raw herring ("Fore-coming herring") meat which was caught off Yoichi of Hokkaido was estimated.

Five specimens each of male and female (4 fishes were 6 years old and one was 7 years old), the average length was 30.4 *cm*, weight was 235 *g* were used. From those herrings, the muscle meat was taken and employed for the determination of the digestibility. Here, for comparison, beef meat was also employed.

The general chemical compositions of the meat used are shown as in Table 33.

Table 33. General chemical compositions of the meat employed

Sample	Sex	Water (%)	Ash (%)	Crude fat (%)	Total-N (%)
Herring meat	♂	70.91	1.19	7.30	3.30
	♀	70.38	1.11	7.84	3.31
Beef meat		71.23	1.21	6.95	3.30

1. Experimental method

To two *g* of the meat employed as the samples, from which fat was removed by ether, was added 430 *cc* of dist. water, 1 *g* of pepsin and 20 *cc* of 10 % HCl solution. After standing, the mixture was kept at 40°C for 48 hours, and then 10 *cc* of 10 % HCl solution was added to the digested mixture to bring the HCl concentration of the mixture to about 1 %. After the digested mixture was filtered, the amount of nitrogen in the remainder was estimated by the usual method, and degrees of digestibility were determined.

2. Experimental result

Results obtained are shown in Table 34.

As seen in Table 34, the digestibility of the herring meat is considerably inferior to that of beef meat, but they are almost the same. As to the digestibilities of fish meat, raw fresh Atka mackerel (water-content 78.26 %) is 97.26 %, flat fish (water-content 79.57 %) was 97.8 %[46]. From those data, the digestibility of herring meat can be stated to be almost as good as that of other fish meat.

X. NUTRITIVE VALUE OF HERRING MEAT

Table 34. Comparison of the digestibility of herring meat with beef meat

Test No.	Herring meat		Beef meat
	♂	♀	
1	97.91 %	97.92 %	98.40 %
2	97.91	97.92	98.40
3	97.89	98.02	98.40
4	98.01	97.92	98.30
5	97.42	98.02	98.30
Average	97.83 %	97.96 %	98.36 %

On the basis of the experimental results which have been obtained and described in the previous Articles, the chemical components and the digestibilties of the herring raw meat, and crude protein of herring meat which was prepared by extracting the fat were estimated to be almost the same as other fish and market beef meat.

In the experiments to be described in this Article, the estimation of the nutritive values of herring raw meat and the crude protein of herring meat was performed by feeding the experimental animals, mice and albino rats, for comparison with the beef meat and its crude protein. Also the comparison was made with the feeding by oats or barley.

A. Feeding experiment with the crude protein of herring meat

1. Experimental method

(1) Preparation of the crude protein

The crude protein of herring meat was prepared by the method described in the previous Article, VIII. Fresh raw meat of "Fore-coming herrings", from which reddish meat ("Chiai-meat") was removed, was crushed and small bones were removed as completely as possible. To the crushed meat was added toluene; the material was extracted three times by water in a cold place, and then extracted twice by 70 % alcohol, by absolute alcohol three times and at last by ether three times. The meat was then dried at low temperature. This was the crude protein of herring meat. The crude protein of beef meat was prepared by the method described above from beef meat bought in the open market.

In some experiments, the crude protein of cod meat which was prepared by the method described above, was used. The proportion of the amount of the crude proteins to be added to the combined feed was 9 % in general, and 11 % in some experiment. Suzuki *et al.*[47] have found that the minimum amount of proteins required for the maintenance of body weight of rats is 5 % and the amount of proteins required for normal growth is 10 % in the combined feed.

(2) Experimental animals

As experimental animals, mice and rats were employed as in usual nutrition experiments. The employed mice and rats were the second generation, of which the parents were fed with standard feed (casein was used as a protein). Before the experiment, the under-developed mice and rats were removed.

(*3*) *Feeding*

Feeding conditions were carefully controlled.

(*4*) *Weighing of the bodies and of the amount of feed eaten*

The bodies of animals were weighed in a container of known weight after the remained feed attached to the bodies had been removed.

It is said that mice or rats eat about 7 *g* of feed per 100 *g* of body weight. In practical case, a somewhat excess amount of feed was given, considering the amount of the scattered feed. The amount of feed eaten was calculated from estimating the given and left-over amounts of the feed.

(*5*) *Proportion of the combined feed*

The proportion of the combined feed was as follows: The crude protein of herring meat or beef meat was 9 % (11 % in some experiment), fat (a mixture of butter and lard) was 15 %, mineral salts were 4 %, carbohydrate (starch obtained from wheat) was 72 %. When the amount of the crude proteins was 11 %, the amount of the carbohydrate was 70 %. Besides, a small amount of vitamin B (oryzanin) was added. Market wheat starch was treated with water, alcohol, ether several times to remove the fat, and then used for the feed. As mineral salts Osborne's salt was used.

2. Experimental results

(*1*) *Feeding experiment of mice*

(i) First experiment

Three mice each male and female, of which the body weights were 15~20 *g*, were used. Those mice were fed with the combined feed containing 9 % of the crude protein of herring meat or beef meat, or with oats only, for 60 days. In the course of feeding, 3 females conceived and were delivered.

The feeding results are shown in Table 35 and Fig. 14.

As seen in Fig. 14, males of the mice fed with the crude protein of herring meat, increased in weight step by step; they gained in the proportion of 10.3 *g* of increased weight per day until 54 days' feeding. On the contrary, males of the mice which were fed with the crude protein of beef meat, gained weight rapidly until 35 days' feeding, but thereafter showed no increase or decrease of weight. In 35 days' feeding by the crude protein of beef meat, the gaining of weight was 0.3 *g* per day. This is 2 times that of the case of feeding by the crude protein of herring meat.

Males of the mice which were fed with oats only, gained 0.27 *g* of weight per day until 15 days' feeding, but thereafter exhibited no increase or decrease of weight. That is

Table 35. First experimental result (Mice)

Sex	Combined feeds used	Body Weight			Feeding period	Increased weight per day
		Initial	Final	Increased weight		
♂	(a) Containing 9 % crude protein of herring	15.0 *g*	22.0 *g*	7.0 *g*	54 days	0.13 *g/day*
	(b) Containing 9 % crude protein of beef	17.0	27.0	10.0	33	0.30
	(c) Oats only	17.0	21.0	4.0	15	0.27
♀	(a) Containing 9 % crude protein of herring	15.0 *g*	26.0 *g* *	11.0 *g*	81 days **	0.14 *g/day*
	(b) Containing 9 % crude protein of beef	16.0	29.0	13.0	45	0.29
	(c) Oats only	20.0	25.0	5.0	15	0.33

* Weight before delivery.
** Elapsed days until the delivery.

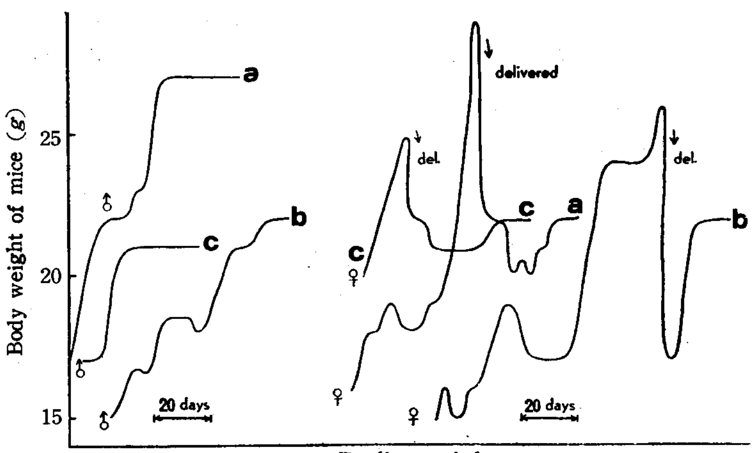

Fig. 14 First feeding results (Mice)
a ···Combined feed containing 9% of the crude protein of beef meat
b ···Feed containing 9% of the crude protein of herring meat
c ···Fed with oats only

to say, in the initial feeding period, mice which were fed with oats grew as well as those with the crude protein of beef meat.

Comparing the gaining of weight per day during the weight-increasing period among the kinds of feeds, the crude protein of beef meat was the largest, the oats were next and the crude protein of herring meat showed the smallest gain. However, in respect of the increasing weight, the crude protein of herring meat was superior to the oats. According to the results obtained in this experiment, from the fact that the gaining of weight of mice which were fed with the crude protein of herring meat showed increase step by step, and considering the difference of the initial weights of the mice employed, the feeding period should be longer. Feeding of female mice was carried out in order to compare the recovery after parturition by the kinds of feeds. Before parturition, the change of weight of bodies was irregular, so it is difficult to compare directly the nutritive value by kinds of feed. As seen in Fig. 14, the recovering state of mice which were given the crude protein of herring meat or of beef meat was superior to that of those fed with oats only.

(ii) Second experiment

In the previous experiment, as the author has observed that the difference of the weights of mice at initial period of feeding influenced somewhat the rate of growth of the mice, here the author has examined how the difference of the weights of male at initial period of feeding, may influence the rate of growth in later period. Two groups each of 13 males which were 13 ~ 14 *g* and 14 ~ 15 *g* by weight were fed with the combined feed containing 9 % crude protein of herring meat for 140 days. The results obtained are shown in Table 36 and Fig. 15 in which the weights of mice were taken as the average of each group.

Table 36. Second experimental result (Mice, Male)

Group	Individual number of mice used	Body weight			Feeding period	Increased weight per day
		Initial	Final	Increased weight		
I (13~14 *g*)	13	14.7 *g*	27.3 *g*	12.6 g	140 days	0.090 *g/day*
II (14~15 *g*)	13	13.6	27.5	13.9	140	0.099

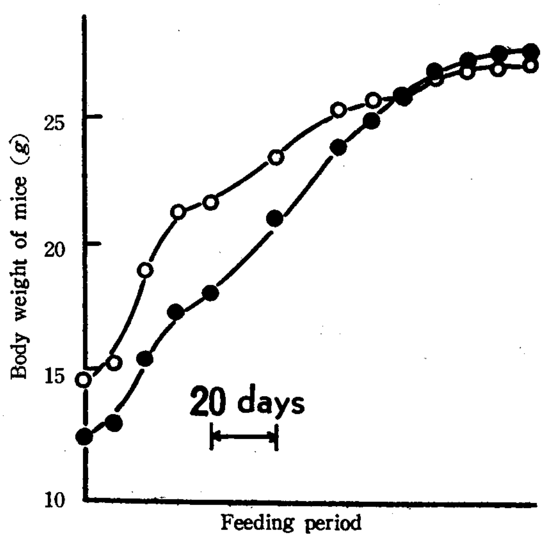

Fig. 15 Second feeding results (Mice, Male)
—●— 13~14 *g* of initial weight
—○— 14~15 *g* of initial weight

As seen in Table 36 and Fig. 15, when the initial weight of mice bodies was small, the rate of growth was pretty good. For the third experiment, the author has taken care to select mice of similar weight and the feeding period was made longer.

(iii) Third experiment

Male mice, of which the body weights were 20 *g*, 18 *g*, 14 ~ 16 *g* and 11 *g* were fed with the combined feed containing 9 % of crude protein of herring meat and beef meat, or oats only. Mice of which weights were 11 *g* at initial period were fed for 60 days, other mice were fed for 280 days. Results obtained are shown in Table 37 and Fig. 16.

Table 37. Third experimental result (Mice, Male)

Combined feed used	Body weight			Feeding period	Increased weight per day (g/day, $\times 10^{-2}$)
	Initial	Final	Increased weight		
a	20 g	31 g	11 g	280 days	39.3
b	20	30	10	280	35.7
a	18	30	12	280	42.8
b	18	30	12	280	42.8
c	18	28	10	280	35.7
a	16	30	14	280	50.0
b	15	30	15	280	53.6
c (1)	15	27	12	280	42.8
c (2)	14	27	13	280	46.4
a	11	18	7	60	117
b	11	17	6	60	100
c	11	13	2	60	33

Note: Combined feed (a)…Feed containing 9% of the crude protein of beef meat, (b)…Feed containing 9% of the crude protein of herring meat, (c)…Fed with oats only.

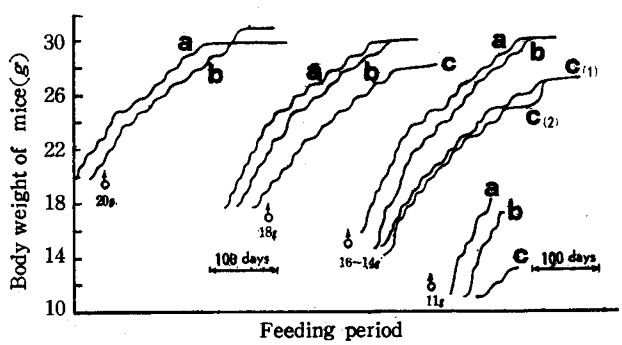

Fig 16 Third feeding results (Mice, Male)

a …Combined feed containing 9% of the crude protein of beef meat.
b …Feed containing 9% of the crude protein of herring meet.
c (1), c (2)…Fed with oats only.

As seen in Table 37 and Fig. 16, the nutritive value of the crude protein of herring meat was inferior to that of the crude protein of beef meat. But the difference was little. The growth of the mice which were given oats only was late.

(2) Feeding experiment by rats (albino rats)

(i) Young rats

Young rats (male and female), of which the weights were 5~11 g were fed under the following conditions.

(a) The young rats were fed with the combined feed containing 9 % of the crude protein of beef meat for the initial 30 days, and then with oats only for the next 60 days. (Each 1 male and female was employed; feeding period was 90 days).

(b) A young rat was given the combined feed containing 9 % of the crude protein of beef meat for the initial 12 days, and then with that containing 9 % of the crude protein of cod meat for the next 36 days. (Male 1, feeding period 48 days)

(c) Rats were given the combined feed containing 9 % of the crude protein of herring meat for 39 days. (Male 15, female 3).

(d) Rats were given the combined feed containing 9 % of the crude protein of cod meat for 84 days. (Male 6, female 5).

(e) Rats were given oats only for 114 days. (Male 6, female 5).
Results obtained are shown in Table 38 and Fig. 17 (1) (2).

As seen in Table 38 and Fig. 17, during the period of feeding with the crude protein of beef meat, the young rats gained about 0.5 *g* of weight per day in average of male and female, that is to say, the rats grew comparatively rapidly. But in the course of the feeding, when the oats were fed, the body weight decreased once and no increase or decrease was afterwards observed. On the contrary, in the course of the feeding with the crude protein of beef meat, when the feed was changed to the crude protein of cod meat, the growth rate fell somewhat, but the rats gained about 0.2 *g* of weight per day.

In the feeding experiment with the combined feed containing the crude protein of herring meat, the rats gained about 0.25 *g* of weight per day. When the young rats were given the crude protein of cod meat, the rats both males and females grew rapidly in the initial several days (the increase of the body weight was about 1.0 *g* per day). But after 7 days, the growth stopped. When the young rats were fed with oats only, they gained about 0.2 *g* per day in average of male and female. The growth rate was as good as with the crude protein of herring meat, but after 50 ∼ 60 days the rats exhibited no increase of weight. But the feeding period with the crude protein of herring meat was short, so the difference of growth rate over a long period between rats which were fed with the crude protein of herring meat and with oats only could not be compared.

Nextly the experiment was carried out by use of adult rats.

(ii) Feeding experiment with adult rats

As the 1st group, 9 males and 6 females, of which the body weight was 100 ± 15 *g* were employed, and as the 2nd group, 8 males and 6 females, body weight 140 ± 10 *g*, were employed. The 1st group of rats was fed with the combined feed containing 9 % of the crude protein of beef meat, or herring meat, or barley only for 195 days. The 2nd group was fed with the combined feed containing each 9 % of the crude protein of beef meat and herring meat for 230 days. The compared results taking an average of male and female are shown in Table 39, Figs. 18 and 19.

As seen in Table 39, all the rats which were fed with the crude proteins of beef meat, herring meat or barley only, gained an increase of weight. Comparing the increasing weight per day during feeding period, from Table 39, one observes no nutritive difference by the kinds of the feeds in the 1st and the 2nd group of rats. But as seen in Figs. 18

Table 38. Feeding results on young rats

Combined feed used	Body weight			Feeding period (days)	Increased weight per day (*g/day*)	Individual number of mice used
	Initial (*g*)	Final (*g*)	Increased weight (*g*)			
(a) Feed containing 9% of the crude protein of beef meat, and then with oats only	♂ 9 ♀ 9	18 17	9 8	90	0.53 * 0~0.02 ** (Av. on ♂ & ♀)	♂ 1 ♀ 2
(b) Feed containing 9% of the crude protein of beef meat, and then with containing 9% of the crude protein of cod meat	♂ 8	22	14	48	0.50 * 0.22 **	♂ 1
(c) Feed containing 9% of the crude protein of herring meat	Av. ♂ 5.2 ♀ 6.3	15.5 15.3	10.3 9	39	0.25 (Av. on ♂ & ♀)	♂ 15 ♀ 3
(d) Feed containing 9% of the crude protein of cod meat	Av. ♂ 11.0 ♀ 10.5	19.5 22.0	8.5 11.5	84	6 days in initial…1.13 6 days after…0.04 (Av. on ♂ & ♀)	♂ 5 ♀ 2
(d) Fed with oats only	Av. ♂ 7.1 ♀ 7.1	17.5 20.0	10.4 12.9	114	Until 54 days (growth period)…0.25 (Av. on ♂ & ♀) Body weight decreased after 54 days	

* In the period of the feeding with the feed containing the crude protein of beef meat
** In the period of the feeding with oats only or with the feed containing the crude protein of cod meat

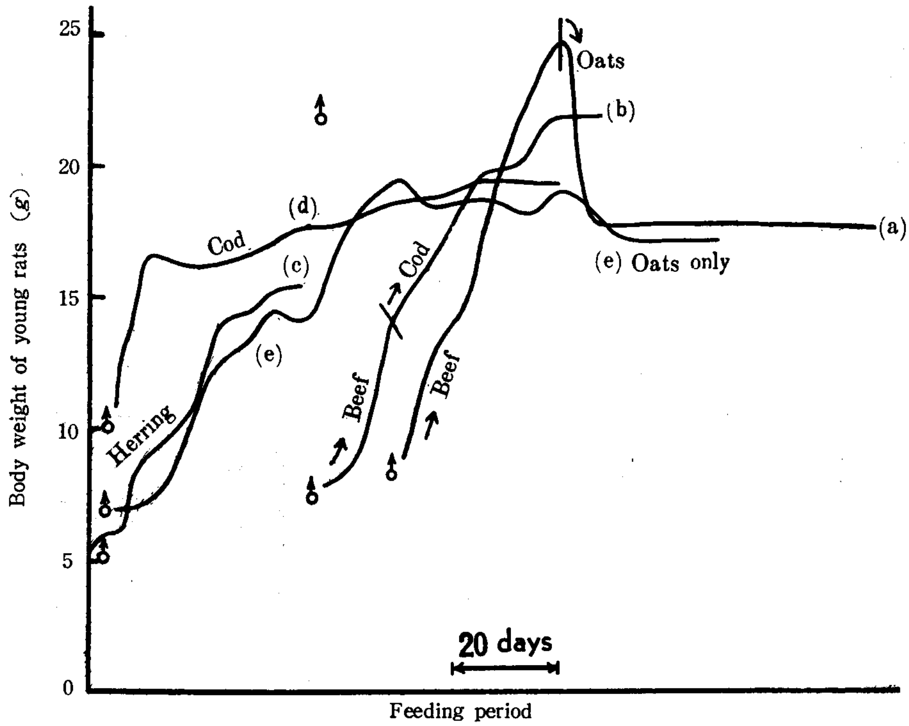

Fig. 17-(1) Feeding results on young rats (Male)

(a)…Combined feed containing 9% of the crude protein of beef meat, and then with oats only.
(b)…Feed containing 9% of the crude protein of beef meat, and then with that containing of 9% of the crude protein of cod meat.
(c)…Feed containing 9% of the crude protein of herring meat.
(d)…Feed containing 9% of the crude protein of cod meat.
(e)…Fed with oats only.

and 19, comparing from the growing curves of rats during feeding period, one observes that the rats which were fed with the crude protein of beef meat or herring meat showed better growth than those fed with barley only.

By comparidon of the increase of body weight per day or the growing curves between the crude proteins of beef meat and herring meat, the nutritive value of the crude protein of beef meat was considered to be pretty superior to that of the crude protein of herring meat. In this experiment, in order to make comparison more accurately between the growth of the rats which were fed with the crude protein of herring meat and with beef meat, the amount of feed eaten was estimated, and the amount of the protein in the feed consumed was calculated.

According to the results obtained in which the increased body weight per 1 *g* of the protein eaten was calculated, when the value of the crude protein of beef meat was 100 %, the value of the 1st and the 2nd groups fed with crude protein of herring meat was

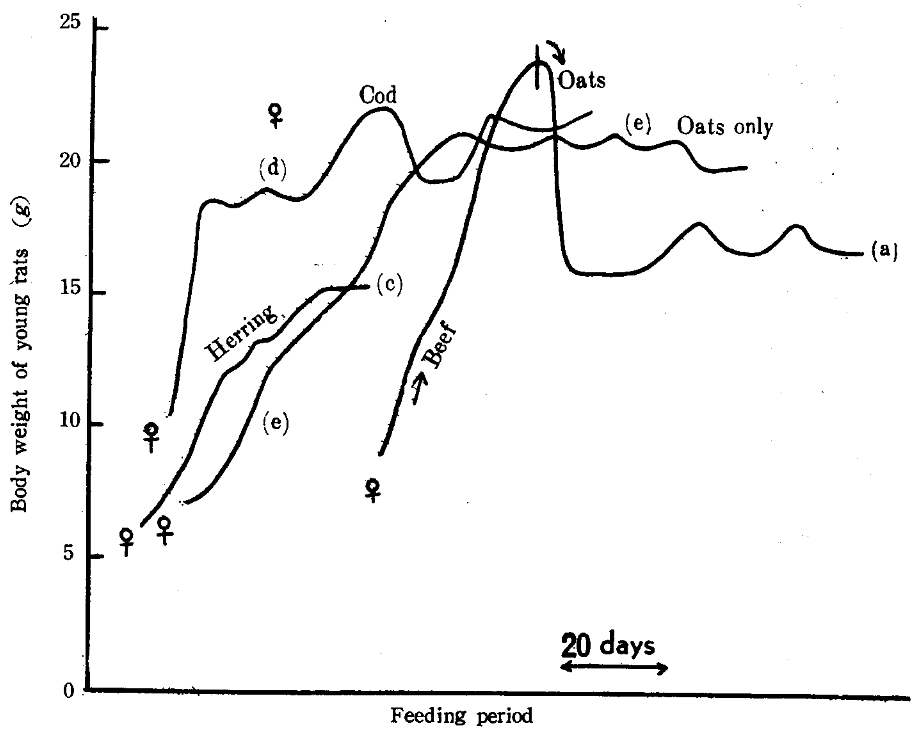

Fig. 17-(2) Feeding results on young rats (female)
Feed signs of (a), (c), (d) and (e) are the same as shown in Fig. 17-(1)

about 96 % taking the average of male and female as shown in the last column of Table 39. That is to say, the nutritive value of the crude protein of beef meat was superior to that of herring meat.

In respect of the difference between the male and female, the male grew better than the female as shown in Figs. 18 and 19.

(iii) Feeding experiment on the increase of mixed amount of the crude protein of herring meat and the adding of amino acids to the combined feed

From the previous experimental results, (ii), the author has ascertained that the nutritive value of the crude protein of herring meat was inferior to that of beef meat. So it was undertaken to examine the growth of rats, when the mixing amount of crude protein of herring meat was increased, or when cystine, histidine (and tryptophane), of which the contents in the crude protein of herring meat are considered to be inferior to that in the crude protein of beef meat, were added.

As seen in Table 30 in the previous article, VIII, the amount of cystine in the crude protein of herring meat was 0.57 % (in ash-free dried matter) compared with 1.2 % in the crude protein of beef meat. Similarly, the amount of histidine was 2.35 % in the

Table 39. Feeding result on adult rats

Group No.	Combined feeds used	Feeding period (days)	Sex	Individual number of rats	Average amount of feed eaten: Total amount (*g*)	Average amount of feed eaten: Protein eaten (*g*)	Average body weight: Initial (*g*)	Average body weight: Final (*g*)	Average body weight: Increased weight (*g*)	Increased weight per day (average, *g/day*)	Increased weight per 1 *g* of the protein eaten (average)	Ratio for the beef meat protein (*g*)
I	a	195	♂	3	1755	159.5	110.3	302.3	192	0.98	1.20	100
			♀	2	1950	177.3	87.5	289.0	201.5	1.03	1.14	100
	b	195	♂	3	1784	162.2	108.3	299.7	191.4	0.98	1.18	93.3
			♀	2	1915	174.1	95.5	287.0	191.5	0.98	1.10	96.5
	c	195	♂	3	—	—	99.0	295.0	196.0	1.01	—	—
			♀	2	—	—	85.5	280.1	194.6	1.00	—	—
II	a	230	♂	4	1840	167.3	134.5	297.0	162.5	0.71	0.97	100
			♀	3	1840	167.3	134.0	294.3	160.3	0.70	0.96	100
	b	230	♂	4	1776	161.5	147.0	298.0	151.0	0.66	0.93	95.9
			♀	3	1947	177.0	129.7	289.0	159.3	0.69	0.90	93.7

Note: Combined feed, (a) Feed containing 9% of the crude protein of beef meet.
(b) Feed containing 9% of the crude protein of herring meat.
(c) Fed with barley only.

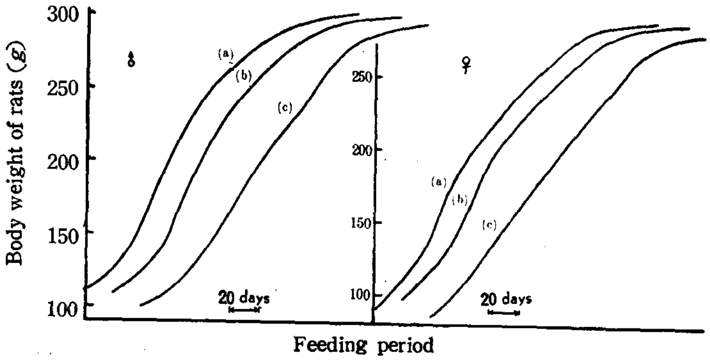

Fig. 18 Feeding results on adult rats (1st group)

(a)…Combined feed containing 9% of the crude protein of beef meat.
(b)…Feed containing 9% of the crude protein of herring meat.
(c)…Fed with barley only.

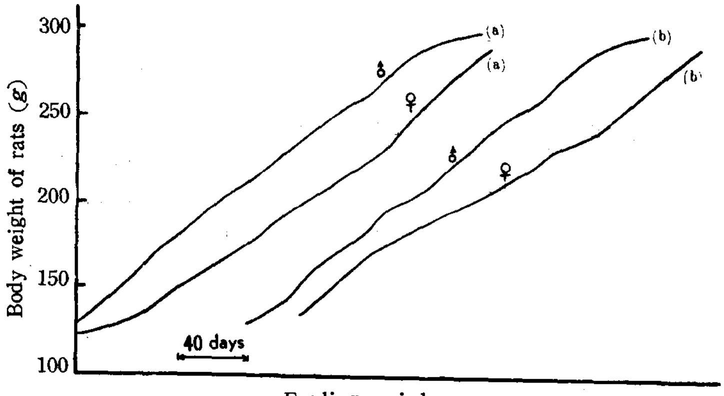

Fig. 19 Feeding results on adult rats (2nd group)
Feed sings of (a) and (b) are the same as in Fig. 18.

crude protein of herring meat for 4.35 % in the crude protein of beef meat.

In this experiment, tryptophane was also added.
The proportion of the components in the combined feed were as follows:

(a) Combined feed containing 9 % of the crude protein of beef meat.
(b) Combined feed containing 11 % of the crude protein of herring meat.
(c) Combined feed containing 9 % of the crude protein of herring meat and each 0.03 g of cystine, histidine and tryptophane.
(d) Combined feed containing 9 % of the crude protein of herring meat and each 0.1 g of cystine, histidine and tryptophane.
(e) Combined feed containing 9 % of the crude protein of herring meat and each 0.1 g of cystine and histidine.

The rats were fed with the five kinds of the combined feed as above described. The experimental results are shown in Table 40 and Fig. 20.

As seen in Table 40 and Fig. 20, when the amount of the crude protein of herring meat was increased from 9 % to 11 %, the growth of rats became better, and became superior in the increase of weight over results obtained by the administration of combined feed containing 9 % of the crude protein of beef meat. According to the results obtained, in which the increased body weights per 1 g of the crude protein eaten were calculated, when the value of the crude protein of beef meat (9 %) was 100 %, the value of the

Table 40. Experimental results on the increase of mixed amount of the crude protein of herring meat and the addition of amino acids to the combined feed

Combined feed	Sex	Individual number of rats	Average body weight			Average amount of feed eaten		Feeding period (days) and mortality	Increased weight per day (average, g/day)	Increased weight per 1 g of the protein eaten (Av.)	Ratio for the beef meat protein (%)
			Initial (g)	Final (g)	Increased weight (g)	Total amount (g)	Protein eaten (g)				
a	♂	2	165.5	300.5	135.0	1350	122.7	180	0.75	1.10	100
	♀	2	148.0	292.5	144.5	1440	130.9	180	0.80	1.10	100
b	♂	3	153.0	298.0	145.0	1210	133.1	180	0.80	1.09	99.1
	♀	2	142.0	290.5	148.5	1260	138.6	180	0.82	1.07	97.3
c	♂	2	151.5	300.0	148.5	—	—	180	0.82	—	—
	♀	2	166.0	298.0	132.0	—	—	180	0.73	—	—
d	♂	1	161	193	32	—	—	died on 53rd days	—	—	—
		1	147	169	22	—	—	died on 35th days	—	—	—
		1	176	190	14	—	—	died on 33rd days	—	—	—
	♀	1	173	201	28	—	—	died on 56th days	—	—	—
		1	170	177	7	—	—	died on 24th days	—	—	—
e	♂	1	214	228	14	—	—	died on 39th days	—	—	—
		1	202	225	23	—	—	died on 61st days	—	—	—
		1*	248	313	65	—	—	survived over 130 days	—	—	—
	♀	1	254	264	10	—	—	died on 32nd days	—	—	—
		1	238	270	32	—	—	died on 71st days	—	—	—

Note: Combined feed, (a) Feed containing 9% of crude protein of beef meat, (b) Feed containing 11% of the crude protein of herring meat, (c) Feed containing 9% of the crude protein of herring meat and each 0.03 *g* of cystine, histidine and tryptophane, (d) Feed containing 9% of the crude protein of herring meat and each 0.1 *g* of cystine, histidine and tryptophane, (e) Feed containing 9% of the crude protein of herring meat and each 0.1 *g* of cystine and histidine.

* Changed the combined feed of 9% of the crude protein of herring meat without adding of amino acids.

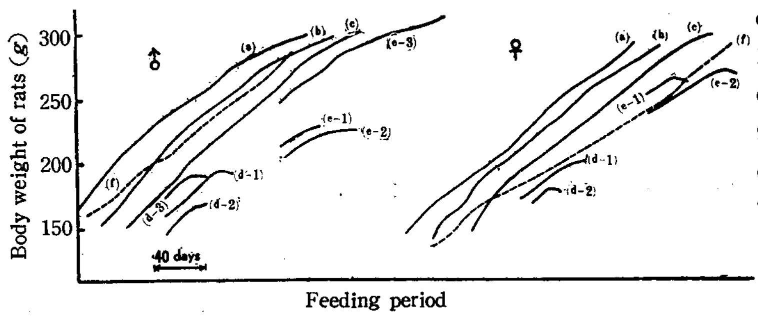

Fig. 20 Experimental results on the increase of mixed amount of the crude protein of herring meat and the adding of amino acids to the combined feed (Adult rats)
Feed signs of (a), (b), (c), (d) and (e) are the same as shown in Table 40. Sign of (f) shows the feed containing 9% of the crude protein of herring meat (No addition of amino acids)

combined feed containing 11 % of the crude protein of herring meat became 98 % (taking an average of male and female). This value was larger than that resulting from the combined feed containing 9 % of the crude protein of herring meat, 96 % as above described in (ii).

Nextly, rats which were fed with the combined feed containing 9 % of the crude protein of herring meat and each 0.03 g of cystine, histidine and tryptophane were found to have grown as well as rats fed with the combined feed containing 11 % of the crude protein of herring meat or 9 % of the crude protein of beef meat. That is to say, the addition of amino acids to the combined feed was clearly effective to increase the weight. However, when the added amounts of amino acids increased to each 0.1 g of amino acids, as in the case of (d) or (e) of the kind of feed, some of the rats died in the course of the feeding. On the other hand, during the feeding by the combined feed containing amino acids, when the feed was changed to the combined feed containing no amino acid, the rats continued to grow without death. That is to say, the excess feeding of amino acids hinders the growth of rats.

In the above described experiments, the author has carried out the feeding of mice and rats with various kinds of crude proteins, and has compared the nutritive values among them. In the same amount of the crude protein in the combined feed, that of the herring meat was pretty inferior to that of beef meat. When the amount of the crude protein of herring meat was properly increased, or the proper amounts of cystine, histidine and tryptophane (the amounts of these amino acids in herring meat are smaller than those in beef meat) were added to the crude protein of herring meat, the nutritive value of the crude protein of herring meat increased to the level of the crude protein of beef meat.

B. Feeding experiment with herring meat

In this experiment, dried herring meat ("Migaki") and canned herring meat (Boiled) were used for the feeding. Here, the beef meat was also used for comparison of the nutritive values. As experimental animals, rats (male) were used.

1. First experiment

Market beef meat, "Migaki" herring meat, canned boiled herring meat and raw sardine meat, of which respectively the chemical components are shown in Table 41, were crushed and dried to powder. These various kinds of meat powder were mixed with white rice powder and wheat flour in the ratio of 20:60:20.

Each 1 *g* vegetable was given to the rats with the combined feeds every day. The feeding results are shown in Table 42 and Fig. 21 (1).

Table 41. Chemical components of feeds used in the first experiment

Sample used	Water (%)	Ash (%)	Crude fat (%)	Crude protein (%)
Beef meat	71.30	1.18 (4.1)	6.92 (24.1)	20.60 (71.8)
"Migaki" (Dried herring meat)	17.50	3.80 (4.6)	17.20 (20.8)	61.5 (74.6)
Canned herring meat (Boiled)	75.52	1.35 (5.5)	5.69 (23.2)	17.44 (71.1)
Sardine meat	76.27	1.96 (8.25)	2.14 (9.0)	19.63 (82.9)

Note: Number in parenthesis shows the percentage of the components to the dried matter.

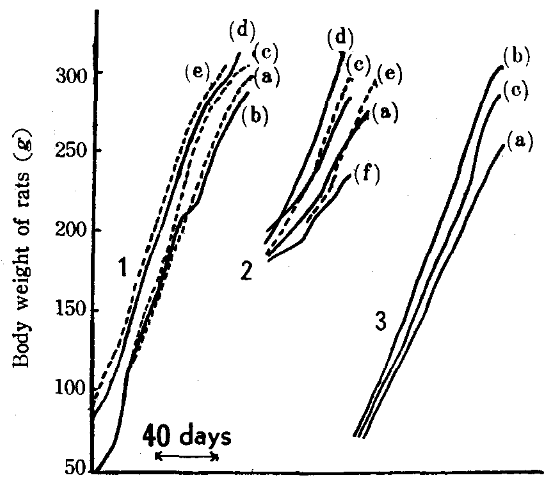

Fig. 21 First, second and third feeding results on rats (Male)

Exp. 1: (a) Beef meat (1), (b) "Migaki" herring meat, (c) Canned boiled herring meat, (d) Beef meat (2), (e) Sardine meat.

Exp. 2: (a) Beef meat, (b) Canned boiled beef meat, (c) Dried herring meat, (d) Canned boiled herring meat, (e) Sardine meat, (f) Oats only

Exp. 3: (a) Beef meat, (b) Canned boiled herring meat. (c) Sardine meat.

As seen in Table 42 and Fig. 21 (1), there is almost no difference in the growth curves of feeding by the various kinds of the meat powder. However, in respect of the increasing of the weight per 1 *g* of the combined feed eaten, the canned boiled herring meat was superior, followed by raw sardine meat, beef meat and "Migaki" herring meat in order.

2. Second experiment

In this experiment, beef meat, canned beef meat (boiled; made in the author's laboratory), canned boiled herring meat, dried herring meat and raw sardine meat of which chemical components are shown in Table 43, were used. Results obtained are shown in Table 44 and Fig. 21 (2). Here, oats were given in contrast with those feeds.

As seen in Table 44 and Fig. 21 (2), in respect of the increase of the body weight per day, the canned boiled herring meat was superior, next dried herring meat, raw sardine meat, canned beef meat, raw beef meat, and oats in order. In this experiment the

Table 42. First feeding results

Sample used	Feeding period (days)	Individual number of rats	Body weight (Average)			Increased weight per day (average, g/day)	Average amount of feed eaten		Increased weight per 1 g of the meat eaten (average)
			Initial (g)	Final (g)	Increased weight (g)		Total amount (g)	Meat eaten (g)	
Beef meat (1)	90	3	54.33	301.33	247.00	2.74	820	164	1.51
"Migaki" (Dried herring meat)	90	3	53.33	293.33	240.00	2.67	815	163	1.47
Canned herring meat (Boiled)	90	3	54.00	310.67	256.67	2.85	810	162	1.58
Beef meat (2)	80	1	83.00	300.00	217.00	2.71	720	144	1.51
Sardine meat	80	4	83.50	303.50	220.00	2.75	725	145	1.52

beef meat was not so good as the crude protein of beef meat, as described in the previous section, **A**, whilst the nutritive value of the dried herring meat was good. This dried herring meat was not secured on the market but was dried from raw meat and immediately employed for the feeding. The fact that this dried herring meat was superior over "Migaki" meat was perhaps due to the period of the preservation after the processing. The nutritive value of canned herring meat or beef meat were superior to that of dried herring meat or beef meat.

3. Third experiment

In the above 1st and 2nd experiments, the nutritive value of the beef meat looked to be inferior to the fish meat. But in the feeding experiment with the crude protein in the previous section, **A**, the crude protein of beef meat was found superior to that of herring meat.

In order to examine the contradiction in the difference, re-examination was made of the feeding with beef meat, canned boiled herring meat and raw sardine meat, of which the respective chemical components are shown in Table 45. The calories of the given samples were almost the same as set down in the last column of Table 45. Experimental animals were rats (male). The results obtained are shown in Table 46, and Fig. 21 (3).

As seen in Table 46 and Fig. 21 (3), even if the sample feeds had the same calorie values, the canned boiled herring meat was superior, next sardine meat and beef meat in order.

4. Fourth experiment

In the 3rd experiment also, the herring meat was found superior to beef meat in nutritive value. The cause of the superiority of the nutritive value of herring meat may be the difference of the amount of pure protein in the meat, the properties of the protein, the composition of the fat contained, mineral salts in the ash or vitamins. The author has tried to find any remarkable difference between the beef meat and fish meat, and has found that the amount of iodine in fish meat is remarkably larger than that of beef meat as shown in Table 47. Rats were fed with the combined feed containing iodine. To obtain the combined feeds containing the same amount of iodine, the author has added adequate amounts of potassium iodide (0.0025 % solution)

Table 43. Chemical components of feeds useds in the second experiment

Sample used	Water (%)	Ash (%)	Crude fat (%)	Crude protein (%)
Beef meat	72.79	1.19 (4.4)	5.33 (19.6)	20.69 (76.1)
Canned beef meat (Boiled)	72.66	1.18 (4.3)	5.41 (19.8)	20.75 (76.0)
Dried herring meat	13.40	3.31 (3.8)	18.10 (20.9)	65.19 (75.2)
Canned herring meat (Boiled)	75.52	1.35 (5.5)	5.69 (23.2)	17.44 (71.2)
Sardine meat	76.27	1.96 (8.3)	2.14 (9.0)	19.63 (82.8)

Note: Number in parenthesis shows the percentage of the components to the dried matter.

Table 44. Second feeding results

Sample used	Feeding period (days)	Individual number of rats	Body weight (Average)			Increased weight per day (average, *g/day*)	Average amount of feed eaten		Increased weight per 1 *g* of the meat eaten (average)
			Initial (*g*)	Final (*g*)	Increased weight (*g*)		Total amount (*g*)	Meat eaten (*g*)	
Beef meat	50	3	185.33	262.67	77.34	1.55	380	76	1.02
Canned beef meat (Boiled)	50	3	199.33	288.67	89.34	1.79	400	80	1.12
Dried herring meat	50	3	181.00	300.00	119.00	2.38	508	102	1.17
Canned herring meat (Boiled)	50	3	192.33	317.33	125.00	2.50	500	100	1.25
Sardine meat	50	3	193.67	297.33	103.66	2.07	450	90	1.15
Oats only	50	3	181.67	237.00	55.33	1.11	—	—	—

or "Kajime" (a kind of seaweed)-meal to the combined feeds. To 20 *g* of beef meat powder, 1.6 *cc* of the potassium iodide solution or 0.01 *g* of "Kajime"-meal were added. To 20 *g* of sardine meat powder, 0.31 *cc* of the potassium iodide solution or 0.002*g* of "Kajime"-meal was added. Those various kinds of meat powder were respectively mixed with white rice powder and wheat flour in ratio of 20:60:20.

The combined feeds were given to respective groups of rats (male). The results obtained are shown in Table 48 and Fig. 22.

To compare the nutritive value, the standard feed was made as follows: starch 72 %, McCollum's salt 4 %, liver oil 4 *cc*, casein 18 %, oryzanin solution 2 *cc*. To this standard feed was also added potassium iodide solution or "Kajime"-meal.

As seen in Table 48 and Fig. 22, the nutritive value of herring meat was as good as the standard feed which was superior to sardine meat or beef meat. But when to the sardine meat or beef meat was added iodine of which the amount was the same as the amount of iodine in herring meat, the nutritive value

Table 45. Chemical components and calorie values of the samples used in 3rd experiment

Sample used	Water (%)	Crude fat (%)	Crude protein (%)	Ash (%)	Calorie value (cal./100 *g* dried matter)
Beef meat	71.30	6.92 (24.1)	20.60 (71.8)	1.18 (4.1)	518.5
Canned herring meat (Boiled)	75.52	5.69 (23.2)	17.44 (71.3)	1.25 (5.5)	508.1
Sardine meat	74.62	5.77 (22.8)	18.19 (71.8)	1.42 (5.6)	506.4

Note: Number in parenthesis shows the percentage of the components to the dried matter.

Table 46. Third feeding results

Sample used	Feeding period (days)	Individual number of rats	Body weight (Average)			Increased weight per day (average, *g/day*)	Average amount of feed eaten		Increased weight per 1 *g* of the meat eaten (average)
			Initial (*g*)	Final (*g*)	Increased weight (*g*)		Total amount (*g*)	Meat eaten (*g*)	
Beef meat	90	1	70	256	186	2.01	684	137	1.36
Canned herring meat (Boiled)	90	1	70	305	235	2.61	720	144	1.63
Sardine meat	90	1	68	287	219	2.43	715	143	1.53

Table 47. Chemical components of feeds used in the 4th experiment

Sample used	Water (%)	Ash (%)	Crude fat (%)	Crude protein (%)	Iodine (γ/100 g of dried matter)
Dried herring meat	13.40	3.31 (3.8)	18.10 (20.9)	65.19 (75.2)	151
Beef meat	72.05	1.18 (4.2)	0.14 (22.0)	20.63 (73.8)	1.8
Sardine meat	73.97	1.72 (6.6)	5.81 (22.3)	18.50 (71.1)	122
Salmon meat	75.05	1.13 (4.5)	3.77 (15.1)	20.06 (80.2)	144
"Kajime"-meal	—	—	—	—	295

Note: Number in parenthesis shows the percentage of the components to the dried matter.

Table 48. Fourth feeding results

Sample used	Body weight (Average)			Increased weight per day (average, g/day)	Average amount of feed eaten		Increased weight per 1 g of the meat eaten (average)
	Initial (g)	Final (g)	Increased weight (g)		Total amount (g)	Meat eaten (g)	
Herring meat	88	305	217	2.41	690	138	1.57
〃	72	299	227	2.52	710	142	1.60
Beef meat	88	271	183	2.03	698	140	1.31
〃	70	249	179	1.99	700	140	1.28
Beef meat + KI	85	298	213	2.33	687	137	1.56
〃	74	293	219	2.43	700	140	1.57
Beef meat + "Kajime"-meal	87	305	218	2.42	690	138	1.58
〃	72	296	224	2.49	706	141	1.59
Sardine meat	90	300	210	2.33	692	138	1.52
〃	70	290	220	2.44	710	142	1.55
Sardine meat + KI	88	303	215	2.39	687	137	1.57
〃	72	296	224	2.49	710	142	1.58
Sardine meat + "Kajime"-meal	87	306	219	2.43	690	138	1.59
〃	72	300	228	2.53	710	142	1.58
Standard feed	88	290	202	2.25	—	—	—
〃	72	278	206	2.29	710	142	1.61
Salmon meat	90	302	212	2.36	687	137	1.55
〃	73	297	221	2.46	700	221	1.58

of the sardine or beef meat became the same as herring meat. It is clear that the nutritive value of beef meat may be increased by the addition of iodine. From these results, the difference of the nutritive values between herring meat and beef meat considered to be due somewhat to the difference of the amounts of iodine.

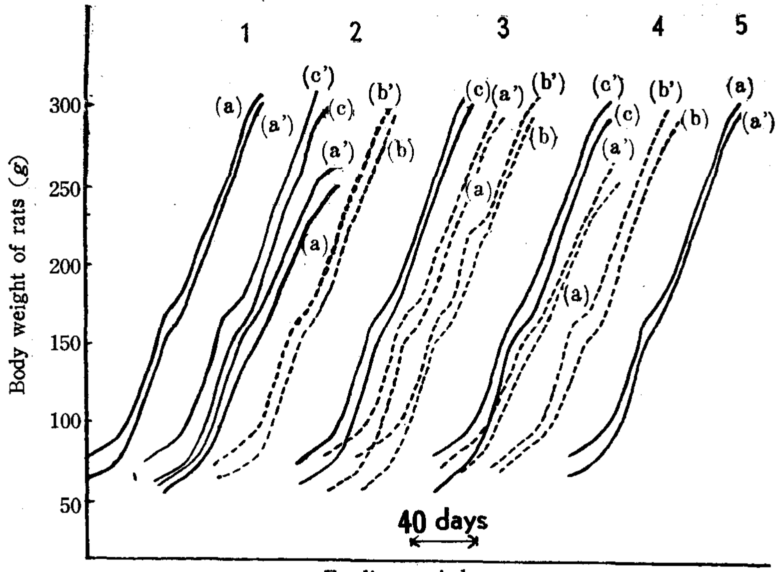

Fig 22 Fourth feeding results on rats (Male)

Exp. 1: (a), (a′) …… Herring meat
Exp. 2: (a), (a′) …… Beef meat
(b), (b′) …… Ditto + KI
(c), (c′) …… Ditto + "Kajime"-meal
Exp. 3: (a), (a′) …… Sardine meat
(b), (b′) …… Ditto + KI
(c), (c′) …… Ditto + "Kajime"-meal
Exp. 4: (a), (a′) …… Standard feed
(b), (b′) …… Ditto + KI
(c), (c′) …… Ditto + "Kajime"-meal
Exp. 5: (a), (a′) …… Salmon meat

C. Consideration

In order to estimate the nutritive value of herring meat, the author has fed mice and rats with the crude protein of herring meat, raw herring meat and canned herring meat. As control, beef mat was also used.

The nutritive value of the crude protein of herring meat was inferior to that of the crude protein of beef meat. When the amount of the crude protein of herring meat was increased, the nutritive value of the crude protein increased to a degree equal of that of the crude protein of beef meat. When adequate amounts of cystine, histidine and tryptophane were added to the crude protein of herring meat, the nutritive value increased, but when an excess amount of amino acids were added to that crude protein, some rats died from the feed, that is to say, the addition of excess amount of amino acids did more harm than good. Nextly, raw or processed herring meat was examined for their nutritive value. Contrary to the crude protein, the raw or processed herring meat was superior to the beef meat. One of the causes of the difference was the difference of the amounts of iodine in herring meat and beef meat. This was clarified by the addition of iodine to the combined feed containing beef meat.

The fact that iodine is an important factor in maintaining the health of human beings has been said from previously[48]. Evard and Culbertson[49] have observed that a group of experimental pigs gained increase of body weight when they are fed with a combined feed containing potassium iodide of 40 *mg* every day for 140 days. Orr and Leitch[49] have shown that the daily feeding of 1 *g* of potassium iodide to pigs did not harm the health. Phillips, Curtis and Erf[49] have fed a group of cows with a combined feed containing iodine of which the forms were potassium iodide, fish meal or kelp meal for 3 years; they have observed that the cows kept their health. Remington, Coulson and Levine[50] have observed that iodine contained in milk, oyster or cod meat is effective to prevent diseases of the thyroid gland and to increase preserved iodine as well as potassium iodide.

Okubo[51] has observed the amount of iodine content in marine products (fish and algae), and has said that fish meat has large value as iodine souce in the nutrition of human beings.

From those results obtained by many investigators, it is clear that the presence of an adequate amount of iodine in herring meat was a cause of its high nutritive value.

Besides iodine, the chemical constitution of fat contained in herring meat or vitamins must be considered. Tadokoro and Kondo[52] have studied the nutritive value of herring oil. According to them, herring fat is rich in oil, therefore it has high nutritive value. They have further observed the fresh dried herring meat ("Migaki" herring meat) is superior in nutritive value to the preserved dried herring meat. This is in agreement with the present author's result. This is perhaps due to the oxidation of the herring fat. At one time, the highly unsaturated fatty acids themselves were considered poisonous, but recently Kaneda, Sakai and Ishii[53] have clarified that the highly unsaturated fatty acid in fish bodies themselves are not poisonous, and rather nutritive, and that the poisonous substance is the auto-oxidized products of the highly unsaturated fatty acids.

The preserved dried herring meat still retains the nutritive value as above stated. This shows that the oil and fat in herring meat are not easily oxidized. This difficulty of oxidation of herring oil was suggested by Kaneda *et al.*[54] In order to prevent the oxidation of the oil, B.H.A. (sustane) has come into use recently.

From previously, the author has been convinced that the nutritive value of the herring meat is not inferior to that of beef or poultry meat; here it has been demonstrated that his belief is correct.

In Japan it is important to eat more fish, because the fish meat including herring has superiority in nutritive value.

SUMMARY

In order to examine the nutritive value of herring meat, various experiments were

carried out and important results were obtained. They are summarized as follows:

(1) In respects of the general chemical composition of the meat of "Spring herring" before and after the spawning and of the same ages, the amounts of water and fat contents in the herring meat vary remarkably. Those components show the reverse relation (Article II).

(2) The general chemical compositions of the meat of "Small herring" and of "Oily herring" were determined. The maximum amount of fat in "Small herring" was below 10 %, and that in "Oily herring" was above 15 %. The larger amount of fat in the "Oily herring" was considered to be due to the plankton containing rich fat which were eaten during the feeding migration (Articles III, IV).

(3) In respects of the chemical components in the extractive of herring meat, creatine, creatinine, hypoxanthine, xanthine, methylguanidine, choline, inosinic acid, taurine were isolated and estimated. In comparison with other fish, the amounts of creatine, creatinine in the extractive of herring meat were smaller than in that of other fish meat, but the amounts of hypoxanthine and methylguanidine were larger (Article V).

(4) The amounts of inorganic components in herring meat and blood were estimated.

(i) In respect of the inorganic components in the herring meat, the amounts of sulfur, calcium, iron, magnesium, sodium, potassium, phosphorus and iodine were estimated. The amounts of Na, K, P and I in the herring meat were as large as in other fish meat (Article VI).

(ii) Among inorganic components estimated, the forms of sulfur and phosphorus were determined. In the total amount of sulfur, about 22 % was water soluble form, and the remainder was insoluble form. In the total amount of phosphorus, about 55 % was water soluble form, 30 % was lipoid form and 12 % was protein form and others.

(iii) In respects of inorganic components in the blood, NaCl is the principal component; equal amounts of phosphorus, iron, potassium and small quantities of calcium, magnesium were present.

(5) When the fresh herring meat was left, the initial pH value being 6.8 descended to the more acidic side, but afterwards ascended to alkali side with the decomposition of the meat; the amounts of volatile basic nitrogen increased. When the amount of volatile basic nitrogen became above 30 *mg* %, the meat became inedible (Article VII).

(6) From fresh herring meat, the crude protein was prepared. The variations in solubility, properties of protein, distribution of amino acids were determined by sex, and maturity (Article VIII).

(i) Nitrogenous components in the herring meat dissolved out abundantly in 0.2 % KOH, 10 % NaCl solutions.

(ii) About half of the amount of 5 % NaCl soluble nitrogenous components was considered to be myosin-form, the rest was considered to be equally myogen-form and non-protein-form nitrogen.

(iii) In the herring meat protein, the amount of organic basic nitrogen was larger than that of monoamino nitrogen. Among monoamino acids, the amounts of leucine and glutamic acid were remarkably larger than the others. Among diamino acids, the amount of histidine was smaller than those of lysine and arginine. But the presence of considerably large amounts of lysine, arginine and proper amounts of histidine, tyrosine, phenylalanine and tryptophane indicated the high nutritive value of herring meat protein.

(iv) Among the amino acids, the amounts of histidine, arginine and non-amino nitrogen decreased after the spawning; on the contrary, it is remarkable that the amounts of monoamino nitrogen and lysine nitrogen increased after the spawning.

(7) The digestibility of herring meat was 98 %; this is almost as good as that of beef meat (Article IX).

(8) From the results as above described, the chemical composition and digestibility of herring meat were considered to be as good as those of beef meat. Next the nutritive value of herring meat was estimated by feeding experiments on mice and rats (Article X).

(i) The crude protein which was prepared from herring meat gave considerably lower nutritive value than the crude protein of beef meat in the same feeding amount (9 % in the combined feed).

(ii) However, when the amount of the crude protein of herring meat was increased (from 9 % to 11 %), or when adequate amounts (each 0.03 %) of cystine, histidine and tryptophane were added to the crude protein of herring meat, the nutritive value of the crude protein increased to the level of that of beef meat.

(iii) When the nutritive values of raw or processed herring meat were compared with those of other fish (sardine, salmon) and market beef meat, the herring meat was superior, whilst the beef meat was inferior.

(iv) As one of the causes of the difference was considered to be the difference in the amounts of iodine content, iodine was added to the beef meat in order to make the same amount of iodine content as in the herring meat. Potassium iodide or "Kajime"-meal (a kind of kelp meal) was used. Then nutritive value of beef meat increased to the level of herring meat.

By the experimental results obtained as above described, the author has been convinced that the nutritive value of herring meat bears comparison with beef meat.

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52) Tadokoro, T. & Kondo, M. (1948). ***Hokusuisi-Geppo* 〔*Jour. Hokkaido Fish. Sci. Inst.*〕**, 5 (8), 1. (Japanese).

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