EFFECT OF WHEAT GLUTEN ON UNDERWATER PELLET STABILITY OF PELLETED CATFISH AND SHRIMP DIETS

by

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INTRODUCTION

The natural harvest areas for warmwater fishes and shellfishes have been unable to meet the recently increased demand. This is creating an opportunity for fish farming which will be a viable form of animal agriculture. Extensive research has been conducted trying to develop economical methods of fish feed production while meeting the nutritional requirements of the fish. Steam pelleting appears to be the most economical method for fish feed production however, with the steam pelleting process, the final product is often susceptible to water degradation. The pellets begin to deteriorate only minutes after being exposed to the water.

The purpose of this research was to determine the effect of vital wheat gluten on underwater pellet stability. Catfish and shrimp diets were formulated with levels of 2.5%, 5%, and 10% wheat gluten. The diets were steam pelleted and all aspects of the process were monitored. Underwater pellet stability tests were conducted with the pellets.

Pelleting of Aquatic Feeds

Steam pelleting is the most common method for agglomerating aquatic as well as all other animal feeds. According to MacGrath (1976), the pelletization process is the most popular method used in the production of aquaculture feeds. Aquatic feeds are processed into pellets to make the feed more available, thus increasing feed efficiency. The advantages of steam pelleting are: it is a well known process in the feed industry, it is relatively inexpensive, and high production rates can be obtained with this method.

A major disadvantage of steam pelleting is that the final product is susceptible to water degradation. Pellet deterioration often begins a few minutes after being exposed to water. A few minutes is not usually sufficient for the feed to be consumed. The disintegration of the pelleted feeds causes significant feed wastage and reduced water quality due to high levels of organic matter present in the feed.

Hastings (1964) conducted a fish feed pelleting study trying to improve pellet durability and acceptability. Different formulations were pelleted and all gave a pellet durability of 90% or more. The study included the effect of different size pellets on acceptability. According to Hastings (1964), the 1/8 in. diameter pellets with length adjusted according to fish size were accepted and retained more readily than other sizes of pellets. Water stability of the pellets was included in the study.

Extrusion, expanded feeds produced with an extruder, is another process that exists for producing aquatic feeds. The process produces better end products but is considerably more expensive than steam pelleting. The extruded product is very water stable. It is not uncommon for the product to remain stable for up to 24 hours.

Hilton et al (1981) conducted a study comparing the effect of extrusion processing and steam pelleting on different diets. Pellet durability, pellet water absorption, and the physiological response of rainbow trout were investigated. Extruded pellets were more durable, more water stable, and showed a higher feed efficiency when fed to the trout. The extruded pellets resulted in a reduction in the amount of feed consumed by the trout. The trout showed a better weight gain from the pellets of the steam pelleting process.

The steam pelleting process could be more useful if underwater pellet stability could be enhanced. Many binders and different diet formulations have been studied to determine their effects on pellet stability. These will be discussed later.

Feed processing techniques are thought to play a major part in increasing underwater pellet stability. Increasing the temperature of the steam conditioning chamber improves pellet stability considerably. This was thought to be due to the increased gelatinization of the starch from the cereals. Stivers (1971) reported that the degree of stability of feeds is believed to be almost directly related to the extent of gelatinization during steam conditioning. Particle size of the pelleted feed seemed to influence underwater pellet stability. According to Stivers (ibid), finely ground material is likely to

dissipate more readily once the pellet starts deteriorating.

Underwater Pellet Stability

It is important for processed aquaculture feeds to remain stable under water until consumed for nutritional and water quality purposes. Improving underwater pellet stability is becoming increasingly important in the feed industry. But, too much stability of the pellets is undesirable since the nutrients may get bound so tightly that they become unavailable to the organism (Balazs et al 1973). There has been a considerable amount of research conducted, involving different binding agents and formula modifications, hoping to enhance underwater pellet stability.

Heinen (1981) conducted a study utilizing eleven different binding agents in crustacean diets - cornstarch, carboxymethyl cellulose, Vitosan HMW, collagen, guar gum, Chitosan-lv, Viscarin, GFS, agar, Kelvis, and Keltone. Sodium hexametaphosphate was added with the alginates, Kelvis and Keltone. The binders were tested with both dry and moist pellets. The results indicated that cornstarch, carboxymethyl cellulose, Vitosan HMW, guar gum, and collagen were undesirable for both the moist and dry pellets. Chitosan-lv, Viscarin, and GFS were undesirable for dry pellets and only desirable to a certain extent for moist pellets. Agar, Kelvis, and Keltone proved desirable for both moist and dry pellets. The agar and the alginates were water stable up to 24 hours.

Alginates as binders achieve enhanced underwater pellet stability. Meyers et al (1972) added calcium alginate and propylene

glycol alginate to crustacean diets. They obtained a stable pellet up to 48 hours. Farmanfarmaian et al (1982) researched the effect of different levels of Kelco HV algin in shrimp diets. Levels of 1% and 2% were most effective. Pellet stabilities up to 22 hours were achieved.

It is known from the literature that underwater pellet stability is directly related to the amount of gelatinized starch available. Hastings et al (1971) studied the effect of adding pregelatinized potato starch to a standard fish feed. Their results showed increased pellet stability with increased amounts of gelatinized starch. They increased the amount of gelatinization by extending the effects of moisture and temperature. This was done by oven drying after the pelleting process which resulted in a low moisture product with increased starch gelatinization.

Studies have been conducted on formula modifications for improving underwater pellet stability. Making aquatic feed more economical to produce is another reason for investigating formula modifications. A happy medium needs to be found so aquatic feeds can be produced economically without lowering the nutritional value.

Jayaram and Shetty (1981) compared two diets: one contained fish meal and the other contained dried silk-worm pupae. The other ingredients in the two diets were adjusted to keep the protein content at 30%. The diet with the silk-worm pupae had a lower pellet stability index during the first hour. The reason was thought to be due to the high fat content of silk-worm pupae which hindered sufficient starch gelatinization. After 3 hours, the silk-worm pupae

had a better underwater pellet stability index because of the larger particles present. The silk-worm pupae could not be ground very fine due to it's high fat content. The diet containing fish meal had a poor underwater pellet stability index after 3 hours. The reason for the unstable pellet was due to the tendency of fish meal to promote water penetration.

Hastings (1964) conducted a study comparing 10 different formulas to determine the effect different ingredients have on underwater pellet stability. Wheat shorts, ground rice chits, and the addition of 10% animal fat showed an improvement in underwater pellet stability. Wheat shorts were more effective in improving pellet stability than the other two. The diet containing 10% animal fat gave a good underwater pellet stability but a poor dry pellet durability.

Hastings et al (1971) reported that a good water stable pellet could be formed by adding enough cereal filler to allow sufficient starch gelatinization. They found the most effective amount of starch filler to be 40%.

Wheat Gluten

To understand how wheat gluten can enhance pellet water stability requires some general knowledge of it's composition and structure. There has been a large amount of research conducted on wheat gluten, but many unanswered questions remain. Following will be a very general discussion on the composition and the primary structure of wheat gluten. Only the primary amino acids and their purposes will be discussed.

Gluten proteins are the storage proteins of wheat. These proteins are insoluble in water. They are easily separated from starch and water soluble materials. Gluten is isolated by kneading the dough under a gentle stream of water. According to Hoseney (1986), this process was first used by Beccari, an Italian chemist, in the mid 1700's. This insoluble residue is a cohesive, viscoelastic mass that is capable of being stretched. The insoluble residue (gluten) makes up about 80% of the total protein of the flour (Khan and Bushuk 1978).

Gluten has two major components, glutenin and gliadin. Lipids and carbohydrates are also present in gluten but make up only a small portion. According to Khan and Bushuk (ibid), lipids make up (5 - 10%) and carbohydrates (5 - 15%) by weight of the total solids of gluten. Bernardin and Kasarda (1973) stated that the elasticity and cohesiveness of the gluten are properties of the protein.

Glutenin comprises approximately 35 to 45% of wheat endosperm protein (Khan and Bushuk 1978). Glutenin is responsible for the elasticity of the gluten proteins. According to Hoseney (1986), the glutenin proteins are multichained and vary in molecular weight from 100,000 to several million with an average molecular weight of about 3 million. They can be broken down into smaller subunits by the addition of a reducing agent. About 80% of the subunits have molecular weights, by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), of 35000MW - 50000MW and are called low-molecular-weight (LMW) subunits, whereas, the remaining subunits are larger, 90000MW - 140000MW, and are termed high-molecular-weight (HMW)

subunits (Flavell et al 1984).

Gliadin comprises approximately 35 to 40% of the flour proteins (Khan and Bushuk 1978). There are many gliadin proteins which are somewhat similar. They have an average molecular weight of about 40000MW, and are extremely sticky when hydrated (Hoseney, 1986). The gliadin proteins are single-chained as compared with the glutenin proteins which are multi-chained. Gliadin is made up of about 50 components. Khan and Bushuk (1978) reported that the molecular weight of these components, determined by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis), range from approximately 12000MW to 80000MW, with the majority of the components having a molecular weight of about 36000MW.

Amino acid composition is one of the main factors influencing the behavior of gluten proteins. There is a large proportion of glutamic acid (Figure 1(a)) in the amino acid composition of glutenin and gliadin (ibid). Glutamic acid is usually in its glutamine (Figure 1(b)) form. This makes several amide (Figure 1(c)) groups to form hydrogen bonds. Lasztizy (1984), reports that hydrogen bonding influences the rheological properties of gluten.

Khan and Bushuk (1978), stated that glutenin and gliadin contain a relatively high proportion of hydrophobic amino acids such as leucine. The side chains of leucine can interact with each other forming hydrophobic bonds. A large number of these relatively weak bonds can influence the function of gluten.

Hoseney (1986) reported that gluten proteins are obviously low in the basic amino acids. This gives a low level of charges causing the

Figure 1. (a) (b) (c)

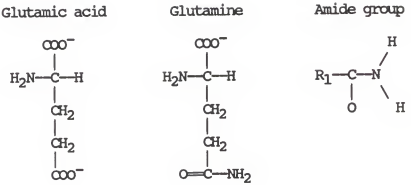
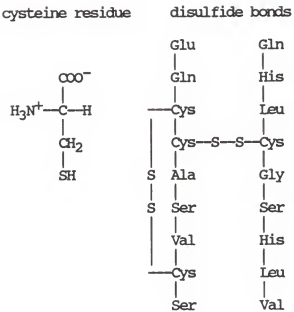


Figure 2.



repulsion forces between the proteins to be low allowing the proteins to associate with each other.

Proline makes up a large proportion (Table 1) of the amino acid composition in gliadin and glutenin. Proline tends to disrupt the secondary structure of the chains and create bends in the chains wherever they occur. This prevents the proteins from readily forming into an α-helix. Hoseney (ibid) said this does not necessarily mean gluten proteins have no ordered structure, only that it is not the common α-helix with which we are familiar.

Huebner et al (1974) and Shewry et al (1984) found high-molecular-weight subunits from reduced wheat glutenin to contain high amounts of glycine. These glycine rich sequences appear to influence the behavior of gluten. The molecular basis of the elastic properties of elastin, a protein of connective tissue, is attributed to the occurrence of sequences rich in glycine (Urry et al 1983). The characteristic conformational feature is a Beta-spiral, that is an α-helix that contains Beta-turns linked by glycine residues(Belitz et al 1986). These glycine rich sequences can be arranged the same way in wheat glutenin as they are in elastin. Tathem et al (1984) and (1985) indicated that this structural similarity between elastin and glutenin suggests that the glycine rich sequences in glutenin are responsible for the elastic properties.

Gluten elasticity is directly related to gluten strength. The role of disulfide bonds has been well established. Disulfide bonds are possible because of the presence of the cysteine residues (Figure 2). These residues are usually located near the N (amino) or C

Table 1. Amino acid composition[1] of glutenin and gliadin.

Amino acid[2]	Glutenin[2]	Gliadin[2]
Lysine	12.5	5
Histidine	13	14.5
Arginine	20	15
Aspartic acid	23	20
Threonine	26	18
Serine	50	38
Glutamic acid	278	317
Proline	114	148
Glycine	78	25
Alanine	34	25
Cysteine	10	10
Valine	41	43
Methionine	12	12
Isoleucine	28	37
Leucine	57	62
Tyrosine	25	16
Phenylalanine	27	38
Tryptophan	8	5
Amide	301	240

[1]Amino acid residues per 100,000g

[2]Kasarda et al. (Table 1)

(carboxyl) terminals of the protein chains. The disulfide bond can connect two cysteine residues in the same chain or in different chains (Figure 2). A reduction in the number of disulfide bonds weakens the gluten.

Ionic bonds are of great importance for the interaction of gluten proteins and gluten strength. Gluten has only a few amino acids with acid and basic side chains where these ionic bonds can occur. Even though they are few, the number of additional crosslinks they create strengthens the protein network. There remains much work to be done to completely determine the exact structure of the gluten proteins.

Gliadin appears to be the main contributing factor to the cohesive properties of gluten. When small sections of wheat endosperm are hydrated, many small fibrils are formed. These fibrils are formed from the protein inside the endosperm cells. This protein is only available to form fibrils when the endosperm cells are broken. By hydrating, the protein streams out of the damaged cell and forms a mass of webbed fibrils. Bernardin and Kasarda (1973) reported that a single flour particle wetted with a drop of water forms protein fibrils with adherent starch granules. These fibrils increase the volume occupied by the particle nearly 20 times; an indication of the amount of webbing capable of being formed by the hydrated protein fibrils.

Nutritional Requirements of Catfish and Shrimp

Studies determining the nutritional requirements of warmwater fishes and shellfishes began in the 1950's. Warmwater fishes and

shellfishes are those species that have optimum growing temperatures of 25° - 30°C (NRC, 1983). Research on the nutritional requirements continues due to the demand for increased fish production. Feeds now are nutritionally more complete resulting in better feed efficiency and increased growth. There is much literature available on the nutritional requirements of catfish due to the amount of attention given to them by the nutritionists. Little is known about the nutritional requirements of shrimp and many studies are being conducted.

The protein requirement in fish diets is influenced by the amino acid composition and availability of the protein source, the environmental conditions, and the culture practices. Garling and Wilson (1976) determined a 32 - 36% protein requirement for young channel catfish using whole egg protein as the protein source. Lovell (1977) indicated the minimum level of protein in balanced diets to be 35 - 40% for fish under 6 inches in size. Forster and Beard (1973) reported that shrimp require a 40% protein level when using fish meal or shrimp meal as the protein source.

Studies show that channel catfish require dietary sources of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (NRC, 1983). Fish generally require a greater percent of amino acids in their diets than most animals. Amino acid composition is very important in preventing depressed appetite and reduced growth rate. Fish diets are frequently deficient in methionine and lysine. Lovell (1977) reported that channel catfish require 2.8% of the protein to be methionine and 5.1%

to be lysine. The exact amino acid dietary requirement has not yet been determined for shrimp.

The dietary energy requirement is relatively low compared to other animals. Fish don't have to maintain a constant body temperature and can easily move through the water wasting very little energy. An energy-protein ratio must be established to prevent reduced growth rate. Studies show that too much energy in the diet will prevent the catfish from consuming the amount of protein required. Garling and Wilson (1977), using purified diets, reported the optimum DE/P (kcal digestible energy/g protein) ratio for small catfish to be 9.6; and Page and Andrews (1973), using practical diets, found the value to be 9.7. Stickney and Lovell (1977) reported that a requirement of 8 to 9 kcal/g of protein is recommended for maximum growth of channel catfish. Again, the digestible energy-protein ratio remains to be determined for shrimp.

Fish raised in their natural culture, for example the ocean, receive an abundant source of vitamins from natural foods. Intensive culture practices, for instance over-stocked ponds, require supplemental vitamins added to the diet because of the limited amount of natural foods. Vitamin requirements can be influenced by size, age, and growth rate of the fishes, environmental conditions, and the diet formula (NRC, 1983). The vitamin requirement for warmwater fishes has been well documented. Only a few vitamins have been found to be required by shrimp; the rest remain to be tested.

NRC (ibid) indicated that catfish require supplements of thiamin, riboflavin, pyridoxine, pantothenic acid, nicotinic acid, biotin,

vitamin B_{12}, choline, ascorbic acid, vitamin A, vitamin D, vitamin E, and vitamin K in their diet. Only inositol and folic acid were found not to be a dietary requirement. Lovell (1983) indicated that inositol is produced in sufficient amounts in the intestine and liver of channel catfish. It was determined that channel catfish fed diets deficient in vitamin C developed what is known as "broken back syndrome" (ibid).

Shrimp require a dietary supplement of thiamine, pyridoxine, choline, inositol, and ascorbic acid (NRC, 1983). The other vitamins remain to be tested for shrimp.

Mineral requirements of fish are very hard to determine. This is due to the difficulty in formulating test diets that are deficient in any one mineral. The water supplies many minerals to the fish, thus, making it difficult to determine mineral requirements.

It was discovered by Andrews et al (1973) that catfish did not require a dietary supplement of calcium since it was absorbed from the water. The dietary phosphorus requirement was determined to 0.33% available phosphorus for channel catfish (Gatlin and Wilson 1986).

Calcium and phosphorus were the only minerals studied to any extent until recently. Gatlin and Wilson (ibid), studied many other minerals determining the levels required for channel catfish. They found magnesium, zinc, selenium, manganese, iron, and copper to be required as a dietary supplement.

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EXPERIMENTAL PROCEDURE 1

Introduction:

The effect of vital wheat gluten on the underwater stability of pelleted catfish diets was investigated. In this phase of the study, four standard catfish diets were formulated and pelleted. Pelleting parameters monitored were production rate and pellet quality. Underwater pellet stability tests were conducted to determine Water Stability Index (WSI%). Data was statistically analyzed using SAS[1] analysis of variance procedure GLM (General Linear Models) using Duncan's test to determine significant differences.

Diet Formulation:

Four catfish diets were formulated to meet their known requirements. Dietary parameters were very similar to most of the commercial catfish diets available.

A control diet plus three diets containing wheat gluten were formulated (Table B1 Appendix B). The levels of wheat gluten studied were 2.5, 5, and 10 percent. The wheat midds were varied to allow for the wheat gluten additions.

Processing (Pelleting):

All diets were processed using a California Pellet Mill Company[2] Master H.D. Model pellet mill equipped with a 4.8mm x 50.8mm straight

[1]SAS Institute Inc., Cary, NC

[2]California Pellet Mill Company, San Francisco, CA

bore die. Each formulation excluding, the trace minerals, vitamin premix, wheat gluten, and oil, was reground through a 1.19mm screen in a Jacobson[3] (P-240) hammermill. The reground ingredients were dry-mixed with the trace minerals, vitamin premix, and wheat gluten for three minutes. Oil was added for a three minute wet-mix. The diets were pelleted at both 70 C and 85 C at a constant motor load. Samples were collected at the pellet mill for the Pellet Durability Index and production rate determinations. Samples for the Water Stability Index were collected off the end of the horizontal double pass cooler.

Pellet quality was determined by percent fines and Pellet Durability Index, a standard test (ASAE A-319), (see appendix C for procedure). The weight of the fines produced during pelleting divided by the weight of the pellets was recorded as percent fines.

Water Stability Index:

Approximately ten grams of pellets were placed in a short length of four inch PVC pipe covered on one end with 8 mesh stainless steel wire cloth. The PVC pipes were suspended from a rack in aquariums exposing the samples to water. Air was piped to an orifice below the PVC containers and bubbled up through the sample to cause a slight turbulence.

The underwater times studied were 0.5, 1, 2, 4, 8, and 16 hours. After the desired underwater time, the containers were removed from the aquariums allowing the samples to air-dry for 48 hours. The samples were then placed in an oven at 110 C for three hours. The

[3]Jacobson Machine Works, Minneapolis, MN

sample dry matter weight after the oven divided by the initial dry matter weight was reported as "Water Stability Index" (WSI %).

Results and Discussion

Processing (Pelleting):

The higher steam conditioning temperature increased the production rate substantially (Table 2). The production rate increased by an average of 25 percent in this study. This was expected due to the lubricating effect of steam.

The fines data are a good indicator of pellet quality. Pelleting the control diet produced twice as many fines as the diets containing wheat gluten at any level (Table 2). Apparently, wheat gluten plays a role in pellet quality enhancement but the level of wheat gluten in the diet had no apparent effect on the amount of fines produced.

This study indicated wheat gluten had no effect on the Pellet Durability Index. All PDI's ranged from 94-96 percent which is considered good (Table 2).

Water Stability Index:

The results of the water stability tests are presented in Tables 3 and 4 and in Figures 1 - 6.

The level of wheat gluten in the diets had a direct effect on the underwater pellet stability. The WSI improved with increasing levels of wheat gluten. There was a significant difference between the control diet and the diets containing gluten in most cases. The 2.5 and 10 percent diets were only significantly different at the 0.5 and 1 hour times as indicated in Table 3. This suggests that there is not much difference between the level of wheat gluten at the longer underwater times.

The 5 percent diet produced some unexpected results. The 5 percent diet showed a significantly better WSI at the 2, 4, and 8 hour times than the 0.5 and 1 hour times. This was probably due to the design of the water stability test device. As the pellets disintegrated the fines material wouldn't fall through the screen due to the lack of turbulence created in the water. The hydration of the gluten was more thorough at the 2, 4, and 8 hour times allowing the gluten to rebond with the fines.

The longer underwater times were not significantly different for the gluten diets (tables 3 and 4). It was thought that wheat gluten had contributed as much as it could to improving WSI after four hours. This was reinforced by the fact that the shorter underwater times indicated significant differences.

Figures 1 - 6 indicate an enhanced WSI due to the elevated steam conditioning temperature. The higher temperature improved the WSI of all the diets with the greatest improvement showing up in the control diet. It is known that starch gelatinization has a direct effect on underwater pellet stability. The WSI results for the 85 C conditioning temperature were substantially better in the control diet than the 70 C which was probably due to the level of gelatinized starch. The wheat gluten diets indicated only a slight difference in WSI between the two steam conditioning temperatures. Apparently, wheat gluten inhibits, to a certain degree, the amount of starch gelatinization. This agrees with the studies conducted by Hastings et al (1971) and Jayaram and Shetty (1981).

The visual difference in the product following the water stability test was substantial. The 5 and 10 percent diets retained their pellet shape through the entire process. The 2.5 percent diet showed very little pellet shape while the control diet pellets showed complete disintegration.

The R-square values (Table 5) indicate there was a problem with the test. The one and two hour R-square values in the 70 C column suggested that the wheat gluten level had a positive effect on the WSI. The low values in the 85 C column indicated that the level of wheat gluten had no effect on pellet disintegration. These results led to the conclusion that there was a problem with the water stability test device.

Table 2. Production Data for All Catfish Diets

	Conditioning Temperature 70 C				85 C			
	Gluten Levels %							
	0	2.5	5	10	0	2.5	5	10
Production[1] Rate (kgs/hr)	1556	1479	1408	1543	1687	1596	1968	2204
Fines (%)	3.0	1.6	1.8	1.5	3.0	1.6	1.8	1.5
Pellet[1] Durability Index (%)	94.0	94.6	95.5	95.6	95.6	95.8	95.5	95.2

[1]Values are means of three replications.

Table 3. Water Stability Index Results for Diets Pelleted at 70 C[5]

Gluten Levels (%)	Water Stability Index[6] Time in Water (hrs) 0.5	1	2	4	8	16
	%					
0	63.30^{d}	68.57^{c}	67.71^{b}	59.80^{b}	58.04^{c}	55.72
2.5	87.60^{b1}	74.98^{b2}	75.30^{ab2}	70.33^{ab23}	69.77^{b23}	65.40^{3}
5.0	77.51^{c2}	72.29^{b4}	80.00^{a2}	82.71^{a2}	90.78^{a1}	74.16^{34}
10.0	94.14^{a1}	88.73^{a2}	82.92^{a3}	78.66^{a4}	78.36^{b4}	77.35^{4}

^{abcd}Column means with unlike letter superscript are different ($P<.05$).

^{1234}Row means with unlike number superscript are different ($P<.05$).

[5]Values are means of two replications.

[6]Product remaining after the underwater test was dried, weighed and reported as %WSI.

Table 4. Water Stability Index Results for Diets Pelleted at 85 C[5]

Gluten Levels (%)	Water Stability Index[6] Time in Water (hrs) 0.5	1	2	4	8	16
	%					
0	86.28[1]	84.34[1]	79.14[2]	77.91[b23]	73.31[c34]	72.30[4]
2.5	93.22[1]	83.24[2]	81.99[2]	78.96[b3]	76.73[b3]	77.36[3]
5.0	81.62[4]	81.55[4]	85.86[3]	92.36[a2]	96.95[a1]	80.13[4]
10.0	92.26[1]	88.86[1]	83.30[2]	78.67[b3]	78.46[b3]	78.27[3]

[abc]Column means with unlike letter superscript are different ($P<.05$).

[1234]Row means with unlike number superscript are different ($P<.05$).

[5]Values are means of two replications.

[6]Product remaining after the underwater test was dried, weighed and reported as %WSI.

Figure 3. Water Stability Index

Time: 0.5hr

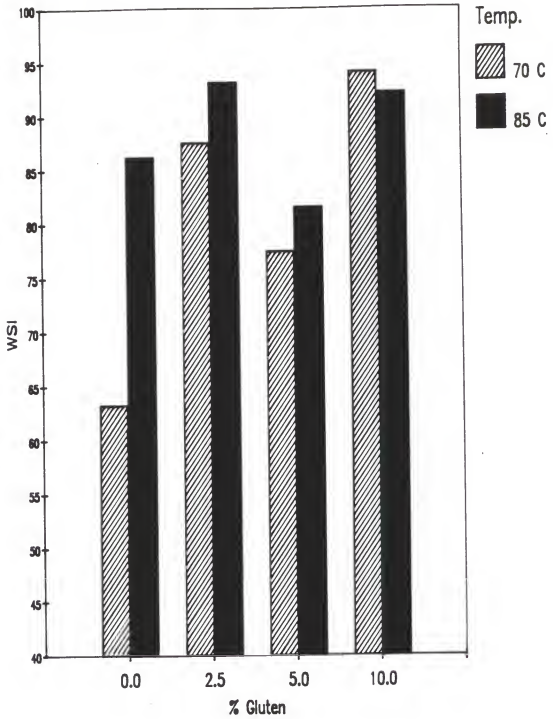

Figure 4. Water Stability Index

Time: 1hr

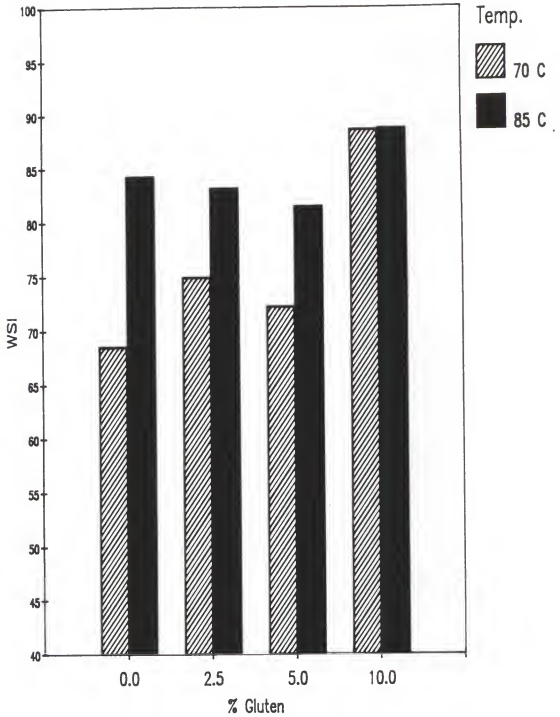

Figure 5. Water Stability Index
Time: 2hr

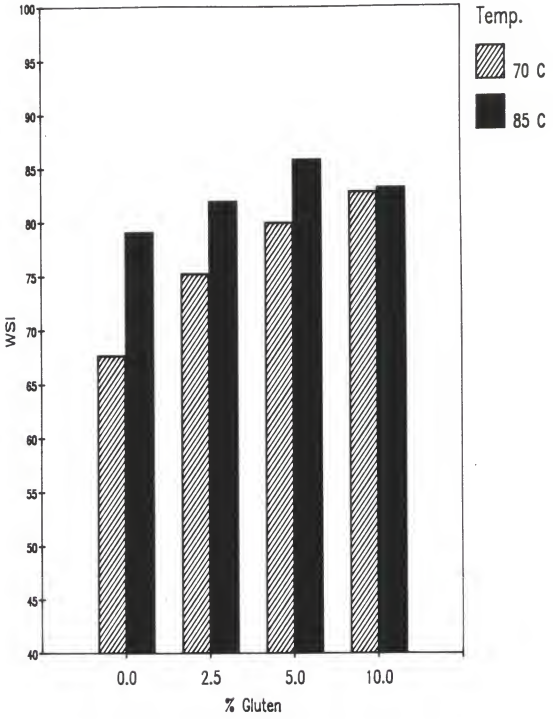

Figure 6. Water Stability Index

Time: 4hr

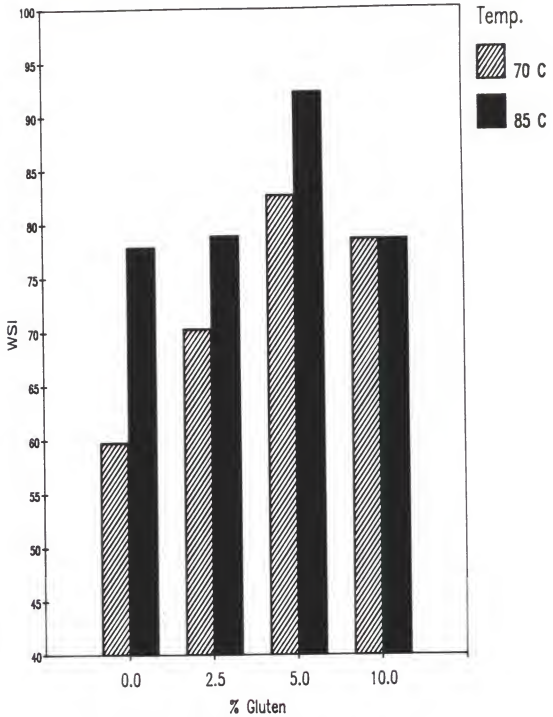

Figure 7. Water Stability Index

Time: 8hr

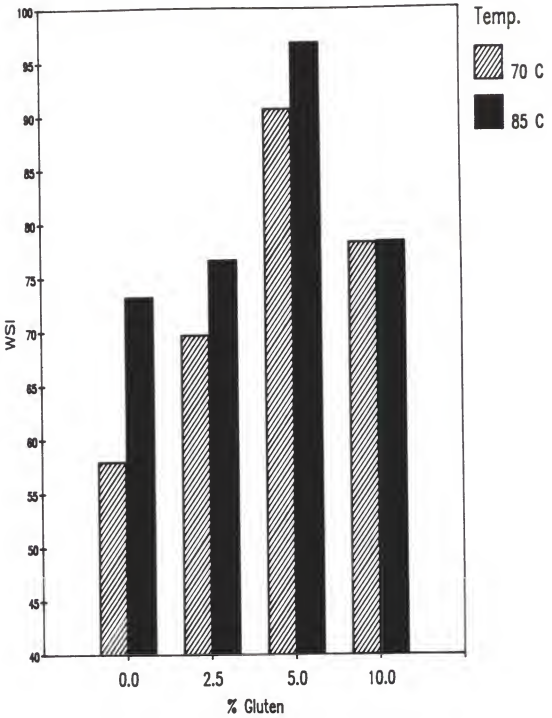

Figure 8. Water Stability Index
Time: 16hr

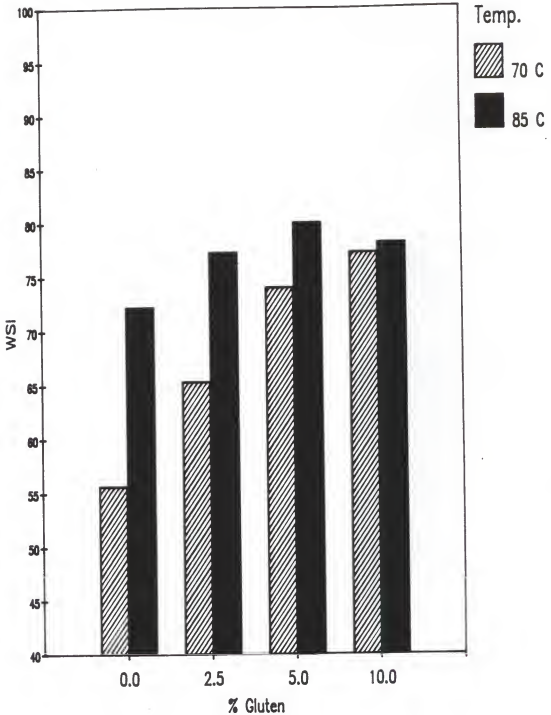

Table 5. Analysis of Variance Over All Gluten Levels

Time	R - Square	
	70 C	85 C
0.5	0.6193	0.0567
1	0.8402	0.2596
2	0.7681	0.2888
4	0.5383	0.0153
8	0.3953	0.0743
16	0.6825	0.3948

EXPERIMENTAL PROCEDURE 2

Introduction:

This experiment investigated the effect of vital wheat gluten on the underwater stability of pelleted shrimp diets. The procedure used in experiment two is basically the same as the procedure used in experiment one except for the water stability test. A diet containing a binder, Nutraflex Mega 40[1], was included in this experiment for further comparison. Basic shrimp feed rations which are currently being used in Latin America were formulated and pelleted. Pelleting parameters monitored were energy consumption, production rate, and pellet quality. Underwater pellet stability tests were conducted to determine the Water Stability Index. Statistical analysis of the data was done using SAS analysis of variance procedure GLM (General Linear Models) using Duncan's test to determine significant differences.

Diet Formulation:

Five shrimp diets (Table B2 Appendix B) were formulated which consisted of three diets containing wheat gluten levels of 2.5, 5, and 10 percent, a binder (Nutraflex Mega 40) diet, and a control diet. The wheat component was adjusted to allow for the wheat gluten and binder additions.

Processing (Pelleting):

The processing procedure in experiment two was the same as

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[1]Swift Adhesives, Downers Grove, IL

experiment one except electrical energy consumption was monitored by a recording amp/volt meter. See experiment one for the procedure.

Water Stability Index:

Approximately ten grams of pellets were placed in square plastic containers 6.35cm^2 x 10.16cm long with 8 mesh stainless steel wire cloth on the bottom. The containers were suspended from a rack which was raised and lowered into aquariums, to create a slight turbulence, at half hour intervals. This was accomplished with a reversible, variable speed DC motor. See Appendix A for a diagram of the water stability test device.

The underwater times studied were 0.5, 1, 2, 4, 8, and 16 hours. After the desired underwater time, the containers were placed in an incubator at 50 C for 15 hours. The samples were then placed in an oven at 110 C for three hours. The dry matter weight after the oven divided by the initial dry matter weight was reported as "Water Stability Index" (WSI %).

Results and Discussion

Processing (Pelleting):

Results for the processing portion of this experiment are presented in Tables 6 and 7.

The results indicated a substantial increase in production rate at the higher steam conditioning temperature. This was expected because of the lubricating effect of steam. The diets containing no gluten showed increased production rate by an average of 5%. The diets with wheat gluten resulted in a 20% increase in production rate at the higher steam conditioning temperature. Apparently, wheat gluten aids in the lubrication effect of steam.

Wheat gluten had no apparent effect on power consumption. The results did indicate more efficient production at the elevated steam conditioning temperature. The KWH/ton were substantially less at the higher temperature. This was also expected because of the lubricating effect of steam.

The fines data indicated a higher quality pellet when wheat gluten was present in the diet. The level of wheat gluten had no apparent effect on pellet quality. This conclusion was consistent with experiment one.

Pellet Durability Index was not significantly improved with the addition of wheat gluten. All PDI's ranged from 93-96 percent as they did in experiment one.

Water Stability Index:

The results of the water stability tests are presented in Tables

8 and 9. Figures 7 - 12 give the water stability test results in graphical form.

Increasing levels of wheat gluten corresponded to an improved WSI. Tables 8 and 9 indicate a significant difference between the control and the wheat gluten diets. The diet that included the binder showed no improvement on WSI. WSI was significantly lower for the binder diet than the control diet at extended underwater times. There was a significance difference between several of the gluten levels at all underwater times. The tables also suggest a significant difference between the underwater times for all the diets in almost every case. The 5 percent diet produced a WSI between the 2.5 and 10 percent diets. This result was opposite of experiment one which is thought to be due to the water stability test device or procedure. The improved test device used in this study appeared to be much more reliable.

Figures 7 - 12 indicate that the WSI was enhanced considerably by the elevated steam conditioning temperature. The improved WSI due to the elevated steam temperature was substantially higher in the control and the binder diets. These results are due to the increased starch gelatinization which is consistent with experiment one, Hastings et al (1971), and Jayaram and Shetty (1981).

The 5 and 10 percent diets retained their pellet shape through the entire test. The 10 percent diet, pelleted at the elevated steam temperature, had the best pellet shape after the study was completed. This agreed with experiment one. There was no visual pellet shape when examining the control and binder diet samples after the study.

Table 13 displays R-square values that are relatively high. This indicates that wheat gluten effects underwater pellet stability at all underwater times. These higher values indicate a good test as opposed to Table 5 in experiment one.

Table 6. Production Data for All Shrimp Diets Pelleted at 70 C

	Gluten Levels %				
	0	2.5	5	10	Binder
Production[1] Rate (kgs/hr)	1510	1335	1290	1417	1419
Fines (%)	3.9	2.7	1.7	1.6	3.4
Pellet[1] Durability Index (%)	95.5	94.5	94.9	95.1	93.9
Power Consumption (KWH/ton)	8.54	9.66	9.95	9.06	9.05

[1]Values are means of three replications.

Table 7. Production Data for All Shrimp Diets Pelleted at 85 C

	Gluten Levels %				
	0	2.5	5	10	Binder
Production[1] Rate (kgs/hr)	1578	1570	1611	1664	1500
Fines (%)	3.8	2.2	2.0	2.8	3.5
Pellet[1] Durability Index (%)	95.1	95.0	95.0	94.4	94.5
Power Consumption (KWH/ton)	7.43	7.15	6.97	6.74	7.48

[1]Values are means of three replications.

Table 8. Water Stability Index Results for Diets Pelleted at 70 C[7]

Gluten Levels (%)	Water Stability Index[8] Time in Water (hrs) 0.5	1	2	4	8	16
	%					
Binder	96.81^{b1}	88.57^{b2}	73.37^{d3}	71.52^{c3}	59.23^{e4}	46.91^{c5}
0	95.96^{c1}	85.88^{c2}	75.33^{c3}	72.95^{c3}	67.35^{d4}	58.20^{b5}
2.5	97.27^{ab1}	88.83^{b2}	76.75^{bc3}	77.35^{b3}	72.74^{c4}	71.56^{a4}
5.0	97.20^{ab1}	90.23^{a2}	78.06^{b3}	77.80^{b3}	75.77^{b4}	71.80^{a5}
10.0	97.47^{a1}	91.36^{a2}	80.46^{a3}	79.74^{a4}	78.75^{a5}	77.82^{a6}

^{abcde}Column means with unlike letter superscript are different ($P<.05$).

^{123456}Row means with unlike number superscript are different ($P<.05$).

[7]Values are means of two replications.

[8]Product remaining after the underwater test was dried, weighed and reported as %WSI.

Table 9. Water Stability Index Results for Diets Pelleted at 85 C[7]

Gluten Levels (%)	Water Stability Index[8] Time in Water (hrs) 0.5	1	2	4	8	16
	%					
Binder	96.90^{b1}	89.30^{c2}	77.59^{e3}	75.89^{c3}	70.27^{e4}	60.76^{c5}
0	96.72^{b1}	89.10^{c2}	78.88^{d3}	78.09^{b3}	76.50^{d4}	72.75^{b5}
2.5	97.21^{ab1}	91.26^{b2}	80.44^{c3}	79.09^{b4}	78.57^{c4}	77.13^{a5}
5.0	97.71^{a1}	92.33^{a2}	82.02^{b3}	80.93^{a4}	80.13^{b5}	77.92^{a6}
10.0	97.83^{a1}	93.08^{a2}	84.60^{a3}	82.12^{a4}	81.42^{a5}	79.45^{a6}

^{abcde}Column means with unlike letter superscript are different ($P<.05$).

^{123456}Row means with unlike number superscript are different ($P<.05$).

[7]Values are means of two replications.

[8]Product remaining after the underwater test was dried, weighed and reported as %WSI.

Figure 9. Water Stability Index

Time: 0.5hr

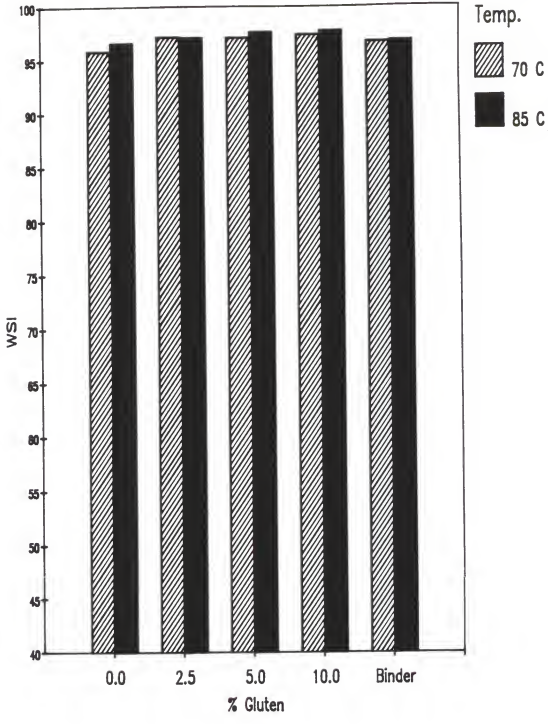


Figure 10. Water Stability Index
Time: 1hr

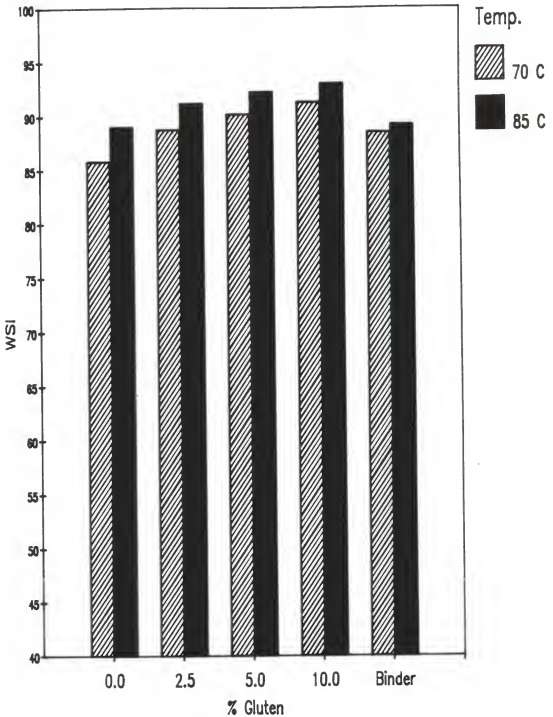


Figure 11. Water Stability Index
Time: 2hr

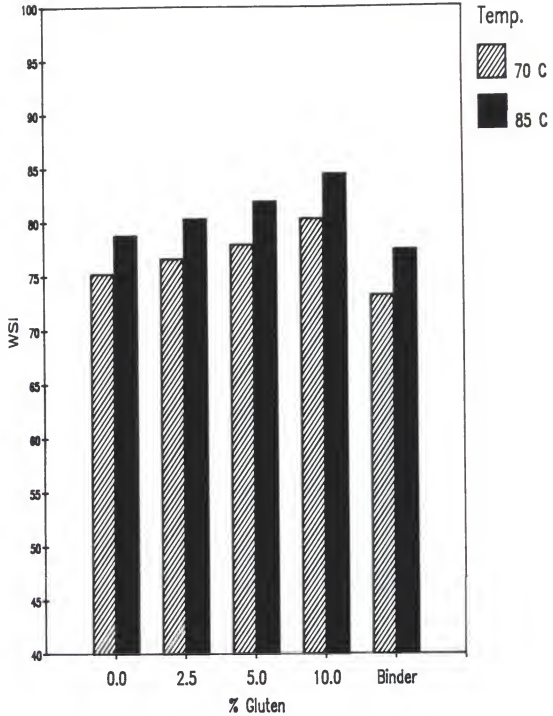


Figure 12. Water Stability Index
Time: 4hr

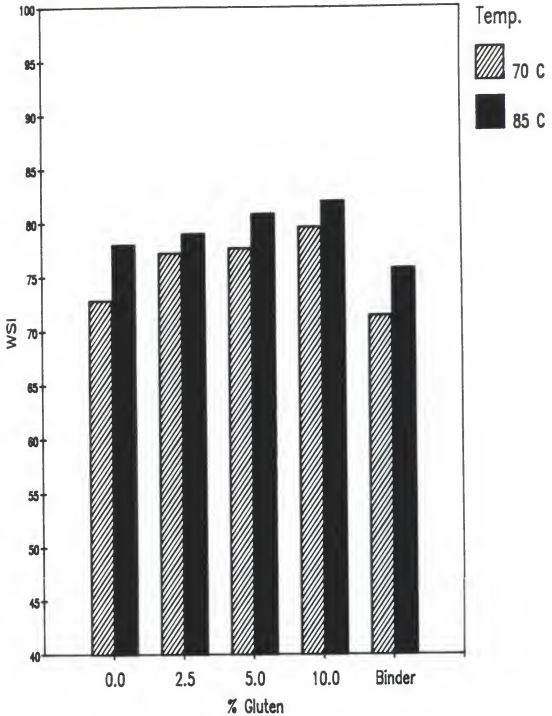


Figure 13. Water Stability Index
Time: 8hr

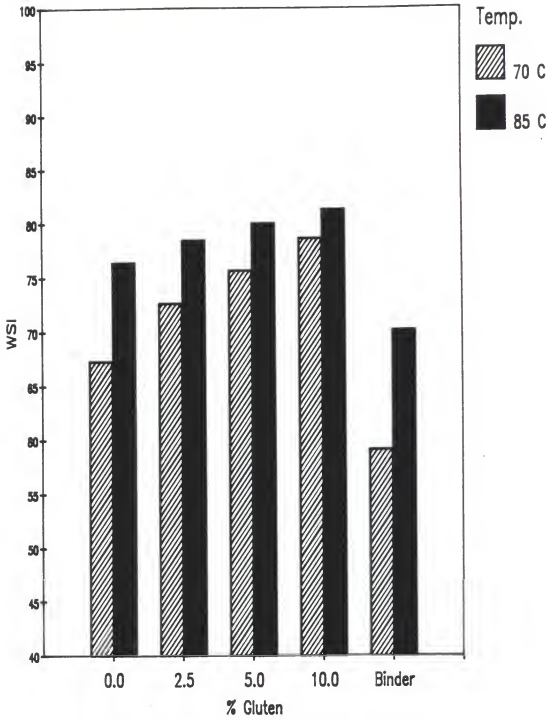


Figure 14. Water Stability Index

Time: 16hr

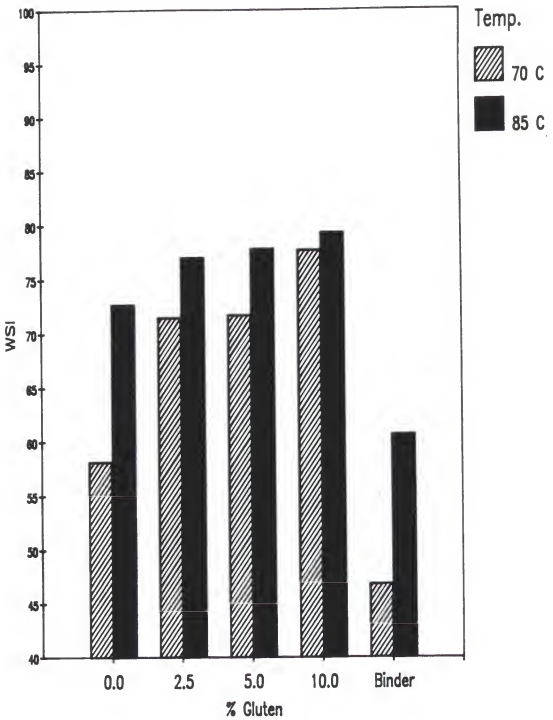


Table 10. Analysis of Variance Over All Gluten Levels (Binder Diet is Included in Analysis)

Time	R - Square	
	70 C	85 C
0.5	0.4565	0.6081
1	0.7582	0.7809
2	0.8160	0.9379
4	0.6988	0.7263
8	0.7398	0.8486
16	0.6946	0.7001

LITERATURE CITED

Hastings, W.H., S.P. Meyers, and D.P. Butler, 1971. A commercial process for water-stable fish feeds. Feedstuffs. 43(47):38.

Jayaram, M.G. and H.P.C. Shetty, 1981. Formulation, processing and water stability of two new pelleted fish feeds. Aquaculture. 23:355-359.

SUMMARY

Different levels of vital wheat gluten were added to catfish and shrimp diets to determine their effect on underwater pellet stability. The pelleting parameters (production rate, pellet quality, and power consumption) were monitored.

The results indicated that wheat gluten improved pellet quality and the production rate. Elevating the steam temperature substantially increased the production rate. Wheat gluten had no apparent effect on power consumption and Pellet Durability Index.

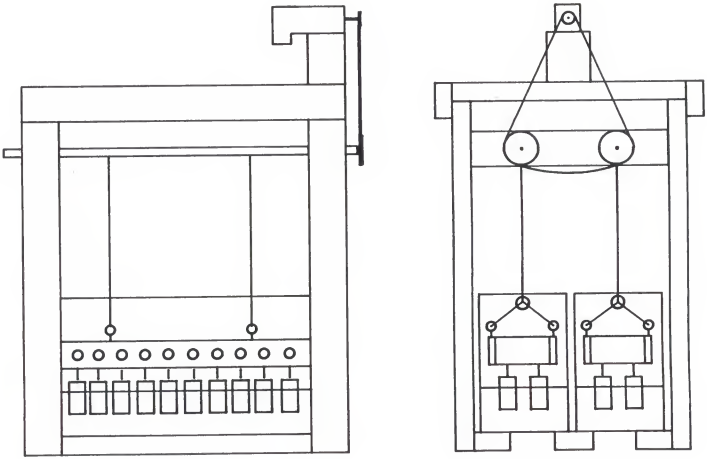
Water Stability Index was significantly better, in most cases, with increasing levels of wheat gluten. This was most evident at the 2, 4, and 8 hour underwater times. The 0.5, 1, and 16 hour times indicated fewer significant differences between the gluten levels. The increasing underwater times were significantly different for each diet in almost every case.

It is evident from this study that wheat gluten can substantially increase underwater pellet stability. Additional research needs to be done to further determine which level of wheat gluten would be more economical. Steam pelleting could become the process of the future if underwater pellet stability could be obtained. Steam pelleting would save aquatic feed manufacturers a great deal of money.

APPENDIX A

This water stability test device (see Figure A1) was designed to simulate the underwater environment. The test device is 1m tall, .9m long, and .76m wide. Two 113.5L fish tanks are positioned at the bottom of the stand.

The racks were designed to hold 20 containers each. The containers are 6.35cm^2 x 10.16cm long with 8 mesh stainless steel wire cloth on the bottom. These racks are connected to shafts at the top of the stand with light weight chain. The shafts are chain driven by a reversible variable speed motor which raises and lowers the containers in and out of the water.



Side View End View

Figure A1. Water Stability Test Device

Appendix B

Diet Formulations

Table B1. Catfish Diets (Experiment one)

Ingredient	Amount			
	0	2.5	5.0	10.0
	%			
Fish Meal	8.0	8.0	8.0	8.0
Soybean Meal	48.2	48.2	48.2	48.2
Corn	31.2	29.2	29.2	29.2
Wheat Midds	10.0	9.5	7.0	2.0
Dical	1.0	1.0	1.0	1.0
Wheat Gluten	----	2.5	5.0	10.0
Tallow/Oil	1.5	1.5	1.5	1.5
Trace Mineral	.05	.05	.05	.05
Vitamin Premix	.05	.05	.05	.05
Nutrients				
Protein (%)	31.1	32.8	34.3	37.4
Ash (%)	6.4	6.4	6.3	6.1
Fiber (%)	4.4	4.4	4.4	4.2
Energy (Kcal/Kg)	2682.0	2696.0	2721.0	2771.0
Amino Acid Composition				
Lysine (%)	1.59	1.62	1.63	1.66
Methionine (%)	.36	.39	.41	.45
Threonine (%)	.87	.92	.96	1.04
Tryptophan (%)	.33	.35	.36	.39

Table B2. Shrimp Diets (Experiment two)

Ingredient	Amount				
	0	2.5	5.0	10.0	Binder
			%		
Fish Meal	20.0	20.0	20.0	20.0	20.0
Shrimp Meal[1]	10.0	10.0	10.0	10.0	10.0
Soybean Meal	30.0	30.0	30.0	30.0	30.0
Wheat	39.5	37.0	34.5	29.5	39.3
Wheat Gluten	----	2.5	5.0	10.0	----
Trace Mineral	.25	.25	.25	.25	.25
Vitamin Premix	.25	.25	.25	.25	.25
Binder[2]	----	----	----	----	.20
Nutrients					
Protein (%)	34.8	36.7	38.0	41.2	34.9
Ash (%)	9.1	9.0	9.0	9.0	9.1
Fiber (%)	4.3	4.4	4.4	4.5	4.3
Energy (Kcal/Kg)	2886.0	2897.0	2908.0	2931.0	2881.0
Amino Acid Composition					
Lysine (%)	1.83	1.85	1.88	1.92	1.84
Methionine (%)	.54	.57	.59	.64	.55
Threonine (%)	1.05	1.09	1.14	1.23	1.05
Tryptophan (%)	.35	.36	.38	.41	.35

[1]Bloom and Bergeron Co., Houma, LA

[2]Swift Adhesives, Downers Grove, IL

APPENDIX C

Pellet Durability[a]

Device:

Durability of pellets and crumbles shall be determined by tumbling the test sample for 10 minutes at 50 rpm, in a dust-tight enclosure. The device is rotated about an axis which is perpendicular to and centered in the 12 inch sides. A 2 by 9 inch plate is affixed symmetrically along one of its 9 inch sides to a diagonal of one 12 by 12 inch side of the can. A door may be placed in any side and should be dustproof. Projections, such as rivets and screws, shall be kept to a minimum and well rounded.

Screens:

Fines shall be determined by screening a sample on a wire sieve having openings just smaller than the nominal pellet diameter. Recommended sieve sizes are shown in Table C1.

Test procedure:

A sample of pellets or crumbles to be tested will be sieved on the appropriate sieve to remove fines. If pellets of .5 inch diameter, or larger, are being tested, select pellets which are between 1.25 inches and 1.5 inches in length. Place a 500 gram sample of sieved pellets or crumble in the tumbling can device. After tumbling for 10 minutes, the sample will be removed, sieved, and the percent of whole pellets or crumbles calculated. Pellet and crumbles durability will be defined as:

$$\text{Durability} = \frac{\text{wt. of pellets or crumbles after tumbling}}{\text{wt. of pellets or crumbles before tumbling}} \times 100$$

Normally pellets will be tested immediately after cooling. When the temperature of the pellets falls within ± 10 degrees F. of ambient, they are considered cool. If tested at a later time, the time, in hours, after cooling will be indicated as a subscript of the durability.

Table C1. Pellet Size and Corresponding Sieve Sizes for Determining Pellet Durability Index

Pellet or Crumble fraction, inches	Size [b]	Required screen size Decimal equiv., inches
3/32	No. 10	.0787
1/8	No. 7	.1110
9/64	No. 6	.1320
5/32	No. 6	.1320
3/16	No. 5	.1570
13/64	No. 4	.1870
1/4	No. 3.5	.2230
5/16	.263	.2650
3/8	5/16	.3125
1/2	7/16	.4375
5/8	.530	.5300
3/4	5/8	.6250
7/8	3/4	.7500
1	7/8	.8750

[a]Wafers, pellets and crumbles-definitions and methods for determining specific weight, durability and moisture content. 1976. In Feed Manufacturing Technology (H. B. Pfost, Ed). American Feed Manuf. Assn., Arlington, VA.

[b]American Society for Testing and Materials, ASTM E 61, Specifications for Wirecloth Sieves for Testing purposes.

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EFFECT OF WHEAT GLUTEN ON UNDERWATER PELLET STABILITY OF PELLETED CATFISH AND SHRIMP DIETS

by

Jed W. McKee

B.S., Kansas State University, 1987

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1988

ABSTRACT

To investigate the effect wheat gluten has on underwater pellet stability, levels of 2.5, 5, and 10 percent vital wheat gluten were added to catfish and shrimp diets. A diet including a pellet binder (Nutraflex 40 Mega) was added to the study for further comparison. The diets were pelleted through a 4.8mm x 50.8mm straight bore die at 70C or 85C. Production rate, power consumption and pellet quality were monitored.

The pellets were subjected to a representative and reliable water stability test. Underwater times investigated were 0.5, 1, 2, 4, 8, and 16 hours. Product remaining after the test was dried, weighed and reported as "Water Stability Index" (WSI%).

The results of the processing portion of the study showed a significant increase in the production rate and a consistently lower power consumption with the higher pelleting temperature. These results were expected due to the lubricating effect of steam. The fines data indicated that pellets with wheat gluten produced fewer fines than the control and binder diets. Apparently wheat gluten plays a role in pellet quality enhancement, however, it had no effect on Pellet Durability Index. All PDI's, including the binder, ranged from 93-96 percent which is considered good to excellent.

It was apparent from the study that both the level of wheat gluten and the level of steam conditioning effected WSI. Increased WSI due to the steam conditioning was obvious in the wheat gluten diets but was substantially better in the control and binder diets.

Results indicated an enhanced WSI corresponding to increasing levels of wheat gluten. The 2.5 percent diets produced a higher WSI than the control and binder diets. The 5 and 10 percent diets retained their pellet shape through the entire process.

This study clearly showed an enhanced underwater pellet stability due to the wheat gluten and elevated pelleting temperature.