Effect of High Salt Content of Indonesian Dried-Salted Fish on Rats

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Skipjack tuna (*Katsuwonus pelamis*) was used to produce Indonesian dried-salted fish (DSF). The flesh was soaked in a 25% NaCl solution (w/v) for 24 h and then dried in an artificial drier at 45 °C for 40 h. A portion of the final product was directly freeze-dried (DSF I), while the remainder was stored for 3 months at 28 °C (DSF II). The effects of high salt content in DSF on protein nutritional quality, internal organs, blood pressure, and mineral loss were evaluated using male Wistar rats. DSF's high salt concentration and 3 month storage period did not significantly affect protein quality, as monitored by protein efficiency ratio, net protein ratio, feed conversion efficiency, protein digestibility, biological value, and net protein utilization. However, DSF's high salt content significantly induced higher blood pressure, hypertrophy of most internal organs, and increased loss of several minerals through urine excretion.

INTRODUCTION

The preservation of fish using salt in combination with drying has been known for thousands of years as a simple method either to prolong its shelf life or to give desired flavors. Some people in developed countries, particularly those with a rice eater's diet, desire the aroma, texture, and other distinct characteristics of dried-salted fish (DSF).

This traditional technique still plays an important role in fish processing, although it varies among countries. The variation includes the method of salting (dry or brine salting), concentration of salt (between 5 and 30%), and drying technique (sun dried or artificial drier). Salting times also vary from 20 to 30 min in the Philippines to 48 h in Indonesia (Wootton and Ismail, 1986). Different countries also prefer salted fish of various moisture contents. Nigerian consumers prefer their products with 33% water, whereas those in the United States prefer a moisture content of 50% (Pigott and Tucker, 1991). With regard to the salt content, Asians prefer saltier fish products than do Europeans.

The Indonesian government has included DSF as one of nine essential food items (Souness, 1988). The consumption rate is much higher in rural areas than in urban areas (Syukur et al., 1992). A major factor preventing a wider acceptance of the Indonesian DSF is probably its strong salty taste, which results from the fish being soaked frequently in saturated salt brines for lengthy periods. In recent years there has been a trend toward Indonesian consumers' preferring lower salt fish (Buckle et al., 1988).

Although Indonesian consumers soak DSF in tap water prior to cooking to reduce the salt content, there is the possibility that the salt content of the prepared product is still high. High intakes of salt from such products could place individuals at risk to certain health problems. To investigate this possibility, the purpose of this study was to evaluate the effects of salt content of DSF on protein quality, internal organs, serum biochemical parameters, and blood pressure of rats fed

20% protein diets for 26 days. In this study, skipjack tuna (*Katsuwonus pelamis*) was used as a model.

PROCEDURES

Preparation of DSF Products. Fresh, nonfrozen, skipjack tuna (*Katsuwonus pelamis*) was purchased from Tsukiji Fish Market, Tokyo, Japan. The fish (mean weight and length approximately 4 kg and 60 cm, respectively) was iced during transportation to the laboratory for processing. The fish were split, gutted, washed in tap water, and filleted. Fillets were cut into 60–70 g slices and divided into two groups. The control group (unprocessed fish) was freeze-dried. The other group (DSF product) was immersed for 24 h in pure NaCl solution (25% concentration, w/v) at ambient temperature (28 °C) with a fish-to-brine ratio of 1:2 (w/v). After salting, the fish was removed, placed on drying trays, and dried in an artificial drier at 45 °C for 40 h.

The DSF product was divided into two groups and sealed with minimal headspace into loose-fitting polyethylene bags. The first group (DSF I) was immediately freeze-dried to represent 0 months of storage. The other group (DSF II), was stored at 28 °C for 3 months. This storage treatment was chosen to simulate the typical storage time used in Indonesia, where dried-salted fish is usually kept at room temperature (around 28 °C) during transportation, storage, and marketing.

Experimental Methods. Three-week-old male Wistar rats (Tokyo Experimental Animal Co., Ltd), weighing approximately 66.1–68.2 g, were used in this study and were fed a pre-experimental diet (a commercial nonpurified diet, type MF, Oriental Yeast Co., Tokyo) for 3 days to allow animals to acclimate to the new environment. After 3 days the animals were weighed and placed in individual wire-bottom metabolism cages. Animals were housed in a room maintained at 22 °C with a relative humidity of 60% and 12 h light–dark cycle beginning at 7:00 a.m. The rats were divided into five groups of eight rats each, and each group received one of four protein or non-protein diets. Diets and water were provided *ad libitum*. Feed intake and body weight were recorded daily for 26 days.

Casein, freeze-dried fresh fish, DSF I (without storage treatment), and DSF II (stored for 3 months) were the sources of protein used in the experimental diet. The chemical compositions of these protein sources are given in Table 1. Diets were prepared as recommended by the Association of Official Analytical Chemists (AOAC, 1990), but the concentration of protein used was adjusted to a 20% level as compared to AOAC's 10% protein level. The main purpose of this treatment was not only to increase the protein content of the

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Table 1. Composition of Fresh and Dried-Salted Fish and Lactic Casein for Rat Bioassay

sample	moisture (%)	ash (%)	fat (%)	protein (%)
fresh fish[a]	2.3	4.1	10.3	83.3
DSF I[b]	2.1	28.9	5.9	63.1
DSF II[c]	2.6	31.0	5.4	60.4
lactic casein	11.4	1.9	1.1	85.5

[a] Freeze-dried sample. [b] Dried-salted fish prepared by 25% salt and then freeze-dried (0 months of storage). [c] Dried-salted fish prepared by 25% salt and then stored for 3 months at 28 °C.

Table 2. Composition of Diet (Percent of Weight) for Rat Experiment[a]

ingredient	casein	fresh fish	DSF I[b]	DSF II[b]	NP[c]
sample	23.4	24.0	31.7	33.1	0.0
cottonseed oil[d]	7.7	5.5	6.1	6.2	8.0
mineral mixture[e]	4.5	4.0	0.4	0.4	5.0
vitamin mixture[f]	1.0	1.0	1.0	1.0	1.0
cellulose[g]	1.0	1.0	1.0	1.0	1.0
water	2.6	4.4	4.3	4.1	5.0
α-cornstarch	59.8	60.1	55.5	54.2	80.0
NaCl (g/100 g of diet)	0.5	1.5	10.3	8.6	

[a] Based on AOAC (1990) to give 20% of protein level. [b] The explanation of DSF I and II is the same as in Table 1. [c] Non-protein diet. [d] Cottonseed oil was obtained from Nacalai Tesque Inc., Kyoto, Japan. [e] Mineral mixture was prepared according to AOAC (1990), but NaCl was removed for DSF I and II groups. [f] AIN-76 vitamin mixture, obtained from Oriental Yeast Co. [g] Cellulose powder obtained from Oriental Yeast Co.

diet but also to increase the salt content. By this treatment we hoped to obtain some interesting information with regard to some biochemical values in serum, the internal organ weights of rats, any loss of minerals, and any effect on the blood pressure of the animals when compared with animals fed fresh fish or casein. The compositions and NaCl concentrations of the experimental diets are shown in Table 2. A non-protein diet was also included to provide metabolic fecal and endogenous urinary nitrogen data.

Protein efficiency ratio (PER) and net protein ratio (NPR) were calculated according to the methods of Bender and Doell (1957). True digestibility (TD), biological value (BV), and net protein utilization (NPU) of protein were calculated as described by Pellet and Young (1980).

Feces and urine from each animal were collected at days 7, 8, 9, and 10. Fecal samples were dried at 105 °C for 3 h, ground, weighed, and analyzed. The urine samples were filtered and stored at −40 °C until they were analyzed.

At the end of the experiment (26 days), all animals were anesthetized with Nembutal (sodium pentobarbital 50 mg/mL, 100 μL/100 g of body weight) and sacrificed between 10:00 a.m. and noon. Serum samples were prepared from blood obtained by cardiac puncture and analyzed immediately. The internal organs were immediately removed, washed with 0.9% saline, and weighed. The stomach was cut open, emptied, and weighed. Liver and kidney were kept at −80 °C until they were analyzed.

Blood Pressure. Prior to sacrifice, the mean blood pressure from each group of animals was measured by the tail cuff method with a programmable PS-100 electrosphygmomanometer apparatus (Riken Kaihatsu Co., Japan). Rats were familiarized with the blood pressure apparatus prior to measurement, and a heating pad was used to maintain animals at an ambient temperature of 35–40 °C. At least five readings were averaged for each animal (Greger and Tseng, 1993).

Chemical Analysis. The DSF products, fresh fish, and casein were analyzed for protein, moisture, ash, water activity, and salt by standard analytical procedures (AOAC, 1990). Protein was determined by the Kjeldahl method, and moisture was determined by oven-drying at 105 °C to constant weight. Ash content was determined using a muffle furnace by heating at 600 °C. Water activity was determined by rotronic hygroskop and salt content by titration against 0.1 M silver

Table 3. Effects of Various Diets on Total Feed Intake, Water Consumption, and Excretion of Feces and Urine of Rats[a]

	lactic casein	fresh fish	DSF I	DSF II
feed (g/day)	20.25a	20.26a	16.26b	15.33b
	(0.67)	(1.80)	(1.30)	(1.56)
water (mL/day)	25.75a	27.87a	92.49b	95.78c
	(1.20)	(2.06)	(0.52)	(1.96)
urine (mL/day)	7.64a	13.07b	62.59c	65.62bc
	(1.77)	(1.28)	(4.37)	(3.18)
feces (g/day)[b]	0.57a	0.53a	0.45ab	0.39b
	(0.13)	(0.09)	(0.15)	(0.10)

[a] Values are means of eight replicates, with SD in parentheses. Different letters in the same line represent significant difference ($p < 0.01$). [b] After drying at 110 °C for 3 h.

nitrate. Total fat was determined by extraction with chloroform and methanol (2:1) as described by Folch et al. (1957).

Mineral analyses of dietary components, feces, urine, and internal organs were determined with an inductively couple plasma (ICP) spectrometer (Model ICPS-1000 II, Shimadzu Co. Ltd., Japan). Sample preparation was conducted by wet ash procedure. A 0.5 g sample was digested in 5 mL of concentrated HNO_3 in an iron jacket oven at 150 °C for 2 h. Digested samples were cooled in an ice bath, diluted to 50 mL with deionized water, and filtered through Whatman No. 5B filter paper. The filtrates were analyzed for Al, Ca, Fe, Cu, K, Mn, Mg, P, Na, S, and Zn. The spectrometer was standardized with the specific mineral standard at each assay time.

Serum biochemical parameters were analyzed by the methods indicated. Total cholesterol and HDL cholesterol were analyzed according to the methods of Allain et al. (1974) and Allen et al. (1979), respectively. The method of Matsumiya (1983) was used for triglyceride determination. Blood urea nitrogen (BUN) was analyzed according to the method of Horn et al. (1966), and creatinine and creatine were determine according to the procedure of Cook (1971). Serum glutamate–oxalate transaminase (SGOT) and serum glutamate–pyruvate transaminase (SGPT) were determined colorimetrically according to the method of Richterich (1969). Serum glucose was measured with a glucose oxidase kit (glucose-B test Wako, Wako Pure Chemical Industry, Ltd., Tokyo). Serum protein was determined by the biuret method, and albumin was colorimetrically measured using bromocresol green as a binding dye (Sugiyama et al., 1991).

RESULTS AND DISCUSSION

Effect of Salt on Protein Nutritional Quality. It can be seen from Table 1 that DSF has a lower protein content than fresh fish. Brining the fish in a 25% NaCl solution for 24 h and drying the fish at a temperature of 45 °C for 40 h reduce the protein content of fish. Sarcoplasmic proteins (myoalbumin, globin, and enzymes) are soluble in water; structural proteins (actin, myosin, tropomyosin, and actomyosin) are soluble in neutral salt solutions of fairly high ionic strength (more than 0.5 M) (Spinelli and Dossow, 1982; Suzuki, 1981). Salt also reduced the total fat and increased the ash contents. The final product had a moisture content, NaCl, and water activity of 33%, 23%, and 0.74, respectively.

The contents of NaCl in the DSF experimental diets were 17–21 times higher than in the casein group and 6–7 times higher than in the fresh fish group (Table 2). The effects of the diets on total feed and water consumption and excretion of urine and feces of rats fed for 26 days are shown in Table 3. The DSF groups had significantly lower feed intake and higher water consumption and urine excretion than either the casein or fresh fish group. The high salt content in the diet caused the rat to easily become thirsty and drink more water (3.7 times more than casein group). Conse-

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Table 4. Protein Quality of Fresh and Dried-Salted Fish Estimated with Rats in Growth and Nitrogen Balance Assays[a]

	lactic casein	fresh fish	DSF I	DSF II
	Growth Assay			
body wt gain[b] (g)	226.39a	226.40a	150.65b	148.12b
	(12.85)	(22.14)	(20.05)	(12.89)
protein intake[b] (g)	105.31a	105.35a	84.55b	79.71b
	(3.48)	(9.38)	(6.76)	(8.12)
FCE[c]	0.51a	0.53a	0.48ab	0.42b
	(0.03)	(0.02)	(0.06)	(0.08)
PER[c]	2.50a	2.58a	2.34ab	2.05b
	(0.16)	(0.10)	(0.31)	(0.37)
NPR[c]	2.91a	3.03a	2.89ab	2.62b
	(0.18)	(0.12)	(0.32)	(0.39)
	Balance Assay			
TD[d] (%)	99.69a	99.69a	99.02b	98.94b
	(0.09)	(0.06)	(0.43)	(0.36)
BV[d] (%)	94.98a	92.45b	92.02b	92.86b
	(1.54)	(1.51)	(0.56)	(1.02)
NPU[d] (%)	94.69a	92.16b	91.12b	91.88b

[a] Values are means of eight replicates, with SD in parentheses. [b] Different letters in the same line represent significant difference ($p < 0.001$) for 26 days of experiment. [c] Different letters in the same line represent significant difference ($p < 0.05$) for 10 days of experiment. [d] Different letters in the same line represent significant difference ($p < 0.01$) for 10 days of experiment.

quently, the urine excretion of the DSF-fed groups was 8.5 times higher than the casein group.

The rats fed fresh fish and casein had similar growth curves, while the DSF groups showed comparably lower growth rates (Table 4). This phenomenon is correlated with the feed intake and directly related to its salt content. It appears that the extra 1.5% salt content of the fresh fish group compared to the casein group did not affect the preference of the rats to eat the diet. As a result, the total feed intake and body weight gain of rats in the two groups were not different. On the other hand, the high salt content of the DSF diet (8.6–10.3%) resulted in a salty taste that caused a reduced feed intake. The fluctuation of the growth curve of rats fed DSF, particularly during the first 6 days of the experiment, indicates that the rats still needed some time to adapt to the salty taste of the diet. Greger and Tseng (1993) also found that rats fed supplemental chloride or sodium had significantly reduced weights compared with rats fed the basal level of cloride or sodium.

The protein quality based on growth and nitrogen balance assays is shown in Table 4. As compared with the casein or fresh fish group, only rats in the DSF II group had significantly lower values of FCE, PER, and NPR. With regard to BV and NPU, no significant difference was seen between the fresh fish and DSF groups. Even though the DSF groups had lower values as compared with casein, their values of digestibility, BV, and NPU were still more than 90%. Therefore, it can be concluded from the rat bioassay that the high salt content of DSF has no measurable effect on the protein quality of DSF. This result is in agreement with other studies that suggest salting and drying *per se* have only a small effect on the nutritional value of fish protein (Cutting, 1962). Although strong salt solutions can denature proteins, this denaturation alone does not appear to be harmful to the nutritional value of the DSF. The present study shows that the strong salt solution treatment only caused the loss of water and salt-soluble proteins in the DSF, and this effect appears to have little nutritional significance.

Results of our study indicate that a 3 month storage period of DSF had no effect on its protein nutritional

Table 5. Effect of Fresh and Dried-Salted Fish on Weight of Internal Organs of Rats

internal organ[a]	lactic casein	fresh fish	DSF I	DSF II
heart	0.36	0.38	0.46[b]	0.46[c]
	(0.02)	(0.04)	(0.04)	(0.03)
lung	0.47	0.47	0.51	0.54
	(0.09)	(0.06)	(0.06)	(0.08)
stomach	0.52	0.52	0.71[c]	0.72[c]
	(0.05)	(0.05)	(0.06)	(0.06)
small intestine	1.97	2.14	3.35[c]	3.69[c]
	(0.21)	(0.21)	(0.20)	(0.21)
colon	0.30	0.24	0.52[c]	0.51[c]
	(0.05)	(0.06)	(0.09)	(0.09)
liver	3.20	3.49	4.12[c]	4.39[c]
	(0.17)	(0.20)	(0.31)	(0.29)
kidney	0.82	0.87	1.24[c]	1.39[c]
	(0.04)	(0.06)	(0.06)	(0.14)
pancreas	0.28	0.26	0.32	0.38[b]
	(0.06)	(0.09)	(0.08)	(0.09)[b]
spleen	0.30	0.30	0.30	0.30
	(0.04)	(0.06)	(0.04)	(0.04)
testis	0.93	1.01	1.24[c]	1.32[c]
	(0.15)	(0.09)	(0.10)	(0.09)
cecum	0.75	1.14[b]	2.04[b]	1.46[b]
	(0.18)	(0.21)	(0.68)	(0.51)

[a] Gram of internal organs per 100 g of body weight. [b] Statistically significant ($p < 0.01$) against casein group. [c] Statistically significant ($p < 0.001$) against casein group.

quality. During the salting process the high salt concentrations around the fish resulted in the osmotic transfer of water out of the fish and of salt into the fish. The removal of water appears to limit bacterial growth and enzyme activity, thus preserving the fish (Wheaton and Lawson, 1985). Because of its low moisture content (33%) and high salt content (23% wet basis), the DSF can have a shelf-life expectancy of more than 6 months, particularly if good packaging technique was used. This condition is viewed as very desirable in geographical areas such as Indonesia, where food distribution requires transportation over long distances.

Salt purity is very important in the salt curing of fish since purity strongly influences the physical character and color of the cured fish. FAO (1981) suggested that salt used for brining should meet the following requirements: contain at least 96% NaCl (dry weight basis), not more than 6% water, and less than 10 ppm of iron and 1 ppm of copper. The NaCl used in the present study was obtained from Wako Chemicals and contains 99.5% NaCl and 2 ppm of iron. In Indonesia, however, some traditional processors reuse the brines for salting up to 20–30 times, which can result in high levels of microbial and insect contamination, especially during the wet season. This contamination usually leads to fly infiltration during the fish processing, and low-quality DSF is produced (Buckle et al., 1988). The quality deteriorates even further if DSF is stored in a poor condition.

Effect of DSF on Internal Organ and Serum Biochemical Values of Rats. The effect of DSF on the internal organs of rats is shown in Table 5. No significant differences in the weights of the internal organs occurred between rats fed casein and fresh fish, except for the cecum. Rats fed DSF products, on the other hand, experienced hypertrophy in almost all of the internal organs. As can be seen in Table 5, cardiac hypertrophy was induced by the source of protein with a high salt content, since the feeding of DSF resulted in significantly ($p < 0.01$) larger hearts than observed in either the casein or fresh fish fed animals. It is also seen that rats fed DSF had significantly ($p < 0.001$) larger kidneys (g/100 g of body weight) than either the

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Table 6. Liver Composition of Rats Fed Casein and Fresh and Dried-Salted Fish for 26 Days[a]

treatment	liver (g)	g/liver			
		moisture	ash	fat	protein
casein	9.2	6.54	0.12	0.63	1.97
	(0.6)	(0.49)	(0.01)	(0.14)	(0.07)
fresh fish	9.9	7.14	0.13	0.63	2.06
	(0.7)	(0.26)	(0.01)	(0.12)	(0.11)
DSF I	9.0	6.60	0.12	0.44	1.82
	(1.0)	(1.00)	(0.02)	(0.06)	(0.31)
DSF II	8.9	6.36	0.12	0.52	1.76
	(0.7)	(0.58)	(0.02)	(0.09)	(0.19)

[a] Values are means of eight replicates, with SD in parentheses.

Table 7. Effect of Diets on Serum Biochemical Values of Rats Fed for 26 Days[a]

component in serum	casein	fresh fish	DSF I	DSF II
total cholesterol	61.71	47.62	50.29	57.50
(mg/dL)	(16.64)	(6.54)	(7.13)	(10.73)
HDL cholesterol	44.00	40.37	41.50	48.87
(mg/dL)	(2.82)	(6.48)	(7.59)	(8.41)
triglyceride (mg/dL)	59.87	71.37	27.37[c]	37.62[b]
	(13.41)	(14.67)	(8.08)	(11.51)
total lipid (mg/dL)	247.57	246.87	200.75[b]	240.75
	(35.72)	(22.30)	(28.90)	(29.33)
glucose (mg/dL)	135.87	143.87	140.12	147.62
	(19.67)	(22.76)	(13.78)	(10.61)
BUN (mg/dL)	13.37	13.62	18.37[b]	21.62[c]
	(1.85)	(2.61)	(3.92)	(2.87)
creatinine (mg/dL)	0.30	0.26	0.22[b]	0.31
	(0.00)	(0.05)	(0.04)	(0.03)
creatine (mg/dL)	2.25	3.56	3.75[b]	4.49[b]
	(1.43)	(1.01)	(0.31)	(0.59)
total protein (g/dL)	5.78	6.05[b]	5.75	5.89
	(0.28)	(0.23)	(0.15)	(0.19)
albumin/globulin	0.71	0.72	0.83[c]	0.79[b]
	(0.04)	(0.03)	(0.07)	(0.04)
albumin (g/dL)	2.39	2.52[b]	2.60[b]	2.60[b]
	(0.14)	(0.10)	(0.11)	(0.11)
GOT (IU/L)	104.17	102.86	106.40	161.80[b]
	(17.96)	(20.55)	(17.11)	(40.89)
GPT (IU/L)	23.43	25.00	44.25[c]	55.40[b]
	(5.74)	(4.75)	(9.78)	(23.17)

[a] Values are means of eight replicates, with SD in parentheses. [b] Statistically significant ($p < 0.01$) against casein group. [c] Statistically significant ($p < 0.001$) against casein group.

casein or fresh fish group. Since it had been proved from the rat bioassay that the DSF quality, even though after 3 months of storage, remained unchanged, the hypertrophy of internal organs of rats fed DSF seems to not be caused by the protein itself. It may be caused by the high content of NaCl in DSF sample, as was noted in studies on rats fed supplemental sodium or chloride that resulted in induced kidney hypertrophy (Kaup et al., 1991; Greger et al., 1993; Greger and Tseng, 1993).

The chemical composition of the rats liver after 26 days of feeding period is shown in Table 6. No significant difference of moisture, ash, fat, and protein contents occurred among the groups. Although the contents of NaCl in the DSF diets were significantly higher than in the other groups, the ash content of liver was the same for all groups. This indicates that there was no accumulation of NaCl in the liver; therefore, the sodium must have been excreted through the urine (see also Table 10).

The effects of DSF on serum biochemical values of rats are shown in Table 7. While the biochemical values such as total and HDL cholesterol, glucose, and protein remained unchanged, creatine and the ratio of albumin to globulin (A/G) were significantly increased on rats

Table 8. Effects of Diets on Rat Mean Blood Pressure after Feeding by 20% Level of Protein for 26 Days

treatment	mean blood pressure (mmHg)	treatment	mean blood pressure (mmHg)
lactic casein	136.83 ± 7.80	DSF I	181.07 ± 0.81[a]
fresh fish	137.93 ± 5.42	DSF II	180.39 ± 3.79[a]

[a] Statistically significant ($p < 0.001$) against casein group.

Table 9. Mineral Contents of Kidney of Rats Fed Various Diets for 26 Days

mineral (mg/100 g db)	treatment group			
	casein	fresh fish	DSF I	DSF II
Ca	533.70	415.80	436.12	434.44
Mg	147.89	105.41	104.74	113.54
K	1263.58	1027.27	986.91	998.10
Na	728.37	573.59	574.72	552.02
P	1542.25	1338.09	1338.15	1398.57
S	1100.60	954.11	986.45	1064.37
Mn	4.06	0.85	0.68	0.53
Fe	134.34	31.19	26.30	27.03
Zn	15.02	10.06	9.11	9.80
Cu	3.97	3.24	3.22	3.44
Al	7.74	3.21	3.07	4.02

fed DSF. On the other hand, triglyceride and total lipid were significantly reduced in rats fed DSF.

Serum GOT and GPT were also significantly increased in rats fed DSF compared to the casein group. The increase in SGOT and SGPT and the enlargement of the liver may imply physiological damage to the liver of rats fed DSF. Further experiments relating to this finding are necessary. Blood urea nitrogen (BUN), a measure of kidney function, was also elevated among rats fed DSF as compared with rats fed casein or fresh fish. This can be understood since it correlates with the hypertrophy of the kidney seen in the DSF groups.

Blood Pressure. The blood pressure of rats fed casein, fresh fish, and dried-salted fish is shown in Table 8. It was observed that rats fed DSF had a blood pressure 45 mmHg higher than did those in the control groups. This result was not surprising, because it is well-known that ingestion of excess NaCl causes hypertension. The association between dietary salt (NaCl) and blood pressure has been assumed to be related primarily to the sodium content of salt (MacGregor, 1985; Tobian, 1991). Some investigators, however, noted that the hypertensive effect of sodium chloride is dependent on the concomitant presence of both cloride and sodium ions (Koletsky et al., 1981; Whitescarver et al., 1986). Greger and Tseng (1993) observed that ingestion of excess chloride appears to induce hypertension in rats through its impact on kidney function. They also conclude that dietary chloride has a more rapid and greater effect on blood pressure than dietary sodium.

We must be careful, however, when we extrapolate this result to humans, because people usually soak the DSF in tap water prior to cooking. This simple method is very effective in reducing the salt content of DSF. During the present study it was shown that overnight soaking of DSF in tap water reduced the NaCl content from 23 to 8%. Thus, protein intake can be increased by use of water-soaked DSF with less concern about producing hypertension. Salt-sensitive individuals, however, would still be at risk of hypertension by its consumption.

Mineral Composition of Kidney and Urine of Rats. The mineral composition of the kidney is shown in Table 9. There was no difference in the kidney mineral composition between rats fed DSF I and II, but

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Table 10. Mineral Excretion in Urine of Rats Fed Various Diets for 26 Days

mineral (mg/day)	treatment group			
	casein	fresh fish	DSF I	DSF II
Ca	7.56	11.82	68.66[b]	64.12[a]
Mg	0.63	0.67	6.25[a]	5.06[a]
K	35.43	49.86	13.34[a]	12.49[a]
Na	11.00	10.12	322.11[b]	286.25[b]
P	19.04	16.02	4.21[a]	4.76[a]
S	9.56	9.57	9.70	9.09
Mn	0.004	0.003	0.006[a]	0.05[a]
Fe	0.02	0.03	0.48[b]	0.48[b]
Zn	0.03	0.04	0.26[a]	0.24[a]
Cu	0.03	ND	0.28[b]	0.09
Al	0.08	0.06	0.77[b]	0.65[b]

[a] Statistically significant ($p < 0.01$) against casein group.
[b] Statistically significant ($p < 0.001$) against casein group.

when compared to the casein group, the kidney's mineral content for rats fed DSF was lower, because some of the minerals were lost through the excretion of urine.

The excretion of minerals through the urine (milligrams per day) is shown in Table 10. It is observed that most of the minerals (Ca, Mg, Na, Mn, Fe, Zn, Cu, and Al) are excreted at significantly higher rates in the urine of rats fed DSF than in the other groups, but the excretion of K and P of rats fed DSF was significantly lower than in the casein group. Kaup et al. (1990), Greger et al. (1991), and Greger and Tseng (1993) also found that rats fed supplemental chloride excreted more Ca and Mg in their urine.

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LITERATURE CITED

Allain, C. C.; Poon, L. S.; Chan, C. S. G.; Richmond,W.; Fu, P. C. Enzymatic determination of total serum cholesterol. *Clin. Chem.* **1974**, *20* (4), 470–475.

Allen, J. K.; Hensley, W. J.; Nicholls, A. V.; Whitfield, J. B. An enzymic and centrifugal method for estimating high-density lipoprotein cholesterol. *Clin. Chem.* **1979**, *25* (2), 325–327.

AOAC. Official Methods of Analysis of the Association of Official Analytical Chemists; AOAC: Washington, DC, 1990.

Bender, A. E.; Doell, B. H. Note on the determination of net protein utilization by carcass analysis. *Br. J. Nutr.* **1957**, *11*, 140–148.

Buckle, K. A.; Souness, R. A.; Putro, S.; Wuttijumnong, P. Studies on the stability of dried salted fish. In *Food Preservation by Moisture Control*; Seow, C. C., Ed.; Elsevier Applied Science: London, 1988.

Cook, J. G. H. Creatinine assay in the presence of protein. *Clin. Chim. Acta* **1971**, *32*, 485–486.

Cutting, C. L. The influence of drying, salting and smoking on the nutritive value of fish. In *Fish in Nutrition*; Heen, E., Kreuzer, R., Eds.; Fishing News (Books): London, 1962; pp 161–179.

Doe, P. E.; Heruwati, E. S. Drying and storage of tropical fish: a model for the prediction of microbial spoilage. In *Food Preservation by Moisture Control*; Seow, C. C., Ed.; Elsevier Applied Science: London, 1988.

FAO. The prevention of losses in cured fish; FAO Fish Technology Paper 219; FAO: Rome, 1981.

Folch, J. M.; Lees, M.; Stanley, G. H. S. A simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497–509.

Greger, J. L.; Murphy, K.; MacNeil, G.; Huttner, O.; Tseng, E. Hypertension in rats: interactions among chloride, sodium and calcium. *J. Agric. Food Chem.* **1993**, *41*, 1292–1296.

Greger, J. L.; Tseng, E. Longitudinal changes during the development of hypertension in rats fed excess chloride and sodium. *Proc. Soc. Exp. Biol. Med.* **1993**, *203*, 377–385.

Horn, D. B.; Squire, C. R. The estimation of ammonia using the indophenol blue reaction. *Clin. Chim. Acta* **1966**, *14*, 185–194.

Kaup, S. M.; Greger, J. L.: Marcus, M. S. K.; Lewis, N. M. Blood pressure, fluid compartments and utilization of chloride in rats fed various chloride diets. *J. Nutr.* **1991**, *121*, 330–337.

Koletsky, R. J.; Dluhy, R. G.; Cheron, R. G.; Williams, G. H. Dietary chloride modifies renin release in normal humans. *Am. J. Physiol.* **1981**, *241*, F361–F363.

MacGregor, G. A. Sodium is more important than calcium in essential hypertension. *Hypertension* **1985**, *7*, 628–637.

Matsumiya, K. Triglyceride. *Med. Technol.* **1983**, *12*, 1201–1206.

Pellet, P. L.; Young, V. R. *Nutritional Evaluation of Protein Foods*; The United Nations University: Tokyo, 1980.

Pigott, G. M.; Tucker, B. W. *Seafood, Effect of Technology on Nutrition*; Dekker: New York, 1991.

Richterich, R. Clinical Chemistry: Theory and Practice; Academic Press: New York, 1969; pp 317–319, 426–428.

Souness, R. A. Reducing post harvest losses associated with dried fish production in Indonesia. *INOFISH Int.* **1988**, *6*, 38–40.

Spinelli, J.; Dossow, J. A. Fish Proteins: Their Modification and Potential Uses in the Food Industry. In *Chemistry and Biochemistry of Marine Products*; Martin, R. E., Flick, G. J., Hebard, C. E., Ward, D. R., Eds.; AVI Publishing: Westport, CT, 1982.

Sugiyama, K.; Iwami, K; Ibuki, F. Nutritive effect of alternate daily ingestion of high-and non-protein diets in growing rats. *Agric. Biol. Chem.* **1991**, *55* (11), 2777–2783.

Suzuki, T. *Fish and Krill Protein: Processing Technology*; Applied Science Publisher: London, 1981.

Syukur, M.; Sunaryanto; Manurung, V. T.; Retnowati, N.; Windarti, H.; Suparno; Fesan, B. Dried fish consumptionin Indonesia and its prospect. *Abstracts of Papers,* 4th Asian Food Conference; Indonesian Institute of Sciences (LIPI) and Indonesian Association of Food Technologists (PATPI): Jakarta, 1992.

Tobian, L. Salt and hypertension: Lessions from animal models that relate to human hypertension. *Hypertension* **1991**, *17*, 152–158.

Wheaton, F. W.; Lawson, T. B. *Processing Aquatic Food Products*; Wiley: New York, 1985.

Whitescarver, S. H.; Holtzclaw, B. J.; Downs, J. H.; Ott, C. E.; Sowers, J. R.; Kotchen, T. A. Effect of dietary chloride on salt-sensitive and renin-dependent hypertension. *Hypertension* **1986**, *8*, 56–61.

Wootton, M.; Ismail, N. The influence of Processing conditions on the properties of dried Nemadactylus macropterus fillets. *J. Sci. Food Agric.* **1986**, *37*; 399–408.