RESEARCH REPORT 1987-1

— 79 —

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INTERNATIONAL ASSOCIATION OF FISH MEAL MANUFACTURERS

REPORT OF PROJECT ON IDENTIFYING FACTORS RESPONSIBLE FOR CAUSING GIZZARD EROSION IN BROILER CHICKS: PART 2

PROJECT A87.1 **CONFIDENTIAL**

BACKGROUND INFORMATION

Occasional parcels of fish meals have been shown to cause erosion of the gizzard lining in broiler chickens when fed at high levels in the broiler diet (7% or greater). Research conducted in Holland, Peru, UK and Japan has been unable to conclusively identify the factors in fish meal which cause the gizzard erosion, and thus no practical measures have been suggested for being able to eliminate the causative agent from fish meals.

Research to date has suggested the following facts, namely:

(1) Gizzard erosion is not uniquely associated with fish meal feeding and can be caused by other components of the animal feed such as vitamin K, mould growths, etc.

(2) High levels of histamine when artificially fed to chickens can cause gizzard erosion, but the active levels are much higher than those likely to be found in practical fish meals.

(3) Heating fish meal for five hours at 130°C or 16 hours at 80°C can result in a product capable of causing gizzard erosion. Thus heating does seem to be an important factor.

(4) The presence or absence of antioxidant in the fish meal does not appear to play a role.

(5) The gizzard erosion factor might be concentrated in the fine particles of fish meal.

In Part 1 of the study (February 1987) commercial samples of fish, presscake or fish meal were taken from five factories in Chile resulting in 29 samples of intermediary or final products which were coded and sent to the Fishing Industry Research Institute (FIRI) in South Africa for gizzard erosion assessment. Fish taken before the cooker, presscake without stickwater concentrate and presscake with concentrate were dried in laboratory ovens in Chile prior the shipment in order to facilitate ease of shipment and ease of mixing of diets in South Africa. Fish meals taken directly after milling before addition of antioxidant, or fish meals with antioxidant manufactured into pellets and stored for three to six months or fish meals with antioxidant stored in bags for three to six months were sent directly to FIRI in South Africa for assessment.

The samples dried in laboratory ovens in Chile all had high gizzard erosion scores caused by the heat treatment in the laboratories. This result lends further support to the theory that excessive heat during drying results in the production of gizzard erosion factors within fish meal.

The data from the meals did not support the theory that the quality of fish meal from a combination of direct-fired dryers and steam dryers was superior to direct-fired dryers alone. It was agreed that these tests should be repeated as Phase 2 of the experiment with certain modifications based on experiences gained in Phase 1.

It was proposed that samples should be taken from less than five factories and the different types of drying equipment would be represented. In addition to the samples taken from the various stages envisaged, a sample of cyclone dust would be taken. Wet samples would be carefully dried at FIRI. FIRI would undertake proximate analysis of samples and analysed for gizzerosine and histamine. The possible of analysing for free histidine and biogenic amines in Cambridge University and Denmark respectively was also discussed.

A protocol was prepared giving guidance on sampling for the second part of the gizzard erosion study and this was circulated to all members of the Scientific Committee on 21st July 1987 (Circular letter no. 1987/52/S). In October 1987 the necessary samples were collected and stored in Chile. The dry samples were returned to FIRI for analysis, some of which showed high gizzard erosion scores. Unfortunately the wet samples were not stored under ideal conditions in error and mould growth was seen on them. It was therefore decided to abort this set of samples and to repeat the sampling procedure.

A further set of samples were prepared and stored in Chile in October 1988. These were successfully air mailed to FIRI in frozen storage and arrived in good condition on 2nd November 1988.

DETAILS OF THE SAMPLES

Samples were taken from two factories (coded 02 and 10) and represented the following products:

	Code
Raw fish	M-1
Cooked fish	M-2
Presscake	M-3
Concentrate	M-4
Presscake and concentrate	M-5
Fish meal without ethoxyquin	M-6
Fish meal with ethoxyquin	M-7
Cyclone solids	M-8
Fish meal pellets	M-9

All frozen (wet) samples were immediately transferred to a cold store at -18°C, while the fish meals with and without ethoxyquin, the pellets and the cyclone fines were kept at room temperature.

FACTORY PROCESSING CONDITIONS IN CHILE

The following details were provided concerning the conditions of the factory processing in Chile

		Factory 02	Factory 10
Raw materials		Anchoveta	Anchoveta
Time between capture and processing		7 hours	8 hours
Ambient temperature		16°C	18°C
Residents time in the cooker		20 minutes	13 minutes
Cooker temperature		92°C	94-98°C
Residents time in the press		10 minutes	10 minutes
Residents time in dryer 1		30 minutes	15 minutes
Temperature of entry gases in dryer 1		500-550°C	508°C
Temperature of exit gases in dryer 1		86°C	85°C
Residents time in dryer 2		25 minutes	15 minutes
Temperature of entry gases in dryer 2		450°C	472°C
Temperature of exit gases from dryer 2		98°C	76°C
Temperature of fines in the cyclone		70°C	58°C
Temperatures in the various stages of the concentrator	Stage 1	120°C	131°C
	Stage 2	105°C	125°C
	Stage 3	86°C	102°C
	Stage 4	47°C	40°C
	Vacuum KG/cm^2	-0.50	-0.90
Percent solids in concentrated liquor		44%	53%

PROCESSING OF WET SAMPLES IN FIRI, SOUTH AFRICA

Almost all of the frozen samples were processed and/or dried within two weeks of arrival with the exception of the raw fish from factory 10 which remained in frozen storage for approximately 20 weeks (see below).

Processing details were as follows:

Raw fish samples (M1) from factory 02 were defrosted on 16th November 1988 and cooked separately in a double walled steam pot for twenty minutes each with constant stirring. While still hot, the fish was transferred to a batch press capable of pressing the fish up to 2,000 lbs/square inch (13,800kPa). The liquor was collected and centrifuged, but no oil could be separated. All the liquor was added back to the fish before drying.

This, and all other wet samples, including those that were not cooked at FIRI, were spread out onto trays in layers of less than 2 cm thick and dried in batches. Drying was in a forced air circulation oven at between 48° and 54°C for the time shown in Table 1. Drying times could be up to 23 hours. All samples were stirred and broken up on the trays at approximately hourly intervals to facilitate drying. All dried samples were ground and returned to -18°C for storage.

In the case of stickwater concentrate (samples M-4) it was hoped to dry these by means of a spray drying process, but due to the disruption as a result of relocation of the Institute, the samples have to be dried in the drying of mentioned above. This resulted in considerable case-hardening, and longer drying times. The final product was first mixed into the ration before an attempt was made to grind it.

Raw fish samples (M-1) from factory 10 were kept frozen at -18°C until 23rd March 1989, the delay being due to the relocation of the Institute. On this date they were defrosted and cooked in a double boiler with constant stirring. As the heat output of this pot was not as efficient as before, each batch was heated for 30 minutes. There was no pressing and no separation of oil before drying. Drying was done as described above.

TABLE 1
Drying times and temperatures

	Code	No. of containers	Factory 02 Temperature (°C)	Time (hrs)	Factory 10 Temperature (°C)	Time (hrs)
Raw fish	M-1	4	48	12.5	54	23
Cooked fish	M-2	4	53	7	50	11
Presscake	M-3	2	53	7	45	8
Concentrate	M-4	1	54	16	54	23
Presscake and concentrate	M-5	2	45	8	45	8

METHOD FOR GIZZARD EROSION TEST

Samples with the same M-code numbers were combined during the last week of March in preparation of the chicken test (see enclosed packing list). The meals prepared above were incorporated into rations at a rate of 70 parts of the test material, 30 parts of yellow maize meal, and 1.5 parts of a chicken vitamin premix. These rations are not balanced for the requirements of the chicken and are designed to accelerate any gizzard erosion effects.

The cages used for these tests are five tier heated battery brooders. The temperature is maintained at 37°C. Broiler chickens were obtained from a local hatchery at day-old. Before the test, the chickens received a balanced ration containing 12% of fish meal that is known not to cause any gizzard erosion for four days.

On the fourth day, the chickens were weighed and distributed to the cages so that all cages contain about the same mass of chickens. There were ten chickens in each of three cages for each fish meal sample. The chickens received the test diets ad lib for seven days. On the eleventh day the chickens were weighed, and killed in chloroform vapour. The gizzards were removed, opened and inspected for signs of lesions. A scoring system is used that assigns zero to a gizzard with no erosion or roughness and three for maximum erosion or roughness. Any gizzard that shows signs of bleeding is given a score of at least one. All scoring is done by one person, who is unaware of the origin of the gizzards.

It follows that the maximum total erosion or roughness score for the thirty chickens in each group is 90. These scores are reported mainly because of the test lay-out permits statistical processing. In addition, to make the figures comparable with results published by others, Janssen counts were derived from the scores as follows:

Erosion score less than 1	- add zero to Janssen count for cage
Erosion score of 1 to 2	- add two to Janssen count for cage
Erosion score greater than 2	- add three to Janssen count.

$$\text{Janssen score} = \frac{\text{(Janssen count for cage) x 100}}{\text{(no of chicks per ration)}}$$

Where possible, the gizzards of chickens that died during the test were also inspected for lesions, but where this was not feasible corrections were made to the treatment scores.

ANALYTICAL METHODS

All samples were measured for the moisture, crude protein, crude fat and salt. The majority of samples were measured for available lysine and the raw fish was measured for total volatile basic nitrogen.

RESULTS AND DISCUSSION

Table 2 gives the TVN values for the raw fish and the chemical analysis of the fish meals that were made in the laboratory or in the factory. Although the ash content of the fish meals was not analysed it can be estimated as a difference from 100% after deducting moisture, protein and fat.

The results show that cooking and pressing undertaken in the laboratory by FIRI could not remove oil to any extent (samples 02-M-1; 10-M-1). It also appeared that the cooked fish received from Chile had already lost some of its oil and liquor, probably through natural drainage, when received in South Africa (samples 02-M-2; 10-M-2).

With regard to the samples from factory 02, the estimated ash figures suggest that samples 02-M-1 through to 02-M-6 were from the same processing run, but that 02-M-7 and 02-M-9 were from a different run.

With regard to factory 10, commercial pressing appeared to be very effective in lowering the fat content of presscake and meal compared with factory 02. Surprisingly the raw fish (sample 10-M-1) seemed to have a low bone content. The estimated ash figures suggest that samples 10-M-2 through to sample 10-M-6 seem to come from the same processing run, but no clear deduction could be made on the sources of sample 10-M-7 and sample 10-M-8.

The available lysine samples of the commercially prepared meals approximately 4% seemed to be reasonable. However the available lysines of the meals prepared from the raw fish or cooked fish in the laboratory in South Africa appeared to be extremely low. At first this suggested that the heating regime used in South Africa had been excessively high, but reference to Table 1 shows that the temperatures never exceeded 54°C although the heating period was long at up to 23 hours. Probably a more reasonable explanation of the low available lysine figures is the possibility that these temperatures and moisture levels encouraged the development of bacteriological, particularly in the samples made from the raw fish which would contain considerable levels of bacteria on the surface of the fish and in the gut. The baterial growth could have resulted in hydrolysis of the protein and the release of free lysine. Free lysine is not normally measured by the Carpenter method for available lysine.

Table shows the results of the chicken feeding trial.

The meal prepared from raw fish from factory 02 (sample 02-M-1) was very poorly consumed, and chicken growth was considerably depressed. This group also experienced the greatest mortality and had severe gizzard erosion. The meal prepared from the raw fish from factory 10 (10-M-1) seemed of a better quality than that from factory 2 but there was some minor depression in growth, low level of mortality and severe gizzard erosion. It is highly likely that these

laboratory prepared meals were unrepresentative particularly as they may have had a high bacteriological level. It is probably wise to ignore these results.

Fish meals prepared from samples of cooked fish, presscake, dry concentrate and presscake and concentrate from both factories showed acceptably low Janssen scores for gizzard erosion (below 50) suggesting that there was no gizzard erosion causing agent in these products prior to drying.

Mortalities in groups that received rations containing dry concentrate (samples 02-M-4 and 10-M-4) were high due to severe diarrhoea in these groups. Growth seems also to have been affected. The gizzards of these chickens were enlarged and stained dark brown. These samples proved difficult to dry and it is suspected that the diarrhoea might have been caused by high salt levels.

Janssen scores for fish meals and cyclone solids from factory 2 proved intermediate between the figure of 50 and below which is regarded as completely safe and the figure of 100 and greater which in South Africa is interpreted as not to be used in poultry diets. In other words these products were probably acceptable. However the fish meals and cyclone solids from factory 10 were unacceptable in terms of Janssen scores and mortality.

Reference to the earlier section in this report on "factory processing conditions in Chile" suggest that the drying conditions in factory 10 were slightly lower than those in factory 2 although the conditions in both factories appeared to be satisfactory and within normal limits. It is possible that factory 2 was utilising a drying technique which prevented radiated heat passing from the burner to the dryer. This technique may not have been employed in factory 10. It has been thought that radiated heat can play a significant role in gizzard erosion factor formation. This should be explored more fully by our Chilean Members in order to assist in the interpretation of these results.

DR. BARLOW - THE FOLLOWING TELEX WAS SENT TO SR J ZALDIVAR AND DR J P A WESSELS

GUIDANCE ON SAMPLING RE GIZZARD LESION STUDY (2ND PART)

ZALDIVAR/WESSELS/DUTHIE MET ON 30 APR 87 TO DISCUSS PROCEDURES FOR TAKING SAMPLES IN CHILE FOR TESTING AT FIRI.

IT IS PROPOSED TO TAKE SAMPLES AT THREE FACTORIES. IT IS UNDERSTOOD THAT THE DRYING EQUIPMENT DIFFERS BETWEEN THESE.

POSSIBLY AS MUCH AS 2000 TE FISH PER PLANT, DISTRIBUTED AMONG UP TO 12 STOCK TANKS, WILL BE AVAILABLE FOR PROCESSING AT EACH PLANT. TO ATTEMPT TO FOLLOW REPRESENTATIVE MATERIAL THROUGH THE PROCESS, IT SEEMS MOST FEASIBLE TO REGARD THE MID SECTION OF THE RAW MATERIAL SUPPLY AS REPRESENTING TIMEWISE THE AVERAGE QUALITY CONDITION, NARROWING SELECTION DOWN TO A PARTICULAR STOCK TANK AND A REPRESENTATIVE QUANTITY OF ABOUT 40 TE.

THE IDEA IS TO TRY TO FOLLOW THIS SORT OF QUANTITY THROUGH THE PROCESS ON A PLUG FLOW BASIS, TAKING ACCOUNT OF THE DIFFERENT RESIDENCE TIMES IN THE VARIOUS ITEMS OF EQUIPMENT, TO ESTABLISH A RELATIONSHIP BETWEEN THE DIFFERENT SAMPLES.

THE SAMPLES IT IS PROPOSED TO TAKE FROM THE VARIOUS STAGES AND QUANTITIES OF WET OR DRY MATERIAL ARE:

NO	SAMPLE	AMOUNT (KG)
1	AFTER BREAKER	8
2	AFTER COOKER	8
3	PRESS CAKE	4
4	STICK WATER CONC.	4
5	PRESS CAKE + STICK WATER CONC.	4
6	MEAL AFTER DRYER	2
7	MEAL AFTER ETHOXYQUIN ADDITION	2
8	CYCLONE FINES	2
9	STORED MEAL (COMMERCIAL CONDITIONS)	2

IT IS ENVISAGED THAT FOR SAMPLES 1, 2, 3 AND 5, THREE SUB-SAMPLES (BUCKETSFULL) TAKEN AT APPROPRIATE INTERVALS (E.G. 15 MINUTES APART IN THE CASE OF SAMPLES 1 AND 2, AND 7 MINUTES IN THE CASE OF SAMPLES 3 AND 5) SHOULD BE MIXED AND SAMPLED TO PROVIDE THE ACTUAL SAMPLE.

ARRANGEMENTS TO CHILL/DEEP FREEZE THE MOIST SAMPLES PROMPTLY AFTER THEY ARE TAKEN ARE OBVIOUSLY NECESSARY.

ALL MOIST SAMPLES WILL BE DESPATCHED TO FIRI IN A FROZEN CONDITION.

DEPENDING ON THE TIME TAKEN FOR FIRI TO RECEIVE THE SAMPLES AND TO PREPARE THEM AND MOUNT THE STUDY, IT MIGHT BE REASONABLE TO AIRMAIL TO FIRI SAMPLES (NO 9) OF THE COMMERCIALLY STORED MEALS TO GAIN THE ADVANTAGE OF INCLUSION IN THE STUDY ALONG WITH THE RELATED SAMPLES (OR EVEN ? TO SAMPLE ON TWO OCCASIONS TO ALLOW BETTER EXAMINATION OF STORAGE EFFECT).

IT IS UNDERSTOOD THAT DUPLICATE SAMPLES CAN BE TAKEN AND HELD IN DEEP FREEZE IN CHILE FOR SUFFICIENT TIME TO COVER FOR ANY PROBLEMS ENCOUNTERED BY FIRI (LIAISON BETWEEN WESSELS AND ZALDIVAR).

SAMPLE 1 IS REGARDED AS ESSENTIAL TO PROVIDE AN INDICATION OF THE STATE OF THE RAW MATERIAL AT THE POINT OF PROCESSING. IT WILL BE ANALYSED BY FIRI FOR TVN, HISTAMINE ETC.

ACCORDING TO DISCUSSION IN THE SCIENTIFIC COMMITTEE FIRI WILL HOLD QUANTITIES OF PREPARED SAMPLES FOR POSSIBLE BIOGENIC AMINE (JENSEN, ESBJERG) AND HISTIDINE (MILLER, CAMBRIDGE) ANALYSES.

RECORDING OF ALL POSSIBLE DETAILS IN CHILE WILL GREATLY ENHANCE THE VALUE OF THE STUDY, E.G. SPECIES OF FISH, TIME FROM CATCHING TO ENTERING PROCESS, AMBIENT TEMPERATURE, PROCESSING TIMES, COOKER AND DRIER TEMPERATURES.

WILL THE RECIPIENTS PLEASE CORRECT ANY MISUNDERSTANDINGS AND CLARIFY ANY POINTS AS NECESSARY.

REGARDS

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INTERNATIONAL ASSOCIATION OF FISH MEAL MANUFACTURERS

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PROJECT NO: A86.1

CONFIDENTIAL

Background Information

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Research to date has suggested the following facts, namely:

(1) Gizzard erosion is not uniquely associated with fish meal feeding and can be caused by other components of the animal feed such as vitamin K, mould growths, etc.

(2) High levels of histamine when artificially fed to chickens can cause gizzard erosion, but the active levels are much higher than those likely to be found in practical fish meals.

(3) Heating fish meal for five hours at 130^{o}C or 16 hours at 80^{o}C can result in a product capable of causing gizzard erosion. Thus heating does seem to be an important factor.

(4) The presence or absence of antioxidant in the fish meal does not appear to play a role.

(5) The gizzard erosion factor might be concentrated in the fine particles of fish meal.

TABLE II

Analysis of laboratory and factory made fish meals and TVBN of raw fish

Bin code	Sample	TVBN (mg/100g)	Moisture content (%)	Crude Protein (%)	Crude Fat (%)	Salt (NaCl) (%)	Estimated Ash	Available Lysine of meal (%)
02 M-1-A	Raw fish	46	9.1)	54.9)	15.2)	2.5)		
02 M-1-B	Raw fish	31	9.6) 9.4	56.1) 56.1	14.6) 15.7	2.3)	18.8	0.83
02 M-1-C	Raw fish	36	9.8)	59.2)	16.3)	2.5)		
02 M-1-D	Raw fish	40	8.9)	54.1)	16.6)	2.2)		
02 M-2-A	Cooked fish	-	7.1)	58.3)	7.4)	2.6)		
02 M-2-B	Cooked fish	-	9.5) 9.2	59.9) 58.7	6.4) 7.3	2.3)	24.8	1.23
02 M-2-C	Cooked fish	-	10.2)	56.8)	8.2)	2.9)		
02 M-2-D	Cooked fish	-	9.9)	59.8)	7.1)	2.5)		
02 M-3-A	Presscake	-	9.2) 8.8	64.5) 66.9	7.3) 5.5	1.2)	18.8	
02 M-3-B	Presscake	-	8.4)	69.3)	3.7)	1.3)		
02 M-4	Dry concentrate	-	18.7	51.6	0.4	11.1	29.3	
02 M-5-A	Presscake and concentrate	-	7.5) 7.8	67.8) 67.3	3.6) 3.3	4.3)	21.6	
02 M-5-B	Presscake and concentrate	-	8.1)	66.8)	3.0)	4.0)		
02 M-6	Fish meal no EQ	-	5.7	70.4	2.9	1.4	21.0	
02 M-7	Fish meal with EQ	-	6.4	74.2	8.6	1.3	10.8	
02 M-8	Cyclone solids	-	9.4	72.4	8.6	1.6		4.39
02 M-9	Fish meal pellets	-	10.9	69.5	9.0	2.1	10.6	4.97
10 M-1-A	Raw fish	31	13.1)	61.9)	22.5)	1.8)		
10 M-1-B	Raw fish	38	14.5) 13.6	49.7) 52.6	21.5) 22.7	2.5)	11.1	1.91
10 M-1-C	Raw fish	35	12.9)	49.4)	24.9)	2.0)		
10 M-1-D	Raw fish	28	13.9)	49.5)	21.8)	2.1)		
10 M-2-A	Cooked fish	-	6.6)	55.7)	10.7)	2.3)		
10 M-2-B	Cooked fish	-	9.4) 8.8	58.2) 56.7	8.6) 11.2	2.1)	23.3	2.78
10 M-2-C	Cooked fish	-	6.8)	58.7)	10.8)	2.1)		
10 M-2-D	Cooked fish	-	12.5)	54.3)	14.6)	2.3)		
10 M-3-A	Presscake	-	8.2) 7.5	69.8) 70.0	2.9) 2.8	0.9)	19.7	
10 M-3-B	Presscake	-	6.7)	70.1)	2.6)	1.2)		
10 M-4	Dry concentrate	-	8.8	56.4	0.6	13.8	34.2	
10 M-5-A	Presscake and concentrate	-	9.4) 8.5	69.1) 68.5	1.6) 2.0	2.6)	21	
10 M-5-B	Presscake and concentrate	-	7.5)	67.8)	2.3)	2.9)		
10 M-6	Fish meal no EQ	-	8.0	66.9	2.4	3.9	22.7	
10 M-7	Fish meal with EQ	-	8.4	67.7	8.3	4.2	15.6	
10 M-8	Cyclone solids	-	11.9	65.6	7.4	5.4		3.76
10 M-9	Fish meal pellets*	-	-	-	-	-		-

*Insufficient sample available for both gizzard test and analysis.

In order to see whether standard commercial processing of fish meal can influence the production of the gizzard erosion factors, commercial samples of fish, presscake, or fish meal were taken from five factories in Chile, resulting in 29 samples of intermediary or final products which were coded and sent to South Africa for gizzard erosion assessment.

Details of the Samples

Five factories provided six samples (1 factory only provided 5 samples) in July 1986 with the samples coded as follows:

Code	Details of Sample
4	Fish before cooker
2	Presscake without stickwater concentrate
3	Presscake with concentrate
1	Fish meal after mill and before addition of antioxidant
5	Fish meal pellet with 3-6 months of storage
6	Fish meal in bags with storage of 3-6 months

Samples 2, 3 and 4 were dried in a laboratory oven prior to shipment in order to facilitate ease of shipment and ease of mixing of diet in South Africa. The drying of the samples in the laboratory had not been closely controlled, but it seemed that these had been dried in a hot air oven at about 110°C for approximately 4 hours.

The details of the samples per factory is as follows:

FACTORY A

Samples 1 to 4 correspond to sardine with 16 hours of time between catching and processing. Samples 5 and 6 correspond to the production of March/86.

They have two concentration plants (evaporators) ATLAS four steps each with

these conditions.

The drying is being performed through a combination of two direct fire dryers followed by two steam dryers as finishers.

FACTORY B

Raw material sardine 9 hrs. old (samples 1 to 4). Sample 5 corresponds to the production of April and sample 6 to May.

They have on ATLAS evaporator and one Chilean made, both with 4 effects.

The drying is being held in a combination of two fire dryers followed by two steam dryers in series and/or parallel.

FACTORY C

Samples 1 to 4 correspond to sardine 14 hrs. old. Sample 5 is from April and 6 from June.

They have one evaporator plant 5 steps Chilean made (type Rock) working with a steam pressure between effects of 4 kg per SQ CM.

Drying is being performed in similar way as factories A and B.

FACTORY D

Samples 1 to 4 correspond to sardine 13 hrs. old. (Between catching and cooking). Sample 5 is April production and 6 is unknown.

They have two concentration plants Rock of 4 steps each. The temperature of the first effect is 120°C and the last is 40°C.

The drying is performed with direct fire dryer.

FACTORY E

Samples 1 to 4 sardine 14 hrs. old. Samples 5 and 6 correspond to fishmeal of April.

They have 4 evaporator plants 4 steps each. Working at 94°C at the inlet and 38°C, at the outlet; pressure between effects is 4 kg per SQ CM. They work with direct fire dryers.

Feeding Tests and Gizzard Erosion Assessment

Twenty-nine fish meals marked by codes only were received by the Fishing Industry Research Institute in Cape Town from Chile. These were mixed into test diets which consisted of 60 parts of each of these meals, 40 parts yellow maize meal and 1,5 parts of a vitamin premix.

Four-day old broiler chickens (Ross, Cornish X White Rock) were placed on experiment for seven days after being starved overnight. The nine chickens per group were selected in such a way that the total masses of groups fell within a narrow-range. Three groups of nine chickens each were allocated at random to each of the thirty treatments, the thirtieth treatment being a South African anchovy meal, made up into a test diet as described above. Food was offered ad lib and food intake as well as initial and final individual chicken masses were recorded.

At the end of the seven-day test period chickens were starved for four hours and after weighing killed with an overdose of chloroform and dissected to remove the gizzards for inspection. Gizzard lesion incidence was scored according to three different systems as follows:

Erosion score

Roughness score

Aggregate "Janssen" score

A score of 3 was allocated to a gizzard with severe erosion or pitting, and in the second system a score of 3 was given for heavy grooving or wrinkling (roughness) that does not penetrate the gizzard lining. The maximum possible treatment score for erosion or roughness of the gizzard lining was therefore 3 x 27 or 81. A gizzard that showed definite signs of bleeding was allotted a score of at least 1.

Gizzard erosion scores were also assigned according to a method described by Janssen, Wiertz & van Dilst[1] which ignores roughness and less severe erosion. Healthy gizzards scored 0 or 1. Clearly eroded gizzards scored 2 and gizzards severely eroded, often with haemorrhage in the linings, scored 3. An aggregate score for a group was calculated as follows:

$$\text{Aggregate score} = \frac{(\text{No with score 2} \times 2) + (\text{No with score 3} \times 3)}{\text{No of gizzards examined}} \times 100$$

These figures are given in the third column of data in the attached Table.

In South Africa fish meals that give rise to aggregate "Janssen" scores of greater than 100 are preferably not used in poultry diets and meals that give scores of less than 50 are regarded as completely safe.

Reference [1] Janssen, W.M.M.A., Wiertz, G. & Van Dilst, F.J.H. Nutritional research on the factor(s) causing gizzard erosion. Proc. of the 3rd Int. Conf. on Production Diseases in Farm Animals, Wageningen, September 1976, 75-76.

Results and Conclusions

The detailed gizzard lesion scores are presented in Table 1, and Table 2 presents a general assessment of the group samples and the aggregate "Janssen" score.

Correlation coefficients for the mean treatment erosion and mean treatment

roughness scores on the one hand, and body mass changes of the chicks on the other, were calculated and found to be, respectively:

$$r = -0.718$$

$$\text{and } r = -0.771$$

From Table 2 it can be seen that samples 2, 3 and 4 taken from all five factories had a high gizzard erosion score, probably caused by the heat treatment in the laboratories in order to dry the samples. This result lends further support to the theory that excessive heat during drying results in the production of gizzard erosion factors within fish meal.

Samples 1, 5 and 6 represent commercially produced fish meals before and after various forms of storage. Most samples were below the gizzard erosion score of 100 likely to result in problems in the field. These fish meals would be found to be acceptable by the poultry industry in South Africa. Samples from factories D and E appeared to be less likely to produce gizzard erosion than samples taken from factories A to C. This is particularly interesting in that factories D and E used only direct fired dryers, whereas A-C used direct fired dryers in combination with steam dryers in order to ensure lower temperatures during the final drying of the fish meal. These data would not support the theory that the quality of fish meal from a combination of direct fired dryers and steam dryers is superior to direct fired dryers alone.

GIZZARD LESION SCORES

TABLE 1

	Erosion	Roughness	Aggregate "Janssen" scores	Body Mass gain (g)
A1	$26,91^{de}$	$27,16^{ghi}$	108	$602,7^{g}$
2	$64,83^{m}$	$57,08^{m}$	268	$409,4^{l}$
3	$69,50^{m}$	$59,50^{m}$	300	$460,2^{jk}$
4	$34,71^{fg}$	$43,26^{ij}$	152	$253,1^{n}$
5	$12,25^{b}$	$14,50^{bc}$	26	$316,2^{m}$
6	$16,54^{b}$	$21,54^{cde}$	39	$716,9^{cd}$
B1	$28,86^{de}$	$28,56^{fg}$	110	$610,9^{g}$
2	$57,13^{kl}$	$46,47^{ijkl}$	233	$563,1^{h}$
3	$49,67^{hij}$	$52,95^{lm}$	192	$517,2^{i}$
4	$61,47^{lm}$	$52,97^{lm}$	246	$349,3^{m}$
5	$19,06^{bc}$	$24,23^{def}$	75	$511,1^{ij}$
6	$34,98^{fg}$	$32,91^{gh}$	126	$703,9^{cde}$
C1	$20,44^{cd}$	$24,47^{def}$	77	$769,4^{ab}$
2	$24,75^{cde}$	$23,50^{def}$	96	$715,5^{cd}$
3	$51,66^{ijkl}$	$48,50^{jkl}$	207	$736,6^{bc}$
4	$25,50^{cde}$	$25,25^{def}$	93	$474,5^{jk}$
5	$28,25^{ef}$	$30,25^{fg}$	141	$545,4^{hi}$
6	$13,90^{b}$	$13,68^{b}$	59	$665,3^{ef}$
D1	$13,75^{b}$	$12,66^{ab}$	46	$734,2^{bc}$
2	$42,94^{h}$	$41,56^{ij}$	180	$777,5^{a}$
3	$35,50^{g}$	$33,94^{gh}$	146	$518,3^{i}$
4	$44,75^{hi}$	$46,25^{ijkl}$	196	$653,5^{f}$
5	$14,00^{b}$	$15,25^{bc}$	30	$319,5^{m}$
E1	$13,38^{b}$	$21,44^{cde}$	15	$789,2^{a}$
2	$54,48^{jkl}$	$53,60^{lm}$	229	$710,2^{cd}$
3	$35,66^{g}$	$39,16^{hi}$	150	$522,6^{i}$
4	$45,50^{hi}$	$45,50^{ijk}$	200	$521,9^{i}$
5	$14,25^{b}$	$19,00^{bcd}$	37	$348,7^{m}$
6	$17,54^{b}$	$23,69^{def}$	46	$728,5^{cd}$
South African	$4,53^{a}$	$6,12^{a}$	8	$690,1^{def}$

a-m Within columns the same letter in superscript next to two means indicates that such means do not differ statistically significantly at the 5% test level.

GENERAL ASSESSMENT OF AGGREGATE "JANSSEN" SCORES

TABLE 2

	FACTORY				
	A	B	C	D	E
FISH BEFORE COOKER (4)	152	246	93	196	200
PRESS CAKE WITHOUT STICKWATER CONCENTRATE (2)	268	233	96	180	229
PRESS CAKE WITH CONCENTRATE (3)	300	192	207	146	150
FISH MEAL AFTER MILL AND BEFORE ADDITION OF ANTIOXIDANTS (1)	108	110	77	46	15
FISH MEAL PELLET WITH 3 TO 6 MONTHS STORAGE (5)	26	75	141	30	37
FISH MEAL IN BAGS WITH 3 TO 6 MONTHS STORAGE (6)	39	126	59	N.A.	46

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MEMORANDUM NO. 310

MARCH 1986

CONFIDENTIAL

REPORT ON VISIT TO THE DEPARTMENT OF AGRICULTURAL CHEMISTRY, UNIVERSITY OF TOKYO - FEBRUARY 1986

W W D Wagener

I. INTRODUCTION

The purpose of this visit was to gain further knowledge of the chemical gizzerosine, reported to cause gizzard erosion in chickens. The International Association of Fish Meal Manufacturers and the Institute were particularly interested in obtaining details of the method of analysis used by Japanese research workers.

At the University of Tokyo I met and talked to Professor Kenji Mori, Professor Tadashi Noguchi and Professor Hiroshi Naito, all of the Department of Agricultural Chemistry. I also attended a short talk on gizzerosine given by Professor Noguchi, and spent about one week in the laboratory observing an analysis for gizzerosine in fish meal. The analysis was done by Mr Yoshinori Ito, a graduate student, assisted by Mr Terao, an undergraduate student.

During the week in Tokyo I also met Dr Yoichi Ozawa (Section Manager, Amino Acids Department) and Mr Yasuhiko Toride (International Feed Additives Department) of the Ajinomoto Co. Inc. (S-8, Kyobashi 1 Chome, Chuo-ku, Tokyo 104, Telex J22690, J24711, Telephone 272-1111).

This company is able to commercially produce synthetic gizzerosine (the dl form) which is presently selling at the following prices:

10 mg	:	50 000 yen
100 mg	:	250 000 yen
1 g	:	1 000 000 yen (approximately 5000 U.S. dollars)

Ajinomoto has a branch office in South Africa called Ajinomoto Interamericana Industria E Comercio Ltda, trading as Super-Seasoning Sales South Africa.

Address: 5th Floor, Total House, Braamfontein, Johannesburg 2001
(PO Box 31509, Braamfontein 2017)
Telephone: (011) 339-3659 Telex 4-27199 SA

The manager is Mr Koji Nomoto.

II. NOTES MADE DURING A TALK ON GIZZEROSINE BY PROFESSOR T NOGUCHI AT THE DEPARTMENT OF AGRICULTURAL CHEMISTRY, FACULTY OF AGRICULTURE, UNIVERSITY OF TOKYO, 17 February 1986

The only active form of gizzerosine is the l-form (also called S- or (-) gizzerosine). The d-form (R- or (+)) has no effect on chickens.

Gizzerosine in fish meal

Fish meals were analysed that contained close to 200 mg/kg gizzerosine. Gizzerosine probably forms in fish meal on heating according to the following scheme:

Histidine (free) + Lysine (bound to protein)

$$\xrightarrow[-NH_3]{-CO_2}$$ Gizzerosine (bound to protein)

Fig. 1 illustrates the formation of gizzerosine in fish meal at 160°C and 130°C. (The ignition temperature of fish meal is more than 200°C.)

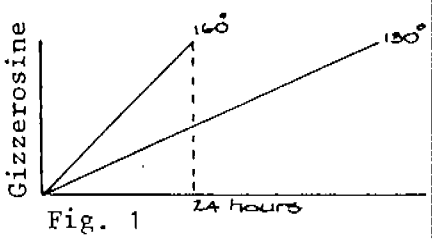

Fig. 1

Fig. 2 illustrates the formation of gizzerosine at 130°C in A: sardine meal plus histidine
and B: casein plus histidine.

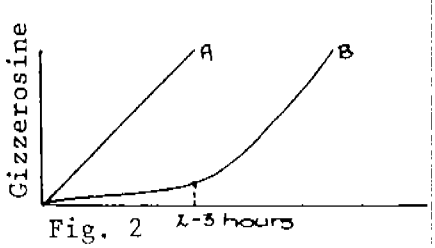

Fig. 2

Casein plus histamine also causes gizzard erosion in chickens, but it has not been established whether gizzerosine is involved.

Adding acetyl histidine to fish meal and heating also results in gizzerosine formation, but slower than with added histidine. Formation at 130°C starts only after 3 hours and reaches 40 mg/kg after 24 hours.

Fig. 3 shows the formation of gizzerosine in relation to the pH of the meal. Adding mercaptoethanol (a reducing agent) to the meal had little effect in inhibiting gizzerosine formation (Fig. 4) and addition of glutamic acid had no effect. Addition of glycine and lysine did result in less gizzerosine being formed, the effect of lysine being greater than that of glycine (Fig. 5 and Fig. 6).

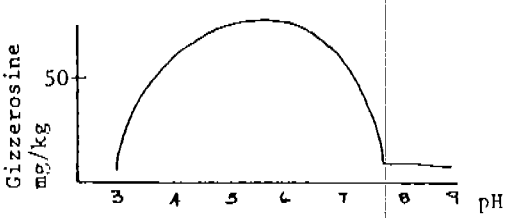

Fig. 3

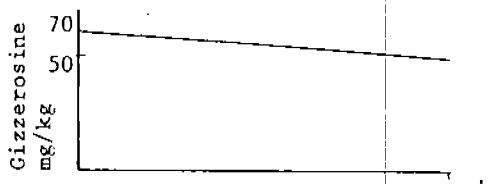

Fig. 4

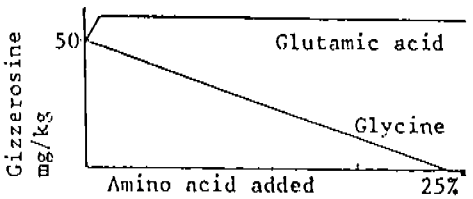

Fig. 5

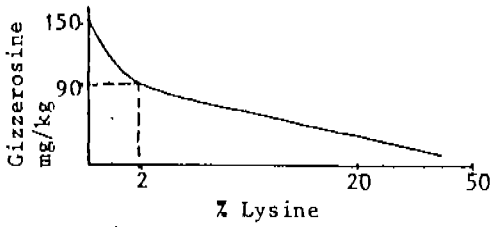

Fig. 6

Fig. 7 shows the effect of the addition of 2% lysine on the formation of gizzerosine at 130°C.

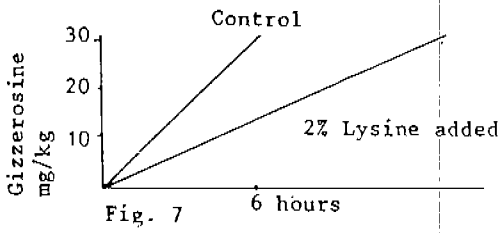

Fig. 7

The effect of the addition of ascorbic acid is even greater than that of lysine (Fig. 8). Addition of only 0,5% reduced gizzerosine formation by about 50%.

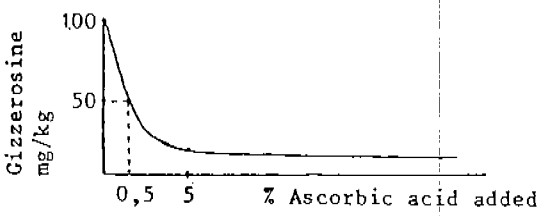

Fig. 8

By adding ascorbic acid to fish meal and heating, and subsequently doing a full amino acid analysis, the results as given in Table I were found. From this it seems as if ascorbic acid reacts with lysine and histidine.

TABLE I

Effect of ascorbic acid addition to fish meal

Amino acid	Control	+ 1% Ascorbic acid	+ 10% Ascorbic acid
Lysine	3,12	3,05	2,65
Histidine	1,65	1,58	1,54

The other amino acids remained unchanged.

The process whereby 2% lysine plus 0,5% ascorbic acid are added to fish meal to prevent gizzerosine formation has been patented by Noguchi *et al.*

After trypson hydrolysis of fish meal no gizzerosine was formed by histidine addition and heating - probably because of the α-amino groups that became available for reaction.

The effect of gizzerosine on chickens

Gizzard erosion effects generally start showing up when the feed as fed to the chickens contains more than 2 mg per kg gizzerosine. The acid secretion stimulatory effect of gizzerosine, as opposed to that of histamine, is probably partly due to the fact that gizzerosine is more stable in solution in the blood (it can be detected for at least 60 minutes) than histamine (only 2 to 3 minutes). When gizzerosine was injected into the stomach of a chicken, it was totally recovered in its blood during the hour following the injection. Determination of gizzerosine in blood is done according to the following scheme:

$$\text{Blood} \xrightarrow{\text{(centrifuge)}} \text{Plasma} \xrightarrow[\text{sulphosalicylic acid)}]{\text{(trichloroacetic acid or}} \text{Sample}$$

$$\xrightarrow[\text{(e.g. 100 } \mu\ell\text{)}]{\text{O P A solution}} \text{HPLC determination}$$

Gizzard erosion was tested using 5 chickens per test (broiler chickens, some strains were found to be more sensitive than others) and scoring from 0 to 3 for combined erosion and roughness (maximum score thus 15). It was found that 6 mg per kg dl-gizzerosine in the feed gave scores of 12 and higher. It was also found that gizzerosine makes the chicken's proventriculus enlarge and the walls get thinner, while the neck gets wider. Chickens fed with 6,25 mg per kg dl-gizzerosine had an average mass of 118 g and a stomach pH of 3,6, as opposed to 142 g and 4,4 for the controls.

Parietal cells of the chicken have two kinds of histamine receptors called H_1 and H_2. Gizzerosine, like histamine, works via the H_2 receptor to stimulate acid secretion, as diagrammatically illustrated in Fig. 9.

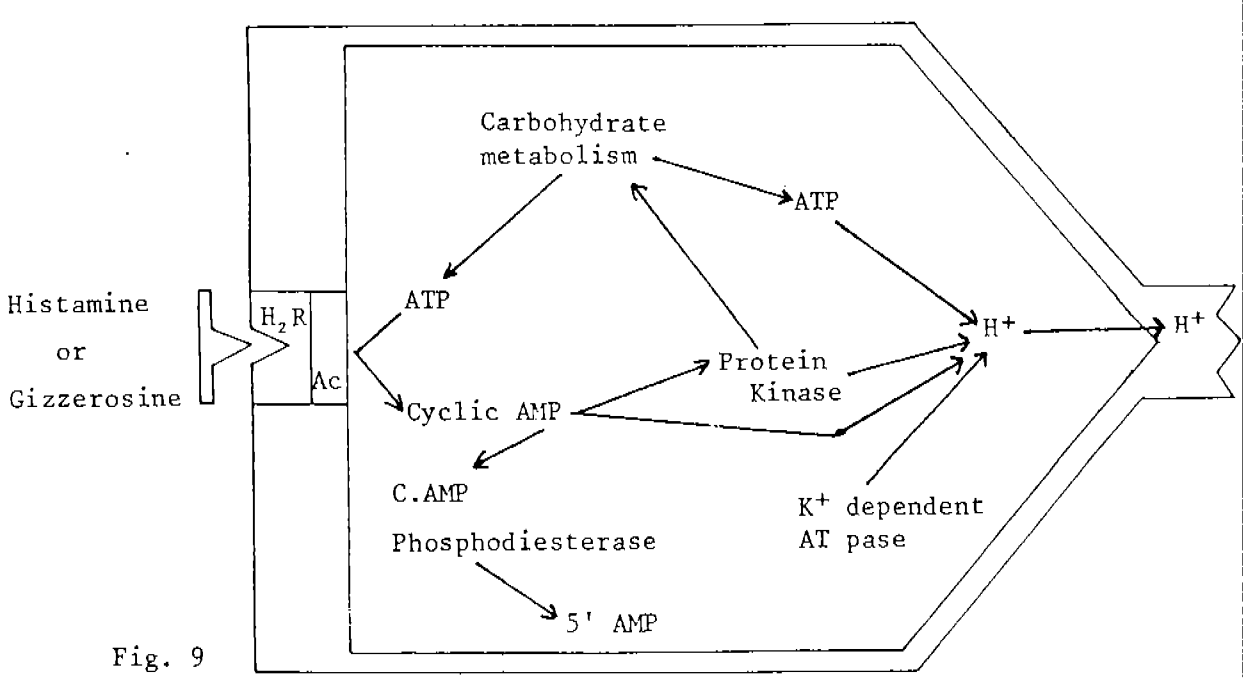

Fig. 9

(See also: Pharmacology of Histamine Receptors, edited by C.R. Ganellin & M.E. Parsons, published by John Wright & Sons, Briston, England, 1982.)

As shown in Fig. 10, cyclic adenosine monophosphate (AMP) formation is stimulated more by gizzerosine than by histamine.

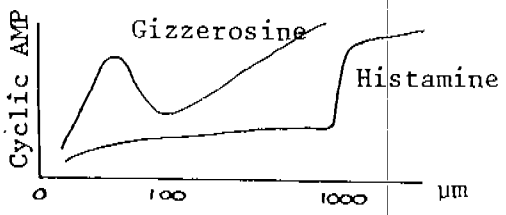

Fig. 10

Fig. 11 shows how the production of cyclic AMP by gizzerosine is suppressed more by cimetidine, an H_2 receptor antagonist, than by pyrilamine, an H_1 receptor antagonist.

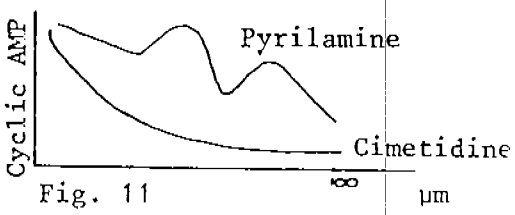

Fig. 11

Analyses of various kinds of fish products for gizzerosine

Three samples of canned mackerel were analysed for gizzerosine and levels of 0, 30 and 80 mg per kg were found. "Mirimboshi", a kind of seasoned sardine, was analysed and found to contain 24 mg per kg. After direct heating for 30 minutes, the level went up to 33 mg per kg.

The following results were found in experiments done in a Japanese factory with sardine meal:

Indirect drying resulted in no gizzerosine being found, while direct drying resulted in a level of 65 mg per kg. In another experiment, an overheated meal was found to contain 68 mg per kg, while meal from the same batch that was not overheated contained only 30 mg per kg. Ten fish meal samples from Thailand were analysed and found to contain 0 to 196 mg per kg gizzerosine.

Reference to Japanese work on gizzerosine

Most of the data and results mentioned in this report are included in the Masters thesis of Mr Yoshinori Ito of the Department of Agricultural Chemistry, Faculty of Agriculture, University of Tokyo (in Japanese).

The effect of gizzerosine on humans is not known.

III. THE DETERMINATION OF GIZZEROSINE IN FISH MEAL

Y. Ito, T. Noguchi and H. Naito

This method was published in *Analytical Biochemistry*, vol. 1, No. 1, 15 November 1985. I have re-written the method for the sake of clarity and also provided certain details not given in the original publication. The original publication should be referred to for details on chemicals and apparatus used, etc.

Method

Accurately weigh about 1 gram fish meal into a 50 mℓ hydrolysis tube and add 20 mℓ 6M HCl. Close tightly and hydrolyse for 24 hours at 110°C. After cooling, add 0,5 - 1 g of active charcoal (Norit), shake well and filter through a sintered glass filter using vacuum. Evaporate the HCl under vacuum, add 10 mℓ distilled water and evaporate the water. Repeat once more and dissolve in 10 mℓ distilled water.

Prepare a column of Amberlite 1R-CG 50 (200-400 mesh), 10 x 150 mm, in the N-ethylmorpholine form by passing at least 200 mℓ freshly prepared 1M N-ethylmorpholine through the column. Put sample on column and wash with 150 mℓ each of 1M and 2M N-ethylmorpholine. Elute the gizzerosine with 200 mℓ 3M NH_4OH. Evaporate the ammonia solution under vacuum, add 10 mℓ distilled water and evaporate again. Repeat once more and dissolve sample in 2 mℓ distilled water. The Amberlite is regenerated by washing with 3M HCl, distilled water, 3M NaOH and distilled water. Equilibrate a LiChroprep RP-8 column (Merck) by passing through at least 100 mℓ 0,05N triethylamine acetate buffer pH 7,0 at a flow rate of 1,5 mℓ/minute (freshly prepared by adding 0,05N acetic acid to 0,05N triethylamine until the pH is 7,0). This is best done by connecting the column to an HPLC pump and injector. Fractions are collected at the column outlet. Inject the sample onto the column and collect the first 50 mℓ of eluate (at 1,5 mℓ/minute with the same buffer). Lyophilise the sample and dissolve in 1 mℓ distilled water. The LiChroprep column is regenerated with iso-propanol. Equilibrate a column of ODS 2201-N (Senshupack) with the same buffer as above (0,05N triethylamine acetate) by passing at least 100 mℓ through at a flow rate of 1,5 mℓ/minute. Inject 100 µℓ of the sample onto the column and collect the first 30 mℓ of eluate (flow rate still 1,5 mℓ/minute). The column can be connected to a U-V detector set at 213 nm. Lyophilise sample and dissolve in 1 mℓ of distilled water.

The abovementioned two column separations can be checked by using a solution of synthetic gizzerosine, e.g. starting with 0,05 mg in 2 mℓ distilled water.

To 200 µℓ (or a suitable quantity) of the sample or 200 µℓ of a 0,01 mg/mℓ solution of gizzerosine in distilled water, add 100 µℓ boric acid buffer (prepared by adding concentrated potassium hydroxide solution to 0,4M boric acid containing 0,1% Brij 35 until the pH reaches 10), 100 µℓ ethanethiol and 100 µℓ O-phthaldehyde reagent (100 mg dissolved in 100 mℓ methanol). Mix well, keep at toom temperature for 10 minutes and add 100 µℓ ethylenediamine solution (10% methanol, v/v) to remove unreacted O-phthaldehyde.

Inject 100 µℓ (or a suitable quantity) of this for HPLC separation with conditions as shown in the publication. First inject the standard gizzerosine to establish its retention time. If no separation or bad separation is found between the gizzerosine peak and the large peak emerging just in front of it (see chromatograms in publication), the elution schedule must be slightly changed (e.g. phosphate buffer 82% to 78% or 82% to 80% or 78% to 76%) until a separation is achieved.

The concentration of gizzerosine in the fish meal is calculated as described in the publication. The lower detection limit for this method is about 2 mg per kg gizzerosine.

Conclusion

The method as published is fairly time-consuming, the analysis time being about one week, although a lot of samples can be done simultaneously depending on the apparatus available.

Work in our laboratory is at the moment directed towards shortening the method by finding alternative clean-up procedures and/or derivatising agents.