Effect of Extrusion Processing on the Utilization of Soybean Meal Diets for Rainbow Trout^{*1}

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The effect of extrusion processing by a twin screw extruder on the nutrient utilization, and the availability of defatted soybean meal (SBM) as partial protein source were studied with rainbow trout of about 4-5 g. In Expt. I, fish were fed the control and 30% SBM diets with and without extrusion processing for 6 weeks. In Expt. II, fish were fed ordinary steamed dry pellets (DP) containing 30% SBM or 30% extruded SBM (ExSBM) as a partial substitute for fish meal, and extruded soft-dry pellets (SDP) with 30% SBM for 8 weeks.

Expt. I: There was no marked difference in growth or feed efficiency between the control and 30% SBM diets. Their dietary values and growth rate were not significantly improved by the extrusion, although the digestibility of starch was markedly elevated. The digestibility of dietary protein was not influenced by extrusion processing, ranging between 88 to 91%. Expt. II: Of the 30% SBM diets, SDP with a higher energy content produced higher growth, PER, protein, and energy retention than DP. Feed consumption was slightly lower in SDP with the higher energy level, leading to improved feed efficiency. Replacement of 30% SBM with the same level of ExSBM did not result in any nutritional improvement of DP.

Many researchers have tried to replace the expensive fish meal component of fish feed with other less expensive plant proteins. Soybean meal as a protein supplement has been used widely in fish feed¹⁻⁶⁾ due to its nutritional quality, lower cost, and market availability. However, the results show great variation in the degree of success for the replacement of fish meal depending on the species of fish, the size of fish, and the types of ingredients used. Our previous study⁷⁾ demonstrated that there was no marked difference in protein quality between defatted soybean meal (SBM) and its extruded meal (ExSBM), and that feed performances were reduced inversely to dietary SBM levels due to reduced protein and energy levels. The elevation of protein and energy levels of SBM diets using extruded soft-dry pellets (SDP) effectively improved the growth and feed performances of rainbow trout.^{*4} Extrusion has become the major processing method

for food and feed production. It has been used to commercially produce other foods, including animal feed, snack foods, and various dairy and meat products. However, this technology has recently been exploited for the production of aquaculture feeds. The effects of extrusion processing on nutritional value have been a major concern. The high temperature and pressure of this process could cause the gelatinization of starch and inactivate protease inhibitors and other antinutritional factors.⁸⁻¹¹⁾ Thus, extrusion can enhance the digestibility of protein through denaturation and by inactivation of protease inhibitors, but the improvement is generally less than 5%.¹⁰⁾ On the other hand, extrusion processes are known to favor the Millard reaction.¹¹⁾ This reaction reduces protein quality by lowering both digestibility and availability of the amino acids involved. However, this amino acid damaging reaction is most likely to occur in

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high carbohydrate foods where protein quality is of secondary importance. Extrusion may have both beneficial and undesirable effects on the nutritional value and physical properties of the feed. Therefore, the effect of extrusion processing on the nutritional values of soybean meal as a substitute for fish meal should be clarified by comparing the utilization efficiency of this protein between steam and extruded pellets. Two experiments were arranged for rainbow trout to investigate: in Experiment I, the effect of extrusion processing on the utilization of soybean meal as a partial replacement for fish meal in the ordinary diet; and in Experiment II, the availability of the SBM or ExSBM as a partial substitution for fish meal in steam dry pellets (DP) in comparison with extruded soft-dry pellets (SDP) with SBM.

Materials and Methods

Experimental Procedure

Two feeding experiments were conducted with fingerling rainbow trout *Oncorhynchus mykiss*. The fish were acclimated in aquaria for 2 weeks using a commercial rainbow trout diet. At the end of acclimation period, the fish were starved for 24 h and weighed individually, after which they were fed with the experimental diets.

At the beginning of each experiment, fifty fish were first collected for whole body analysis. The pooled samples of fifty fish in each experiment were analyzed for crude protein, crude lipid, moisture, and crude ash using the same methods as described previously.¹²⁾

At the end of each experiment, fish were starved for 24 h before individual weighing (final body weight). Ten fish were collected from each of two aquaria from each dietary treatment for whole body analysis. The fish from each aquarium were pooled and analyzed for body composition by the methods described for the initial fish samples.

All the experimental diets were analyzed for crude protein, crude lipid, gross energy, moisture, and crude ash contents using the methods described above.¹²⁾

Feeds and Feeding Conditions

All the experimental diets used in this study were kindly prepared and pelleted by Sakamoto Fish Feed Company into two types of feed, steam dry pellets (DP) and extruded soft-dry pellets (SDP).

The SDP were prepared using atwin screw extruder (Buhler Ltd.) by the same preparation methods as described elsewhere.¹²⁾

Experiment I: two kinds of extruded pellets containing 0% SBM (control) and 30% SBM, and a feed ingredient mixture of these two diets (without extrusion pelleting process) were tested. For digestibility determination, these two experimental pellets and their feed ingredient mixtures were supplemented with 0.5% chromic oxide and repelleted by being forced through a garlic press, cut to suitable size for fish, and later dried in a vacuum dryer machine at 20°C for 24 h at Tokyo University of Fisheries. The diets in which the fish meal was not substituted were taken as control diets, containing 55% fish meal and 10% krill meal as protein sources. The fish meal portion in the control diets of both extruded and non-extruded types was substituted with SBM at a level of 30%. The diet composition and chemical analysis are shown in Table 1. The extrusion conditions of diets used in this experiment are summarized as follows: screw speed (rpm), 260; feed rate (kg/h), 1650; water injection (kg/h), 330; material temperature (°C), 75; material press (kg/cm²), 52; cutter speed (rpm), 900; no. of cutter, 2; die plate (ϕ : mm), 13; no. of holes, 12.

Fish with an average weight of about 5.2 g were divided into 8 groups of 20 fish each in 45 l aquaria, with a continuous water supply at a rate of 0.6–1.0 l/min at $14 \pm 1^\circ\text{C}$. Fish in duplicate aquaria were fed with one of the varieties of dietary preparations. They were fed three times daily to satiation for 6 weeks.

In this experiment, the apparent digestibility of crude protein, crude lipid, crude starch, and gross energy was determined by the indirect method using chromic oxide as an inert indicator.¹²⁾ After the last meal, the experimental aquaria were cleaned and feces were collected for digestibility determination. The pooled samples of feces from each aquarium were analyzed for crude protein, crude lipid, crude starch, gross energy, and chromic oxide.¹²⁾

Experiment II: the dietary value of three ordinary dry pellets containing 0 and 30% SBM, or 30% ExSBM, and extruded pellets with 30% SBM were evaluated on the basis of growth and feed performance. The 0% SBM (control) diet was formulated to contain 54% fish meal and 10% krill meal as protein sources. The diets containing 30% SBM were prepared by replacing the

Table 1. Ingredient and nutrient compositions of the experimental diets used in Experiments I and II

SBM in diet (%)	Experiment I				Experiment II			
	0	0	30	30	0	30	30* ¹	30
Ingredient (%)	Non-Ext.* ²	Ext.* ³	Non-Ext.	Ext.	DP* ⁴	DP	DP	SDP* ⁵
Fish meal* ⁶	55	55	25	25	54	21	21	25
Krill meal	10	10	10	10	10	10	10	10
Soybean meal	—	—	30	30	—	30	30	30
Wheat flour	8	8	8	8	19	19	19	7
Potato starch	3	3	3	3	—	—	—	3
Wheat gluten	3	3	3	3	—	—	—	3
Mineral mix.* ⁷	3	3	3	3	2	2	2	2
Vitamin mix.* ⁷	3	3	3	3	2	2	2	2
Feed oil* ⁸	15	15	15	15	11	14	14	18
Binder	—	—	—	—	2	2	2	—
<i>Nutrient content (% on dry matter basis)</i>								
Moisture	6.7	6.6	6.6	6.7	7.6	7.7	6.8	7.9
Crude protein	48.7	48.5	40.9	42.3	50.0	43.5	42.8	45.8
Crude lipid	25.7	21.7	23.2	25.6	20.2	20.2	20.2	23.1
Crude ash	13.0	13.6	11.4	11.9	11.7	8.9	8.9	9.0
Crude starch	11.4	12.6	17.5	18.2	16.0	21.0	16.9	24.1
GE (kcal/100 g)	584.9	550.2	559.7	576.4	543.3	541.7	538.6	560.2
DE (kcal/100 g)	491.9	491.0	441.3	465.6	—	—	—	—
CP/GE	83.2	88.1	73.1	73.4	92.0	80.3	79.4	81.7
DCP/DE	89.1	90.1	83.2	80.2	—	—	—	—

*¹ Extruded SBM.*² Non-extruded diet.*³ Extruded diet.*⁴ Ordinary steam dry pellet.*⁵ Extruded soft-dry pellet.*⁶ Local sardine meal.*⁷ See Watanabe *et al.*¹³⁾*⁸ Mixture of sardine oil: pollack liver oil (1:1).

same level of fish meal in the control diet. The extrusion conditions of diet used in this experiment are summarized as follows: screw speed (rpm), 260; feed rate (kg/h), 1650; water injection (kg/h), 330; material temperature (°C), 62; material press (kg/cm²), 55; cutter speed (rpm), 900; no. of cutter, 2; die plate (ϕ : mm), 13; no. of holes, 12. The extrusion conditions of SBM (ExSBM) have been given elsewhere.⁷⁾ The compositions of experimental diets and nutrient contents are shown in Table 1. The pellets were broken into pieces of suitable size for the experimental fish.

Fish with an initial weight of about 5.0 g were stocked in 45 l aquaria with 25 fish each. Duplicate tanks were randomly assigned to each experimental diet, which was distributed to satiation twice daily for a period of 8 weeks. The water temperature and flow rate were kept the same as in Experiment I.

Statistical Methods

Statistical analysis of the experimental results was carried out using analysis of variance (ANOVA). Duncan's multiple range test was used to compare the individual treatment mean differences at the 0.05 significance level.

Results and Discussion

Growth and Feed Performance

Experiment I: the effect of extrusion processing on growth response and feed performance is presented in Table 2. Although a slight reduction in growth and feed efficiency was obtained for diets containing 30% SBM, there was no significant difference between the dietary treatments for these parameters ($P > 0.05$). Growth and feed efficiency were not significantly influenced by the extrusion processing, although the absolute feed efficiency values were slightly

Table 2. Effect of extrusion processing and partial replacing fish meal with soybean meal on growth and feed performance in Experiments I and II

Dietary treatment		Av. body wt. (g)±SD		Weight gain (%)	Feed efficiency (gain/feed)	Feeding rate (%)
		Initial	Final			
<i>Experiment I:</i>						
0% SBM	: Non-extruded	5.3±0.4	22.3±3.7	321.3 ^{a*}	1.12 ^{ab}	2.74
	: Extruded	5.3±0.3	22.3±3.2	321.6 ^a	1.25 ^b	2.47
30% SBM	: Non-extruded	5.2±0.4	20.6±3.1	298.2 ^a	1.02 ^a	2.94
	: Extruded	5.2±0.4	21.5±3.0	312.5 ^a	1.08 ^{ab}	2.82
<i>Experiment II:</i>						
0% SBM	: Dry-pellet	5.1±0.3	43.1±7.2	753.8 ^x	1.22 ^x	2.46
30% SBM	: Dry-pellet	5.0±0.4	40.8±6.5	715.9 ^x	1.10 ^y	2.70
30% ExSBM	: Dry-pellet	5.1±0.4	40.5±6.5	697.4 ^x	1.06 ^y	2.76
30% SBM	: Extruded (SDP)	5.1±0.4	42.5±5.1	732.7 ^x	1.26 ^x	2.36

* Values within column with the same superscript are not significantly different ($P > 0.05$).

Table 3. Effect of extrusion processing of SBM and diets containing 30% SBM on PER and retention of protein and energy in rainbow trout

Dietary treatment	Daily intake/fish		PER	Retention (%)		
	Protein (mg)	Energy* ¹ (cal)		Protein	Energy	
<i>Experiment I:</i>						
0% SBM	: Non-extruded	184.3	1863	2.31 ^{a*2}	35.2 ^a	48.5 ^{a,b}
	: Extruded	165.4	1673	2.57 ^a	38.6 ^a	55.5 ^b
30% SBM	: Non-extruded	155.3	1673	2.48 ^a	37.5 ^a	43.2 ^a
	: Extruded	159.5	1756	2.55 ^a	39.0 ^a	45.4 ^{a,b}
<i>Experiment II:</i>						
0% SBM	: Dry-pellet	295.3	3208	2.43 ^x	38.8 ^x	52.9 ^x
30% SBM	: Dry-pellet	269.5	3354	2.51 ^x	39.0 ^x	48.0 ^y
30% ExSBM	: Dry-pellet	269.3	3386	2.49 ^x	38.6 ^x	47.7 ^y
30% SBM	: Extruded (SDP)	256.8	3143	2.75 ^y	42.4 ^y	55.9 ^x

*¹ Values are presented as digestible energy for Experiment I, and gross energy for Experiment II.

*² Values within column with the same superscript are not significantly different ($P > 0.05$).

better for the extruded diets than for the non-extruded ones. The final abody weight and percent gain of fish fed the 30% SBM diet with or without extrusion were comparable to those fed the control diets. These results indicate that the inclusion of 30% SBM in place of fish meal did not reduce growth and feed efficiency when the diet contained proper amounts of protein and energy. It was also confirmed that the markedly lower growth of rainbow trout fed the diet containing 30% SBM observed in our previous study⁷⁾ was mainly due to the lower digestible crude protein (DCP)/digestible energy (DE) intake. The DCP/DE of the control and 30% SBM diets in the former study were 88 and 77 mg/kcal; but

higher values of 89 and 83 mg/kcal were noted in this experiment (Table 1).

Regardless of the extrusion process, the diet with 30% SBM gave feed consumption as good as the control groups (Table 2). This result indicates that the diet containing 30% SBM was palatable to rainbow trout as the fish meal diet. The extruded diets showed a slight reduction in feeding rate compared to the non-extruded diet. This result was also supported by Hilton *et al.*¹⁴⁾ who reported that rainbow trout reared on the extruded pellets had a prolonged gastric emptying time, and that this reduced the total daily feed consumption.

The PER values varied from 2.31–2.57 (Table 3).

There was no significant difference between PER values of fish fed the control diets and those fed diets containing 30% SBM. The PER of the extruded diets was also not statistically elevated, although it was slightly higher in them.

The retention of protein and energy was estimated on the basis of the increment of body protein and protein intake, as well as the increment of body energy and gross energy intake, respectively. In spite of the fact that there was no significant difference ($P > 0.05$) between the extruded and non-extruded diets, the protein and energy retention showed a tendency to increase slightly in the extruded diets. The retention of protein and energy in the 30% SBM diet was also not significantly different from those of the control diet ($P > 0.05$). Consequently, protein retained in fish fed diets containing 30% SBM (37–39%) was comparable to those fed the control diets (35–38%), suggesting that SBM protein was efficiently utilized.

Experiment II: the growth performance, feed utilization, and feed consumption in the experimental fish are shown in Table 2. The results showed that there was no significant difference in weight gain between treatments. Nevertheless, the final body weight of fish fed DP with 30% of SBM or ExSBM was slightly lower than those fed SDP with 30% SBM, which was comparable to fish fed the control diet. The slightly higher final body weight and percent gain obtained in fish fed the control diet may be attributed to the higher dietary CP/GE ratio, leading to an increased protein intake. Of the 30% SBM diets, the SDP produced a higher growth and feed efficiency than DP, demonstrating that extruded pellets with higher energy levels improved feed utilization. The feed efficiency in fish fed SDP containing 30% SBM (1.26) was approximately equal to that of fish fed the control diet (1.22), and was significantly greater than those fed DP with 30% SBM (1.10) or ExSBM (1.06). Hilton *et al.*¹⁴⁾ stated that extruded pellets were observed to be more durable, had superior water stability, and absorbed more water than steam pellets. They also reported that extruded pellets were retained longer in the stomach of rainbow trout than steam pellets, and they suggested that this may have been responsible for the observed higher feed efficiency with the extruded pellet.

Feed consumption was reduced in 30% SBM-SDP with the highest energy level. It was pos-

tulated that the fish may have eaten to satisfy their energy requirement, as has been pointed out by Page and Andrews.¹⁵⁾ This was also supported by Hilton *et al.* who found that rainbow trout reared on extruded pellets had prolonged gastric emptying time, and that this reduced the total daily feed consumption of the fish. This also agreed with the results of the first experiment, that the feeding rate of fish fed extruded diets tended to decrease for both the control and the 30% SBM diets. Therefore, the lower feed consumption and excellent growth of fish fed 30% SBM-SDP indicated that feed and protein utilization was markedly improved in fish fed with this diet compared to those fed with DP with SBM or ExSBM. This result was also supported by the previous study⁴⁴ which concluded that increasing the SBM level up to 50% in SDP could give excellent growth and feed performance in rainbow trout.

PER values were generally high, ranging from 2.43 to 2.75 (Table 3). The best PER was observed in fish fed 30% SBM-SDP. As the growth rate of fish fed the control diet was not greatly different from fish fed 30% SBM-SDP, the lower PER of the control diet may be partly due to the effect of the higher dietary protein level. It has been reported that increasing the dietary protein level improved growth but caused a reduction in PER.^{12, 16, 17)}

The same tendency was observed for protein and energy retention. These values were estimated on the basis of the same method as in Experiment I. Protein and energy retention varied from 39–42% and 48–56%, respectively. Fish fed 30% SBM-SDP gave the best protein and energy retention. However, the protein retention of fish fed DP with 30% SBM or ExSBM was not markedly different from those fed the control diet, while the energy retention was slightly lower than the control group.

SBM was found to be unpalatable to some other fish, such as chinook salmon, causing growth reduction and high mortality.¹⁸⁾ However, this protein meal was acceptable to rainbow trout, as fish fed 30% SBM diets showed palatability as good as those fed fish meal diets. Tacon *et al.*¹⁹⁾ reported that commercial hexane extracted SBM could be used to replace fish meal for up to 50% (26% SBM in the diet) in the diet for rainbow trout, with no adverse effects on growth performance or feed utilization efficiency. The same authors also found that extrusion of that SBM did

not improve growth or feed utilization. Similar to the present study, growth or feed performance of fish fed DP with 30% ExSBM were not improved. These results were also supported by our previous result⁷⁾ that there was no marked difference in growth or feed efficiency between diets with SBM and ExSBM. Dabrowski *et al.*²⁰⁾ noted that the growth rate of rainbow trout was reduced significantly when 50% of fish meal was replaced by SBM (25% SBM in diet). They speculated that the availability of methionine in soybean meal was one of the major factors responsible for the reduction in growth performance.

Apparent Digestibility

The apparent digestibility of dietary nutrients determined in Experiment I is presented in Table 4. The apparent digestibility of dietary protein and lipid was not markedly different between the various dietary treatments. The protein digestibility was relatively high, ranging from 88 to 91%. The inclusion of 30% SBM did not cause any reduction in protein digestibility. Likewise, protein digestibility was not influenced by extrusion processing, while starch digestibility was enhanced. The starch digestibility of the non-extruded diets was less than 32%, while it improved to over 69% for the extruded diets. It has been found that the extrusion process caused an expansion of the gelatinization of starch granules with an immediate opening-up of molecular chains, leading to increased digestibility.⁹⁾ Although the starch digestibility of extruded diets was clearly improved, the growth and feed efficiency were not effectively elevated in fish fed extruded diets. This could be attributed to the low dietary starch content. This is also supported by a slight increment in energy digestibility. The diets containing 30% SBM gave a lower energy digesti-

bility than the control. This could be attributed to the effect of the undigested polysaccharides in SBM. It has been supported by previous studies^{21,22)} that the carbohydrates of soybean meal are largely in the form of higher polysaccharides which are expected to be undigestible.

Likewise, it has been found that soybean meal protein was well digested by rainbow trout.^{7,23)} Sandholm *et al.*²⁴⁾ reported a high protein digestibility of 80% in rainbow trout. Similarly, Grabner and Hofer²⁵⁾ found a digestibility of about 93% for both rainbow trout and carp. It is believed that nonheated soybean meal contains several anti-nutritional factors, such as trypsin inhibitors which have been shown to decrease protein digestibility and reduce growth in fish.^{26,27)} Trypsin inhibitors, which constitute approximately 6% of the total protein of soybeans, are known to increase the secretion of pancreatic enzymes into the intestine, which in turn causes hypertrophy of the pancreas and the loss of undigested pancreatic enzyme into the feces.²⁸⁾ Pancreatic enzymes such as trypsin and chymotrypsin are rich in sulfur amino acids. An excessive loss of these enzymes through the feces is believed to be responsible for growth depression due to a drain on the body tissue of sulfur-containing amino acids.²⁹⁾ Fortunately, these protease inhibitors are inactivated by adequate heat treatment. Consequently, the heating process received during commercial extraction of SBM (105°C for 10–20 minutes) used in this study might destroy most of these factors. Thus, excellent growth of the experimental fish in both experiments was observed, as well as high protein digestibility (more than 89%).

Whole Body Composition

The whole body composition of the initial and final fish in Experiments I and II is given in Table 5. The body protein, moisture and ash contents were influenced by the dietary treatments in both Experiments I and II. In general, a high body protein reflects good health and excellent utilization of the feed. All fish in both the experiments increased body lipid levels from the initial stage after 6 and 8 weeks of feeding, respectively. In Experiment II, the lipid content of fish fed 30% SBM-SDP was higher than those of fish fed with all other diets; this is reflected by dietary lipid and energy levels. The body lipid concentration of fish has been shown to increase with increasing levels of dietary lipid and energy.^{30–32)} Likewise, Hilton *et al.*¹⁴⁾ noted that

Table 4. Effect of extrusion processing on apparent digestibility of dietary protein, lipid, starch and energy (Experiment I)

Dietary treatment	Apparent digestibility (%)			
	Protein	Lipid	Starch	Energy
0% SBM diet:				
Non-extruded	90.1	95.9	26.6	84.1
Extruded	91.2	96.0	86.9	89.2
30% SBM diet:				
Non-extruded	89.7	92.3	32.7	78.9
Extruded	88.3	91.2	69.7	80.8

Table 5. Whole body composition of the experimental fish fed diets containing 0 and 30% SBM with or without extrusion and extruded soft-dry pellets (SDP)

Dietary treatment	Protein	Lipid	Moisture	Ash
<i>Experiment I:</i>				
Initial fish	15.16	5.71	76.45	2.64
0% SBM : Non-extruded	15.24	15.33	67.77	2.20
: Extruded	15.04	14.85	68.22	2.21
30% SBM : Non-extruded	15.13	14.07	68.62	2.33
: Extruded	15.29	14.46	68.38	2.27
<i>Experiment II:</i>				
Initial fish	15.52	5.17	76.69	2.64
0% SBM : Dry-pellet	15.92	15.46	66.77	2.08
30% SBM : Dry-pellet	15.55	15.40	67.50	2.02
30% ExSBM : Dry-pellet	15.56	15.41	67.68	2.06
30% SBM : Extruded (SDP)	15.47	16.17	66.86	2.10

there was no difference in the body lipid content of trout reared on extruded or steam pelleted diets which contained the same amount of energy.

The nutritional quality of SBM diets was not significantly improved by the extrusion process, although the digestibility of starch was markedly elevated. Moreover, the extrusion of SBM in DP could not improve growth or feed utilization, but SDP with high energy levels improved growth and feed performance. It has been concluded that the inclusion of 30% SBM in diet had no adverse effect on rainbow trout in terms of growth, feed efficiency, feeding activity, PER, and protein digestibility.

Thus, feed efficiency and protein utilization of SBM diets were found to be improved on preparation as high protein, high energy pellets.

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