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Availability of Genetically Modified Feed Ingredients for Rainbow Trout *Oncorhnchus mykiss*

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Abstract：The feeding studies were conducted to assemble the information on the availability of gene-modified (GM) feed ingredient for rainbow trout, using diet containing GM defatted soybean meal (SBM) as an alternative protein source to provide and ensure good protein accessibility and product safety. The utilization of genetically modified defatted soybean meal (GM SBM) as feed by rainbow trout was investigated, in comparison with non genetically modified defatted soybean meal (non-GM SBM). The nutrient utilization showed that there was no significant difference in growth and feed performance between GM and non-GM SBM groups in 12 week feeding experiment. However, the cauliflower mosaic virus 35S promoter fragment of the GM SBM was detected in the muscle of fish receiving GM SBM diet by Nested-PCR. Additionally, the promoter fragment vanished by the 5th day after changing the diet to non-GM diet. Subsequently, the study was carried out to examine the degradation and the possible carry over of foreign DNA fragment by means of measuring it from transgenic plant and host plant contained in GM or non-GM SBM and evaluate the safety for fish. These foreign DNA fragments were not completely degraded in stomach and intestine and might be taken up into organ via the garsrointestinal (GI) tract. However, foreign DNA was not detected after the withdrawal period. Judging from these findings, the novel feed ingredients derived from GM SBM could be considered as having equivalent nutritional quality and verifying the safety as feed ingredient.

Aquaculture is increasing as an important contributor to economic development and to the global food supply. Nearly one-third of the fish consumed by humans is the product of aquaculture, and that percentage will only increase as aquaculture expands and the world's conventional fish catch from the ocean and freshwater continues to decline because of overfishing and environmental damage (FAO, 2000; OECD, 2001). Consequently, the needs for commercial feeds for intensively cultivating fish are increasing.

Most prepared fish feeds use soybean meal as a good quality plant protein (Halver and Hardy, 2002). Since genetically modified (GM) soybean meal (SBM) has been developed, it might be used as a feed ingredient for prepared feeds. Thus, the consequences of changing fish diet formulations on final product safety and quality need to be investigated. There are two important issues considered in the safety assessment of GM crops used as fish feed ingredients. First is fish safety which is assessed through feeding studies to evaluate the equivalence of nutritional performance. The second is food safety that is determined by the digestibility of the transgenic protein and its incorporation within the fish (Brown *et al.,* 2003; Sanden *et al.,* 2004).

The present article reviews information on the usefulness of GM feed ingredients for rainbow trout through formulations combining GM SBM as an alternative protein source that provides good protein availability and product safety. A series of studies

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were conducted to assess various combinations of soy protein from GM SBM and non-genetically modified defatted soybean meal (non-GM SBM) as substitutes for fishmeal. Subsequently, a study was conducted to determine the effect of GM SBM diets as a means to possibly transfer foreign DNA from the GM SBM protein to fish.

Availability of genetically modified soybean meal in rainbow trout diets

The availability of SBM as a replacement for fishmeal has been practiced for many years. Feeding studies have shown that SBM is a good protein supplement for fishmeal and can be incorporated in diets for growing rainbow trout (Cho *et al.*, 1974; Pongmaneerat and Watanabe, 1993; Tacon *et al.*, 1983). Rainbow trout were able to grow at a similar rate with a fishmeal based diet replaced with 30% defatted soybean meal (Pongmaneerat and Watanabe, 1992; Refstie *et al.*, 2000). The effects of soybean meal inclusion in diets for rainbow trout showed that no differences were observed at up to 40–50% replacement (Refstie *et al.*, 2000).

Research has been conducting showing that soybean meal produced from GM soybeans is comparable in chemical composition to conventional soybean meal (Padgette *et al.*, 1996). Other feed ingredient studies showing the nutrition equivalency of glyphosate-tolerant and conventional maize have been conducted with dairy cattle (Folmer *et al.*, 2000), sheep (Donkin *et al.*, 2000), and poultry (Brake and Vlachos, 1998). One study examined the nutrition bioequivalence of soybean meal prepared from non-GM or GM soybeans on a short-term basis in several species (Hammond *et al.*, 1996), though nutrient utilization studies in various aquatic animals are insufficient.

Utilization of genetically modified feed ingredient

The results of feeding studies, as measured by growth, feed conversion and composition, showing the nutrition equivalency of herbicide-tolerant and conventional soybean meals have been reported for catfish (Hammond *et al.*, 1996) and Atlantic salmon (Sanden *et al.*, 2004). Moreover, GM soybean meal, at the 12% inclusion level, was as safe as conventional soybean meal, at least in terms of its effect on histological parameters in the Atlantic salmon intestinal tract (Sanden *et al.*, 2005) and on health (Hemre *et al.*, 2005). GM maize has been studied in Atlantic salmon (Sanden *et al.*, 2005), poultry (Aeschbacher *et al.*, 2005; Rossi *et al.*, 2005; Tony *et al.*, 2003b), and cattle (Erickson *et al.*, 2003). The results showed that there are no existing reports where significant differences between conventional and genetically modified feeds were found.

Consequently, Chainark *et al.* (2006) investigated the utilization of genetically modified defatted soybean meal (GM SBM) as feed for rainbow trout in comparison with non-genetically modified defatted soybean meal (non-GM SBM). Both meals were included at levels of around 15 and 30% in four diets (42% protein). The diets were fed to juvenile fish (48.3 g on average weight) for 12 weeks. Table 1 shows the results of the feeding experiment. There was no significant difference in growth and feed performance between the GM and non-GM SBM groups at either inclusion level at the end of 12th week. The cauliflower mosaic virus 35S promoter fragment (220 bp) of the GM SBM was detected in the muscle of fish receiving both levels of GM SBM diet by Nested-PCR, with the frequency of detection being greater at the higher inclusion level (Table 2 and Fig.1). Additionally, the promoter fragment was not detected by the 5th day after changing the diet to a non-GM ration. Conversely, the promoter fragment was not detected from fish fed with non-GM SBM formulations. The results demonstrated that the availability of protein in GM SBM was similar to that of non-GM SBM, and the promoter fragments which were found in the muscle of fish were not detectable after changing the diet to non-GM diet, verifying the availability of the GM SBM in rainbow trout feed.

Investigations of ingested foreign DNA in rainbow trout

A number of studies have now been conducted in which foreign DNA derived from GM feed ingredients has not been detected in food products derived from livestock receiving GM feed. Studies have been conducted on poultry (Ash *et al.*, 2000),

Table 1. Growth and feed performance in rainbow trout fed diets graded levels of non-GM and GM SBM for 12 weeks.

Diet	Body weight (g)		SGR*[1]	FGR*[2]	Protein Retention(%)*[3]	PER*[4]
	Initial	Final				
non-GM SBM 15%	48.2±0.5	143.6±0.3	1.30±0.01	0.99±0.01	39.5±0.1	2.37±0.01
non-GM SBM 30%	47.8±0.2	140.8±0.6	1.28±0.01	1.03±0.01	38.3±0.4	2.28±0.03
GM SBM 16%	48.7±0.8	143.5±1.3	1.29±0.01	0.98±0.01	39.8±0.1	2.39±0.01
GM SBM 31%	48.5±1.0	141.6±0.1	1.28±0.02	1.01±0.02	39.0±0.7	2.32±0.05

The values were not significantly different ($P<0.05$)

*[1] SGR (Specific Growth Rate) = {(Ln Final body weight (g) - Ln Initial body weight (g)) / Experimental period (days)} × 100

*[2] FGR (Feed Gain Ratio) = Feed consumption (g) / Weight gain (g)

*[3] Protein retention = {(Final body weight (g) × Protein (%) - Initial body weight (g) × protein (%)) / Feed consumption (g) × Protein (%)} × 100

*[4] PER (Protein Efficiency Ratio) = Weight gain (g) / Protein consumption (g)

Table 2. Detectable CaMV 35S promoter fragment of recombinant DNA (220 bp) by Nested-PCR in muscles of rainbow trout fed diets graded levels of non-GM and GM SBM for 15 weeks and after withdrawing GM SBM.

Sampling day (week) /Fish number sampled	Fed with Non-GM and GM SBM diets		Fed with Non-GM SBM diet		
	84 (12) / 20	105(15) / 5	2 / 5	5 / 5	8 / 5
non-GM SBM 15%	0	0	0	0	0
non-GM SBM 30%	0	0	0	0	0
GM SBM 16%	1	1	0	0	0
GM SBM 31%	4	2	1	0	0

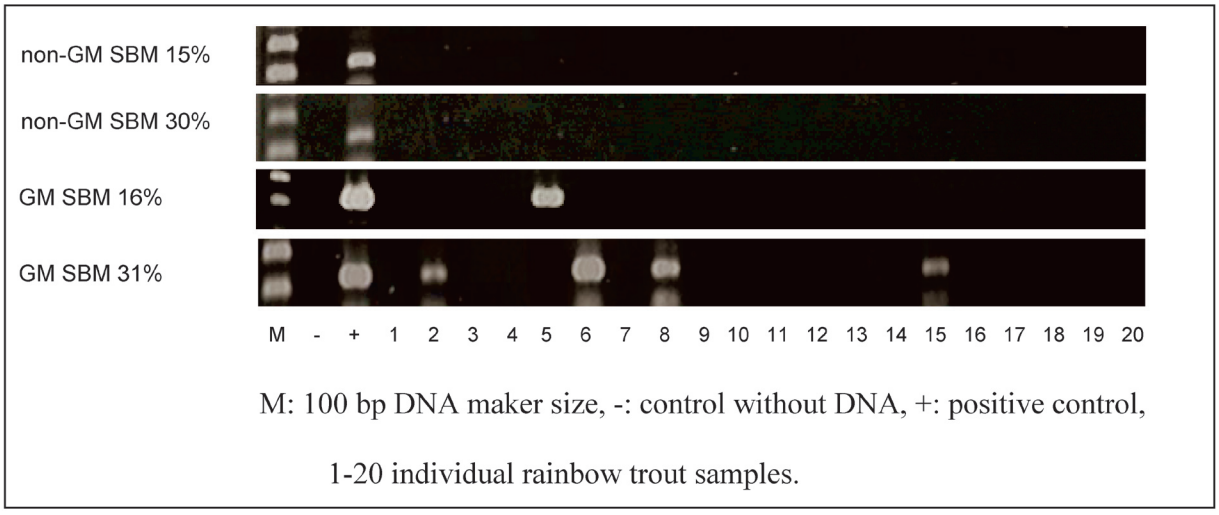

Fig. 1. Detectable CaMV 35S promoter fragment of recombinant DNA (220 bp in length) by Nested-PCR in muscles of rainbow trout fed graded levels of non-GM and GM SBM diets at the end of 12th week.

swine (Weber and Richert, 2001), and dairy cows (Phipps *et al.,* 2002). Interestingly, small fragments of plant DNA have been detected in various animal gastrointestinal tracts, e.g. the intestines of fish (Sanden *et al.*, 2004), in some animal tissues from fish (Nielsen *et al.*, 2006; Nielsen *et al.*, 2005), swine (Klotz *et al.*, 2002; Reuter and Aulrich., 2003) chickens (Chambers *et al.*, 2002; Einspanier *et al.*, 2001), cattle (Einspanier *et al.*, 2001), and in bovine saliva and rumen fluid (Duggan *et al.*, 2000). However, until now, few studies have been conducted on aquatic animals.

Chainark *et al.* (2008) reported degradation and the possible carryover of foreign DNA fragments by means of measuring it from transgenic plants and host plants contained in GM or non-GM SBM formulated diets and evaluated the safety for fish. For that study, the experimental diets were formulated with GM and non-GM SBM at a level of 30%. The two experimental diets, also included fishmeal to achieve a 42% protein level. Initially, 240 rainbow trout averaging 50.5 g were fed the non-GM SBM diet for two weeks. Thereafter, the fish were divided into two groups, each of which was fed one of the experimental for an additional two weeks, then sampled. Fish fed the GM diet were then given the non-GM diet and sampled 1st, 3rd, 5th and 7th day after being placed on that diet. The degradation of digesta in the gastrointestinal (GI) tract (stomach, anterior and posterior intestine) and possible transfer of foreign DNA into various organs (blood, head kidney, spleen, liver, muscle and brain) were examined. Foreign DNA fragments, such as CaMV 35S promoter (220 bp) and *Glycine max* chloroplast (257 bp) were traced by Nested-PCR and located by *in situ* hybridization (ISH). The chloroplast DNA fragment was amplified in non-GM and GM SBM diets, but promoter DNA fragment was detected only in the GM SBM diet, indicating that cross contamination of the non-GM SBM could be ruled out. The promoter DNA fragment was detected in the contents of the GI tracts of fish fed the GM SBM diet, but chloroplast DNA fragment was amplified from fish fed non-GM or GM SBM diet. Moreover, promoter fragment was not detected on the 3 rd day after changing the diet (Table 3). The promoter DNA fragment was detected in the blood, head kidney and muscle of fish fed the GM SBM diet, but not in the spleen, liver or brain. Promoter fragment was not detected on the 5 th day after the switch over (Table 4). No promoter DNA fragment was detected in blood or other tissues of fish fed the non-GM SBM diet. Chloroplast DNA fragment was detected in blood and some tissues of fish fed either the non-GM or GM SBM diet. ISH analysis confirmed that the promoter and chloroplast DNA were found in tissues. These results suggested that foreign DNA fragments were not completely degraded in the stomach and intestine and might be taken up into organs via the GI tract. However, foreign DNA was not detected after the withdrawal period. Thus, the uptake of DNA from GM SBM might be regarded as safe as non-GM SBM.

These series of studies have demonstrated that an appropriate combination of GM and non-GM SBM could be a good protein source without leading to a significant loss in growth performance. In addition, the DNA from GM SBM found in fish fed a diet

Table 3. Detection of CaMV 35S promoter DNA fragment (220 bp) in contents of GI tract from fish (n=20) fed GM SBM diet at the end of the 2nd week and after changing the diet to non-GM SBM diet by Nested-PCR.

Fed with	GM SBM diet	non-GM SBM diet			
Sampling day	15	1	3	5	7
Stomach	8	2	0	0	0
Anterior intestine	5	3	0	0	0
Posterior intestine	2	5	0	0	0

Table 4. Detection of CaMV 35S promoter fragment (220 bp) in leukocyte and tissues of fish (n=20) fed GM SBM diet at the end of the 2nd week and after changing the diet to non-GM SBM diet by Nested-PCR.

Fed with	GM SBM diet	non-GM SBM diet			
Sampling day	15	1	3	5	7
Leukocyte	2	3	1	0	0
Head kidney	1	1	2	0	0
Spleen	0	0	0	0	0
Liver	0	0	0	0	0
Muscle	1	1	0	0	0
Brain	0	0	0	0	0

containing it vanished after a certain period of time. Furthermore, gene expression initiated by the promoter derived from GM SBM was not observed in fish cells. Judging from these findings, the novel feed ingredients derived from GM SBM could be considered as having equivalent nutritional quality and verify their safety. Therefore, it is considered that the GM SBM might be potentially useful in developing diets for rainbow trout and the other fish species though the required withdrawal period should be determined on a species-by-species basis.

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