ニジマスの亜鉛およびマンガン利用に及ぼす大豆油粕エクストルーダー処理の影響

誌名	水産増殖 = The aquiculture
ISSN	03714217
著者	佐藤, 秀一 秋元, 淳志 Porn-Ngam, N.
巻/号	45巻2号
掲載ページ	p. 275-284
発行年月	1997年6月

(水産増殖 45巻2号 275−284
SUISANZOSHOKU (1997-H 9))

Effect of Substitution of White Fish Meal with Extruded Soybean Meal in Diets on Zinc and Manganese Availability to Rainbow Trout*1

Shuichi SATOH*2, Nantiga PORN-NGAM*2, Atsushi AKIMOTO*3,
Toshio TAKEUCHI*2, and Takeshi WATANABE*2

*2 *Department of Aquatic Biosciences, Tokyo University of Fisheries, Konan, Minato, Tokyo 108, Japan*
*3 *Research Station, Nippon Formula Feed MFG.Co.Ltd, Yaizu, Shizuoka 425, Japan*

Abstract

A series of feeding experiments was conducted to determine the effect of extruded defatted soybean mean (SBM) on mineral availability to rainbow trout. SBM supplemented with graded levels of Zn and Mn, and were fed to rainbow trout juvenile. The growth and Zn and Mn contents of fish decreased with increased content of 100 ℃ extruded SBM in diet. These effects were reversed by supplementation with Zn and Mn at the levels of 80 and 40 μg/g, respectively. Growth equivalent or better than that on fish meal was obtained by feeding the diet containing 145 ℃ extruded SBM at 30 %.

The results of this study demonstrated that high temperature extrusion strongly influences the quality of SBM, and that SBM extruded at around 145 ℃ might be a very suitable material for substitution of fish meal in terms of Zn and Mn utilization.

It has been reported that the phosphorus (P) portion of tri-calcium phosphate in white fish meal (WFM) mainly inhibits bioavailability of zinc (Zn) in fish meal diets which containing P at levels of two or three times higher than P requirement.[1-4] Furthermore, improved Zn utilization was achieved by reducing the dietary P content by deboning the WFM to reduce the proportion of hard tissue contained in the meal. Alternative method to reduce dietary P content is the partial replacement of WFM with adequate plant protein sources containing lower amount of P. In this regard, soybean meal (SBM) was chosen as the substitutional ingredient due to its suitability in terms of protein content, cost, availability. Watanabe *et al.*[5-8] reported that SBM has been successfully used in diets for rainbow trout and yellowtail. However, it is reported that SBM contained nutritional inhibitors such as tripsin inhibitor and phytic acid which reduces Zn utilization. On the other hand, recently extrusion cooking has been applied in many areas not only for processing of human food, but also fish feed. The extrusion cooking easily processes any feed ingredient with high temperature and high pressure. For instance, tripsin inhibitor in SBM is effectively inactivated and raw starch is gelatinized easily by extrusion.

Thus, the experiment was conducted to determine the effect of reduction of P content by partial replacement of fish meal with extruded SBM on mineral

Received : September 2, 1996
Key words : Rainbow trout, Zinc, Manganese, Extrusion, Soybean
*1 Mineral nutrition in Fish-XXXIII.

availability in rainbow trout feed. In Experiment I, effect of graded levels of extruded SBM substitution for white fish meal was examined, and effect of extrusion condition such as temperature on Zn and Mn bioavailability was determined in experiment II.

Materials and Methods

Conditions of Soybean Meal Extrusion

SBM used in this experiment was an ordinary defatted feed ingredient. SBM was processed by extrusion cooking (Bühler twin-screw extruder DNDG-62) at the research station of Nippon Formula Feed MFG. Co. Ltd. The condition of extrusion is shown is Table 1. In Experiments I and II, SBM was extruded in ordinary condition (100℃ ; EX-SBM) for fish feed, and was additionally cooked in a higher barrel temperature (145℃ , HT-EX-SBM) in order to decrease phytic acid content in SBM in Experiment II. Proximate and mineral composition of SBM is shown in Table 2. Proximate composition was not changed by extrusion in comparison with the original SBM, however gelatinized starch content was increased by extrusion in any condition. Phytic acid content in EX-SBM was not lowered by the cooking at 100℃ , but almost 20% of phytic acid in HT-EX-SBM was degraded by 145℃ extrusion. The mineral composition of SBM was not influenced by any extrusion conditions, showing much lower content of P and higher content of manganese (Mn) than white fish meal.

Table 1. Conditions of extrusion cooking for soybean meal

	EX-SBM	HT-EX-SBM
Feed rate (kg/h)	80	80
Water rate (kg/h)	27	27
Steam rate (kg/h)	0	10
Barrel temperature (℃)	100	145
Pressure (Bars)	65	40
Die diameter (fm/mxl)	4	4

Experimental Diets

Nine experimental diets were formulated to contain graded levels of lower temperature extruded SBM (EX-SBM) as a partial replacement of WFM in Experiment I (Table 3). Diets 1-3 were formulated as control groups, and contained 55% WFM as the sole protein source. WFM was reduced to 43% in diets 4-6, and 30% in diets 7-9 on inclusion of 15% and 30% EX-SBM, respectively. The mineral mixture used was the Ogino salt mixture which satisfies the requirements of rainbow trout at a level of 5% in the diet.[9] Zn was deleted from the mineral mixture in order to adjust Zn content of the experimental diets. Incremental levels of Zn (0, 20, and 40 μg/g diet) were added to the diets at each EX-SBM substitution level. The amounts of α-starch added to the diets were adjusted to give isocaloric diets. Seven diets were formulated to be isonitrogenous and isocaloric in Experiment II (Table 4). Diet 10 was arranged as a control diet with 52% WFM as the sole protein source. The WFM used in Experiment II was different from that of Experiment I, contaning lower ash. Diets 11-16 contained 30% WFM and nonextruded SBM was added to diets

Table 2. Proximate and mineral compositions of white fish meal and soybean meal

		WFM		SBM	EX-SBM	HT-EX-SBM
		Expt. I	Expt. II			
Moisture	(%)	9.1	8.7	8.4	5.7	6.8
Protein	(%)	63.2	69.6	44.5	46.4	47.1
Lipid	(%)	9.3	9.5	3.0	2.2	2.7
Starch	(%)			18.2	21.1	20.4
(α-Starch)				12.4	18.1	18.6
Ash	(%)	18.4	14.0	6.1	6.3	6.2
Phytic acid	(%)			1.2	1.3	1.0
Ca	(mg/g)	66.7	39.2	2.9	3.0	2.8
P	(mg/g)	40.7	25.6	6.4	6.4	6.6
Na	(mg/g)	8.4	8.2	3.9	4.2	4.2
K	(mg/g)	4.0	5.2	12.2	12.2	12.7
Zn	(mg/g)	81.5	60.8	43.9	46.4	45.9
Mn	(mg/g)	3.6	4.0	39.7	38.9	37.6

Table 3. Formulation and proximate compostion of the diets for Experiment I (%)

Ingredient	Diet no.								
	1	2	3	4	5	6	7	8	9
White fish meal	55	55	55	43	43	43	30	30	30
EX-SBM*[1]	0	0	0	15	15	15	30	30	30
α-Starch	20	20	20	19.2	19.2	19.2	16.4	16.4	16.4
Dextrin	5	4.9	4.8	5	4.9	4.8	5	4.9	4.8
Lipid*[2]	10	10	10	10.7	10.7	10.7	11.5	11.5	11.5
Vitamin mixture	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin E (50 %)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Zn-free mineral mix.*[3]	5	5	5	5	5	5	5	5	5
Zn premix.*[4]	0	0.1	0.2	0	0.1	0.2	0	0.1	0.2
Cellulose	2.9	2.9	2.9	0	0	0	0	0	0
Proximate composition (%)									
Moisture	4.1	4.3	4.1	4.3	4.3	4.4	4.2	4.2	4.5
Crude protein	33.2	33.5	33.4	33.3	33.4	33.4	32.9	33.0	33.0
Crude lipid	14.5	14.4	14.7	14.5	14.3	14.5	14.6	14.5	14.5
Crude ash	13.1	13.0	13.1	12.0	12.2	12.1	11.1	11.1	11.0

*[1] Extruded soybean meal.
*[2] Soybean oil: pollock liver oil = 3:2.
*[3] Zinc free Ogino salt mixture.
*[4] 20 mg Zn/g (880 mg $ZnSO_4 \cdot 7H_2O$/9.12 g Dextrin).

Table 4. Formulation and proximate composition of the experimental diets for Experiment II (%)

Ingredient	Diet no.						
	10	11	12	13	14	15	16
White fish meal	52	30	30	30	30	30	30
Soybean meal*[1]	0	33	33	0	0	0	0
EX-SBM*[2]	0	0	0	30	30	0	0
HT-EX-SBM*[3]	0	0	0	0	0	30	30
α-Starch	18	13.8	13.8	12	12	12	12
Dextrin	5	5	4.6	5	4.6	5	4.6
Lipid*[4]	10	11.1	11.1	11.2	11.2	11.2	11.2
Vitamin mixture	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin E (50 %)	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Mineral mixtrue*[5]	5	5	5	5	5	5	5
Zn premix.*[6]	0	0	0.2	0	0.2	0	0.2
Mn premix.*[7]	0	0	0.2	0	0.2	0	0.2
Cellulose	7.9	0	0	4.7	4.7	4.7	4.7
Proximate composition (%)							
Moisture	4.1	4.8	5.1	4.2	3.6	4.7	4.5
Crude protein	36.9	37.7	37.2	36.6	36.5	36.1	36.8
Crude lipid	15.0	14.4	14.5	14.2	14.5	14.6	14.5
Crude ash	11.2	10.1	9.9	9.6	9.8	9.5	9.6

*[1] Defatted soybean meal (SBM).
*[2] Extruded soybean meal by the same condition as Experiment I.
*[3] Extruded soybean meal in high temperature.
*[4] Soybean oil: pollock liver oil = 3 : 2.
*[5] Complete Ogino salt mixture.
*[6] 20 mg Zn/g (880 mg $ZnSo_4 \cdot 7H_2O$/9.12 g Dextrin).
*[7] 10 mg Mn/g (275 mg dried $MnSO_4$/9.725 g Dextrin).

11 and 12 at a level of 33 %, EX-SBM was added to diets 13 and 14 at 30 %, HT-EX-SBM was also added to diets 15 and 16. A complete mineral mix was added to all diets providing Zn and Mn at 40 and 20 μg/g, respectively. Additional Zn and Mn were supplemented to diets 12, 14, and 16 at levels 40 and 20 μg/g, respectively. The amount of α-starch in the formulated diets was adjusted to keep the total dietary α-starch level constant.

The mineral compositions of the experimental diets used in Experiments I and II are given in Tables 5 and 6, respectively. The amounts of Ca and P in the WFM diets (diets 1-3) were approximately 39 and 30 mg/g, respectively. As elevation of EX-SBM inclusion, the contents of Ca and P decreased to be about 32 and 28 mg/g, respectively (diets 4-6), and about 24 and 21 mg/g, respectively (diets 7-9). Zn content in the diets without supplemental Zn (diets 1, 4, and 7) was approximately 40 μg/g, originating from WFM and/or EX-SBM. The Zn content in other diets was increased by an amount approximately equal to the supplemental levels. The Mn content of the diets with WFM as the sole protein source was approximately 20 μg/g. The replacement of WFM with EX-SBM resulted in slight increase in the Mn content of the diets to about 25 μg/g by 15 % EX-SBM and 30 μg/g by 30 % EX-SBM. The control diet in Experiment II (diet 10) contained lower Ca and P than Experiment I due to usage of the different lot of WFM which contained lower Ca and P. Zn content of diet 10, 11, 13, and 15 was approximately 73-75 μg/g and was increased in other diets in accordance with Zn supplementation levels. The control diet (diet 10) contained 20 μg Mn/g and the diets with any types of SBM increased the content to about 29 μg/g in diets 11, 13, 15 and addition of Mn to diets 12, 14, 16 elevated to about 44 μg/g.

Table 5. Mineral composition of the test diets for Experiment I

Diet no.	EX-SBM in diet (%)	Added Zn (μg/g)	Ca (mg/g)	P (mg/g)	Mg (mg/g)	Na (mg/g)	K (mg/g)	Fe (μg/g)	Zn (μg/g)	Mn (μg/g)
1	0	0	38.6	30.2	2.0	6.7	7.5	353	44.8	20.9
2	0	20	39.2	31.1	2.1	6.8	7.3	345	61.8	20.8
3	0	40	38.5	30.6	2.1	6.8	7.0	350	83.0	20.2
4	15	0	31.7	27.9	2.4	5.9	9.0	352	40.9	24.3
5	15	20	32.0	28.0	2.4	6.0	9.1	348	65.7	24.6
6	15	40	32.4	28.3	2.4	5.8	9.0	340	80.3	23.5
7	30	0	24.5	22.6	2.5	4.9	9.5	340	41.2	28.1
8	30	20	24.2	21.5	2.5	5.0	9.8	345	59.5	31.2
9	30	40	23.8	21.6	2.5	4.8	9.6	348	79.6	30.1

Table 6. Mineral compositions of the test diets for Experiment II

Diet no.	Type of diet*	Supplemental		Ca (mg/g)	P (mg/g)	Mg (mg/g)	Na (mg/g)	K (mg/g)	Fe (μg/g)	Zn (μg/g)	Mn (μg/g)
		Zn (μg/g)	Mn (μg/g)								
10	WFM	40	20	24.2	25.0	2.1	6.2	7.7	340	74.2	20.8
11	SBM	40	20	15.2	20.6	2.6	5.0	9.2	327	75.0	30.8
12	SBM	80	40	15.3	20.0	2.6	5.3	9.4	333	110.2	44.3
13	EX-SBM	40	20	15.4	20.8	2.5	5.0	8.9	335	73.4	29.7
14	EX-SBM	80	40	14.9	20.4	2.5	5.1	9.0	332	109.7	43.9
15	HT-EX-SBM	40	20	15.2	19.9	2.5	5.1	8.4	330	72.8	27.9
16	HT-EX-SBM	80	40	15.2	20.0	2.5	5.2	9.0	338	109.1	43.4

* WFM, white fish meal based diet; SBM, the diets containing defatted soybean meal; EX-SBM, the diets containing low temperature extruded soybean meal; HT-EX-SBM, the diets containing high temperature extruded soybean meal.

Feeding and Chemical analyses

Fingerling rainbow trout *Oncorhynchus mykiss* were used as the experimental animals. Eyed eggs were obtained from the Okutama Branch of Tokyo Fisheries Experimental Station and hatched at Laboratory of Fish Nutrition, Tokyo University of Fisheries. The fry were kept on a commercial rainbow trout diet for two months. They were divided into 9 lots of 35 (1.2 g on average), and 7 lots of 30 (1.6 g on average) fish each, respectively in Experiments I and II. Then they were fed the diets shown in Tables 3 and 4 for 18 and 21 weeks, respectively, at water temperature of 14 to 20 ℃. At the termination of feeding experiment, five fish from each tank were randomly selected and pooled for whole body mineral analysis, and seven fish for vertebral mineral analyses. Phytic acid content was determined by the method of Thompson and Erdman[10]. Preparations of analytical samples and analytical conditions for minerals in the test diets, whole body, and vertebrae were the same as those described previously[4].

Data were analyzed using an analysis of variance to determine significant ($p<0.05$) differences among treatment means. Duncan's multiple range test was used to evaluate[11].

Results and Discussion

Experiment I

The results of feeding in Expriment I were shown in Table 7. Growth was lowest in fish fed diets containing any levels of EX-SBM without Zn supplement. Elevation of supplemental Zn from 0 to 20 μg/g significantly improved the growth at each level of EX-SBM substitution, however further increase to 40 μg Zn/g did not produce any significant difference in growth when the EX-SBM level was either 15 or 30 %. Feed efficiency showed a same trend as the growth, namely inclusion of EX-SBM and unsupplementation with Zn decreased feed efficiency, however supplementation of Zn at levels of more than 20 μg/g improved it of any inclusion levels of EX-SBM. Cataracts were induced by all diets without Zn supplementation, occurring in 35, 50, and 42 % of the fish for EX-SBM levels of 0, 15, and 30 %, respectively. Supplementation of 20 μg Zn/g was sufficient to avoid the occurrence of cataracts except fish fed 30 % EX-SBM which exhibited 25 % of cataracts. Dwarfism was observed in the fish fed all diets without supplemental Zn, the fish fed the diet without EX-SBM showing a much greater incidence than either the 15 or 30 % EX-SBM groups. Increasing Zn supplementation to 20 μg/g reduced dwarfism to 7 % in the 0 % EX-SBM group and dwarfism was completely eliminated in the 15 % EX-SBM group, while further Zn supplementation was necessary to avoid the occurrence of dwarfism in the 0 % EX-SBM group. In the fish fed the 30 % EX-SBM diets, dwarfism was not avoided by Zn supplementation at the levels of even 40 μg/g.

Mineral composition of vertebrae is shown in Table 8. Both P and Ca contents showed almost no difference amongst dietary treatments although the 30 % EX-SBM diet without supplemental Zn resulted in the lowest P

Table 7. Effect of substitution of white fish meal with extruded soybean meal on growth, feed efficiency, and the occurrence of cataracts in Experiment I

Diet no.	EX-SBM in diet (%)	Zn added (μg/g)	Av.body wt.(g)		SGR (%)	Feed efficiency*1	Cataracts (%)	Dwarfism (%)
			Initial	Final				
1	0	0	1.2	25.1 bc*2	2.41	1.02	35	26
2	0	20	1.2	32.2 d	2.61	1.14	0	7
3	0	40	1.2	45.1 e	2.88	1.13	0	0
4	15	0	1.2	20.7 b	2.26	0.89	50	5
5	15	20	1.2	34.9 d	2.67	1.15	0	0
6	15	40	1.2	35.6 d	2.69	1.02	0	0
7	30	0	1.2	8.6 a	1.56	0.82	42	4
8	30	20	1.2	23.9 bc	2.37	0.90	25	8
9	30	40	1.2	26.8 c	2.47	0.91	0	4

*1 g growth/g feed.

*2 Values within a column followed by the same superscript letter are not significantly different ($P>0.05$).

and Ca contents. Vertebral Zn content was increased by the addition of supplemental Zn at all EX-SBM levels. The 30 % EX-SBM diet showed lower Zn content than other diets at any given Zn supplementation levels. There was little difference in vertebral Mn content between fish fed the 0 and 15 % EX-SBM diets. However, the Mn content was lowered in fish fed the 30 % EX-SBM diets at all Zn supplementation levels. There was no influence of dietary treatment on vertebral Mg, Na, and K contents.

Mineral contents of the whole body are shown in Table 9. Ca content showed relatively high values in the 0 % EX-SBM diet groups, and was increased slightly by Zn supplementation. In general, P content was not affected by dietary treatment although there was a slight increase in P with increasing Zn supplementation for the 0 and 30 % EX-SBM diets. Zn content showed results similar to those of the vertebrae. Namely, Zn in whole body was increased by the Zn supplement to all EX-SBM levels. The 30 % EX-SBM diets produced lower Zn contents than others. Mn contents were almost similar to those of Zn, however the Mn content was lowered by the inclusion of EX-SBM more severely than Zn content.

In Experiment I, dietary P content was decreased by the substitution of WFM with EX-SBM and the ratio of Ca to P seemed to be optimum for rainbow trout to utilize Zn in a high P diet. However, inclusion of EX-SBM depressed the growth and lowered not only Zn but also Mn content in whole body. Phytic acid was one of the inhibitors to Zn utilization. Phytic acid content of EX-SBM used in Experiment I was almost same amount as to that of the original defatted SBM, and was not reduced by the extrusion condition employed in Experiment I. Watanabe *et al.*[5-8] reported that soybean meal has been successfully used in diets for yellowtail and rainbow trout and that no influence was observed in protein utilization. Thus, the result of

Table 8. Effect of substitution of white fish meal with extruded soybean meal on the mineral composition of vertebrae in Experiment I (dry basis)

Diet no.	EX-SBM in diet (%)	Added Zn (μg/g)	Ca (mg/g)	P (mg/g)	Mg (mg/g)	Na (mg/g)	K (mg/g)	Zn (μg/g)	Mn (μg/g)
1	0	0	171.2	112.0	3.8	2.5	3.5	47.2	16.7
2	0	20	170.1	110.1	3.6	2.5	3.5	71.2	19.4
3	0	40	174.7	113.7	3.8	2.5	3.6	110.7	22.1
4	15	0	167.5	105.2	3.7	2.0	3.0	31.5	13.5
5	15	20	170.9	108.1	3.6	2.4	2.9	80.0	20.2
6	15	40	171.2	111.6	3.8	2.3	3.2	92.4	20.6
7	30	0	165.2	94.3	3.5	1.8	2.8	28.6	6.4
8	30	20	171.1	104.4	3.7	2.1	3.3	42.8	11.6
9	30	40	170.7	109.2	3.7	2.1	2.8	69.9	13.5

Table 9. Effect of substitution of white fish meal with extruded soybean meal on the mineral composition of whole body in Experiment I (wet basis)

Diet no.	EX-SBM in diet (%)	Added Zn (μg/g)	Ca (mg/g)	P (mg/g)	Mg (mg/g)	Na (mg/g)	K (mg/g)	Zn (μg/g)	Mn (μg/g)
1	0	0	6.0	4.0	0.3	0.9	3.0	6.8	0.7
2	0	20	6.3	3.9	0.3	1.0	2.9	8.4	0.8
3	0	40	6.5	4.5	0.3	1.0	2.9	10.6	1.0
4	15	0	5.5	4.0	0.3	1.1	2.8	4.6	0.2
5	15	20	5.2	4.1	0.3	1.1	2.8	8.6	0.6
6	15	40	5.4	4.3	0.3	1.2	2.9	10.1	0.9
7	30	0	5.0	3.6	0.2	1.1	2.7	3.5	0.1
8	30	20	5.2	4.0	0.2	1.0	2.8	5.5	0.3
9	30	40	5.5	4.5	0.2	1.2	3.0	8.4	0.6

Experiment I may suggest that phytic acid or another component in soybean meal inhibits Zn and Mn utilization.

Experiment II

The results of feeding in Experiment II are shown in Table 10. Substitution of WFM with the orginal SBM with or without additional Zn and Mn supplements significantly lowered the growth compared to the fish fed the control diet (diet 10) containing WFM as a sole protein source. The diet containing EX-SBM without additional supplemental Zn and Mn (diet 11) also produced a growth significantly lower than that on the control diet (diet 10) in a manner similar to Experiment I, the growth being not significantly different from those receiving the SBM diets. However, the double amount supplement of Zn and Mn to the EX-SBM diet significantly improved the growth to a level comparable to that on the control diet. The use of HT-EX-SBM (diet 15) resulted in growth better than the control (diet 10). Supplementation of double amount of Zn and Mn to the HT-EX-SBM diet slightly improved growth. SGR in Experiment II showed a similar a similar number between the treatments which produced good growth, especially in the control fish (diets 1 and 10) in Experiment I and II (2.8-2.9). There were only some minor differences in feed efficiency. The diets which significantly reduced growth also induced eye lens cataracts with the highest incidence in the fish fed the SBM diets without additional Zn or Mn.

Mineral composition of vertebrae is shown in Table 11. Partial replacement of white fish meal with SBM or EX-SBM reduced the vertebral Zn content, a large reduction of 44 % being given by the SBM diet. The vertebral Zn content of the fish fed HT-EX-SBM diets was slightly higher than that on the control diet (diet 10). The double supplementation with Zn and Mn to diets resulted in about two fold increase in the vertebral Zn content of the fish fed SBM and EX-SBM diets.

Table 10. Effect of substitution of white fish meal with various soybean meals on the growth, feed efficiency, and the occurrence of cataracts in Experiment II

Diet no.	Type of diet*	Supplemental		Av. body wt. (g)		SGR (%)	Feed efficiency*	Cataracts (%)
		Zn (μg/g)	Mn (μg/g)	Initial	Final			
10	WFM	40	20	1.6	79.7[b]*	2.66	1.04	0
11	SBM	40	20	1.6	60.6[a]	2.47	0.96	8.5
12	SBM	80	40	1.6	66.3[a]	2.53	1.00	0
13	EX-SBM	40	20	1.6	63.9[a]	2.51	0.98	2.9
14	EX-SBM	80	40	1.6	78.6[b]	2.65	0.99	0
15	HT-EX-SBM	40	20	1.6	83.4[b]	2.69	1.01	0
16	HT-EX-SBM	80	40	1.6	85.8[b]	2.71	1.06	0

* See the footnote of Tables 6 and 7.

Table 11. Effect of substitution of white fish meal with various soybean meals on the mineral composition of vertebrae (dry basis)

Diet no.	Type of diet*	Supplemental		Ca (mg/g)	P (mg/g)	Mg (mg/g)	Na (mg/g)	K (mg/g)	Zn (μg/g)	Mn (μg/g)
		Zn (μg/g)	Mn (μg/g)							
10	WFM	40	20	166.0	94.5	2.8	2.6	3.1	79.5	11.0
11	SBM	40	20	160.5	92.9	2.9	2.6	3.0	44.3	4.6
12	SBM	80	40	157.4	91.8	2.8	2.3	2.9	98.9	13.7
13	EX-SBM	40	20	163.4	90.4	3.2	2.5	3.1	58.0	6.4
14	EX-SBM	80	40	171.7	86.0	2.9	2.6	2.9	110.5	13.6
15	HT-EX-SBM	40	20	159.6	87.6	3.0	2.4	3.0	87.2	13.6
16	HT-EX-SBM	80	40	170.7	88.5	3.0	2.5	3.0	110.5	14.2

* See the footnote of Table 6.

but in only a small increase in the HT-EX-SBM group. Mn content in vertebrae showed a same tendency as Zn content.

Mineral composition of whole body is shown in Table 12. The whole body mineral contents showed a similar trend as those of vertebrae. Namely, the whole body Zn content in the fish fed SBM or EX-SBM diet (diet 11 or 13) was markedly lower, and that on HT-EX-SBM diet (diet 15) was higher than that on the control fish (diet 10). The fish fed SBM or EX-SBM diet (diet 12 or 14) showed an increase in whole body Zn content when the diets were supplemented with double amount Zn and Mn, whereas there was little change in the fish fed HT-EX-SBM diet (diet 16) with additional Zn and Mn. The whole body Mn content of fish receiving the SBM diet (diet 11) was markedly lower that any other group, whereas it was highest in the fish fed HT-EX-SBM diet (diet 16). A double amount supplementation of Zn and Mn to diets containing SBM or EX-SBM resulted in distinct increases in whole body Mn content to levels markedly higher than that of the control group (diet 10).

In Experiment II, the diet containing EX-SBM also produced low growth in a manner similar to Experiment I, but not significantly different from that on the SBM diet. However, the addition of Zn and Mn to the EX-SBM diet improved the growth significantly to a level comparable to that the WFM diet. This finding may suggest that additional Zn and Mn supplementations to a EX-SBM diet are necessary to achieve comparable growth to that on the WFM diet.

The dietary phytic acid content calculated from the content of phytic acid of the raw ingredients was about 0.4 % in both SBM and EX-SBM inclusion diets and less than 0.3 % in the diet containing HT-EX-SBM. It is suggested that these levels were not high enough to have an inhibitory effect of phytic acid, and that a higher temperature during the extrusion processing of SBM might resulted in inactivation of other nutritional factors[12-15] apart from the gelatinization of starch since the fish fed the HT-EX-SBM diet exhibited better growth performance.

The reduction in vertebral Zn by diets with higher content of phytic acid coincided with the findings of Satoh *et al.*[16] and Zhou *et al.*[17] The latter workers found a similar response in rats for which reduction of phytate in soybean flour significantly increased bone Zn level and they further indicated that phytic acid is the primary inhibitory factor in soybean products that results in reduced Zn availability in rats.

In current experiment, the protein source in both Experiments I and II were partially replaced by treated soybean meals at the level of 40 % in the protein derived from white fish meal. P content in both Experiments I and II was successfully decreased from 30 or 25 mg/g to 20 mg/g due to the partial substitution of white fish meal with soy bean meals, the ratio of Ca to P in the experimental diets seemed to be ideal for rainbow trout to have a high Zn availability in a high P diet according to the results of previous study with semi-purified diet[2]. However, the formulation with the original SBM or SBM extruded at 100 ℃ resulted in poor growth and lower Zn and Mn content in vertebrae. These diets contained 0.4 % phytic acid. Spinelli *et al.*[18] reported that phytic acid *per se* did not induced

Table 12. Effect of substitution of white fish meal with various soybean meals on the mineral composition of whole body (wet basis)

Diet no.	Type of diet*	Supplemental Zn (μg/g)	Supplemental Mn (μg/g)	Ca (mg/g)	P (mg/g)	Mg (mg/g)	Na (mg/g)	K (mg/g)	Zn (μg/g)	Mn (μg/g)
10	WFM	40	20	4.5	3.6	0.2	1.0	3.0	11.5	0.7
11	SBM	40	20	5.4	4.5	0.3	1.0	3.0	9.0	0.3
12	SBM	80	40	5.7	4.7	0.3	1.0	3.1	14.1	1.0
13	EX-SBM	40	20	5.4	4.5	0.3	1.0	3.1	9.1	0.6
14	EX-SBM	80	40	4.5	3.9	0.2	1.0	3.1	13.8	1.2
15	HT-EX-SBM	40	20	4.6	4.1	0.3	1.0	3.0	13.3	1.1
16	HT-EX-SBM	80	40	5.1	4.3	0.3	1.1	3.0	15.6	1.3

* See the footnote of Table 6.

poor growth of rainbow trout, however suggested that phytic acid might influence Zn availability. Richardson *et al.*[19] reported that phytic acid together with calcium phosphate induces poor growth and inhibit Zn availability to chinook salmon. The experimental diets in which WFM was partially substituted with SBM in the current study contained both phytic acid and tricalcium phosphate at levels of 0.4 % and 4 %, respectively. Thus, judging from the results of both Richardson *et al.*[19] and the current study, it seemed that combination of phytic acid and tricalcium phosphate might induce severe inhibitory effect. It is necessary to conduct further study to clarify combining influence of phytic acid and tricalcium phosphate.

The results of this study have demonstrated that phytic acid content in ingredient is very important when fish meal is substituted with plant protein sources, and that high temperature extrusion cooking influences the quality of plant protein source very efficiently, and that the soybean meal extruded at around 150 ℃ might be very a suitable material for substitution of fish meal in term of Zn and Mn utilization.

Acknowledgements

We express here our sincere thanks to the Okutama Branch to Tokyo Fisheries Experimental Station, which kindly provided eyed eggs of rainbow trout.

This study was supported inpart by a Grant-in-Aid for Scientific Research of the Ministry of Education, Science, Culture, and Sport.

References

1) Satoh, S., N. Porn-Ngam, T. Takeuchi, and T. Watanabe (1993): Effect of various types of phosphates on zinc availability to rainbow trout. *Nippon Suisan Gakkaishi*, 59, 1395-1400.

2) Porn-Ngam, N., S. Satoh, T. Takeuchi, and T. Watanabe (1993): Effect of the ratio of phosphorus to calcium on zinc availability to rainbow trout in high phosphorus diet. *Nippon Suisan Gakkaishi*, 59, 2065-2070.

3) Satoh, S., N. Porn-Ngam, T. Takeuchi, and T. Watanabe (1996): Effect of excess amount of dietary phosphorus on growth and mineral availability to rainbow trout. *Fisheries Sci.*, 62, 483-487.

4) Satoh, S., T. Sasaki, N. Porn-Ngam, R. Ishida, T. Takeuchi, and T. Watanabe (1997): Effect of deboning of white fish meal on the zinc availability to rainbow trout. *Suisanzoshoku*, 45(2), 267-273.

5) Watanabe, T., V. Viyakarn, H. Kimura, K. Ogawa, N. Okamoto, and N. Iso (1992): Utilization of soybean meal as a protein source in a newly developed soft-dry pellet for yellowtail. *Nippon Suisan Gakkaishi*, 58, 1761-1773.

6) Pongmaneerat, J. and T. Watanabe (1992): Utilization of soybean meal as protein source in diets for rainbow trout. *Nippon Suisan Gakkaishi*, 58, 1983-1990.

7) Viyakarn, V., T. Watanabe, H. Aoki, H. Tsuda, H. Sakamoto, N. Okamoto, N. Iso, S. Satoh, and T. Takeuchi (1992): Use of soybean meal as a substitute for fish meal in a newly developed soft-dry pellet for yellowtail. *Nippon Suisan Gakkaishi*, 58, 1991-2000.

8) Pongmaneerat, J. and T. Watanabe (1993): Nutritional evaluation of soybean meal for rainbow trout and carp. *Nippon Suisan Gakkaishi*, 59, 157-163.

9) Ogino, C., L. Takeuchi, H. Takeda, and T. Watanabe (1979): Availability of dietary phosphorus in carp and rainbow trout. *Nippon Suisan Gakkaishi*, 45, 1527-1532.

10) Thompson, D. B. and J. W. Erdman Jr. (1982): Phytic acid determination in soybeans. *J. Food Science*, 47, 513-517.

11) Duncan, D. B. (1955): Multiple range and multiple *F* tests. *Biometrics*, 11, 1-42.

12) Bjorck, I. and N. G. Asp (1983): The effects of extrusion cooking on nutritional value: A literature review. *J. Food Eng.*, 2, 281-308.

13) Kiang, M. J. (1989): Extrusion technology for the aquaculture industry. In: Proceedings of the People's Republic of China Aquaculture and Feed Workshop (ed by D. M. Akiyama), Sept. 17-30, pp 310-315.

14) Phillips, R. D. (1989): Effects of extrusion cooking on the nutritional quality of plant proteins. In: Protein Quality and the Effects of Processing (ed by R. D. Phillips and J. Finley). Marcel Dekker, Inc., New York, U.S.A., pp. 219-246.

15) Carmire, M. E. (1991): Protein functionality modification by extrusion cooking. *J. Am. Oil Chem. Soc.*, 68, 200-205.

16) Satoh, S., W. E. Poe, and R. P. Wilson (1989): Effect of supplemetal phytate and/or tricalcium phosphate on weight gain, feed efficiency and zinc content in vertebrae of channel catfish. *Aquaculture*, 80, 155-161.

17) Zhou, J. R., E. J. Fordyce, V. Raboy, D. B. Dickinson, M.-S. Wong, R. A. Burns, and J. W. Erdman (1992): Reduction of phytic acid in soybean products improves zinc bioavailability in rats. *J. Nutr.*, 122, 2466-2473.

18) Spinelli, J., C. R. Houle, and J. C. Wekell (1983): The effect of phytates on the growth of rainbow trout (Salmo gairdneri) fed purified diets containing varying quatities of calcium and magnesium. *Aquaculture*, 30, 71-83.

19) Richardson, N. L., D. A. Higgs, R. M. Beames, and J. R. McBride (1985): Influence of dietary calcium, phosphorus, zinc and sodium phytate level on cataract incidence, growth and histopathology in juvenile chinook salmon (*Oncorhynchus tshawytscha*). *J. Nutr.*, 115, 553-567.

ニジマスの亜鉛およびマンガン利用に及ぼす大豆油粕エクストルーダー処理の影響

佐藤秀一[1]・ナンティガ ポンナム[1]・秋元淳志[2]・
竹内俊郎[1]・渡邉　武[1]
([1]東京水産大学資源育成学科, [2]日本配合飼料㈱水産開発センター)

ニジマスの亜鉛（Zn）およびマンガン（Mn）利用に及ぼすエクストルーダー処理大豆油粕の影響を調べる目的で，大豆油粕を100℃あるいは145℃でエクストルーダー処理し，北洋魚粉と代替し，Zn および Mn の添加量を調整した試験飼料を作製し，ニジマス稚魚を飼育した。その結果，100℃で処理した大豆粕で魚粉を代替すると，その代替率が高くなるに従い，成長が劣り，魚体中の Zn および Mn 含量が低くなった。その飼料に Zn および Mn を80および40 μg/g それぞれ添加すると魚粉飼料に匹敵する成績が得られた。一方，145℃でエクストルーダー処理した大豆油粕を30％配合すると，魚粉飼料と同等以上の飼育成績が得られた。