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International Fishmeal and Oil Manufacturers Association

THE VITAMIN D AND VITAMIN A CONTENT OF FISH MEALS FOR FISH FEEDS

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STRICTLY CONFIDENTIAL

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Summary and Recommendations

The EC limit for maximum content of vitamin D in fish feeds is believed to be too low (3,000 iu/kg). Fish meals which may be included at up to 70% of some fish feeds, are believed to contain high levels in some instances. As a result, the EC limit might be exceeded. In the absence of reliable data, and doubt about the reliability of the chemical method for determining vitamin D in fish meals, both chemical and biological tests have been compared. Samples of fish meal (16) representing commercial production world-wide were obtained to compare chemical HPLC with chick bioassay methods. Satisfactory agreement was found between the two methods.

Vitamin A content was also determined (chemically) to see if it might be used to predict vitamin D content. No relationship was found between the content of the two vitamins. Wide variations were found in the vitamin D and vitamin A content of the fish meals. Vitamin D levels found ranged between 182 iu/kg and 3,705 iu/kg, and vitamin A between 2,240 iu/kg and 243,710 iu/kg.

It is recommended that the current practice of supplementing fish feeds with both vitamin D and vitamin A should continue. This will in many instances result in the vitamin D content of the feed exceeding the EC limit. It is further recommended that a case be made to get the maximum limit on vitamin D content of fish feeds in the EC raised.

Background:

The EC has set a maximum limit on the vitamin D content of fish feeds of 3,000 iu/kg. This limit applies in feeds to which supplementary vitamin D is added, and it includes both the added and naturally occurring vitamin D. It is intended to protect the human consumer of the fish from excessive vitamin D intake.

Data on vitamin D content of fish meals, though limited, indicates it could be high, and may result in the EC limit being exceeded. More information on vitamin D content of fish meal is needed.

Earlier the EC set a maximum limit of 2,000 iu vitamin D in fish feeds. This is below the requirement of some fish, for example, salmonids. Details of this original limit, and the need to raise it are contained in the report "The Feeding Stuffs Regulations 1988 - Maximum Content of Vitamin D permitted in Fish Feeds" (Appendix 1). This report also considers the efficacy and safety of vitamin D.

The EC limit has now been raised to 3,000 iu/kg largely as a result of a Danish group showing that the previous limit could cause vitamin D deficiency to occur in some farmed fish. This new limit could still be exceeded in fish diets with a high fish meal content (over 50%) as already mentioned. Furthermore, the chemical method to determine vitamin D in feedstuffs is believed to be unreliable when applied to fish meal. Consequently a project was initiated at the Institute of Nutrition in Bergen to investigate the analytical method and survey the vitamin D content of fish meals in commercial trade.

The Work Undertaken

Sixteen fish meal samples representing the main types in world trade were obtained for the project. Vitamin D was determined by chick bioassay, based on the anti-rachitic properties of the vitamin, and also by a chemical procedure (HPLC). The bioassay method is considered a reliable though laborious procedure. In addition vitamin A was determined chemically by the company Roche to see if there is a relationship between vitamin A and vitamin D. As the former is more easily determined chemically, if a relationship exists, it may be possible to estimate vitamin D from vitamin A content.

Details of the vitamin determinations are given in the attached report from the Institute of Nutrition (Fiskeri - Direktoratets Ernaeringsinstitut) (Appendix 2). The type of fish used in the manufacture of the fish meals was as follows:

Sample No.	Fish
1	capelin
2	blue whiting
3	herring
4	sand eel
5	unknown[1]
6	herring/sprats
7	Norway pout
8	herring
9	capelin
10	white fish/mackerel
11	scad
12	anchovy, pilchard
13	anchovy, pilchard
14	jack mackerel
15	pilchard/E.whiteheadi
16	menhaden

[1]likely to be anchovy type

The Results

The results of the analyses are summarised in Table 1. A plot of vitamin D determined chemically and by chick bioassay is given in Figure 1.

From these results, the following points can be made:

i) the chick bioassay and chemical methods of determining vitamin D in fish meals give values which agree satisfactorily.

ii) the vitamin A and D content of the fish meals were very variable.

iii) there appeared no to be no relationship between vitamin A and vitamin D content in fish meals.

iv) there did not appear to be any relationship between the type of fish used in producing the meal and the content of vitamin A and vitamin D, though the number of samples from the same or similar fish was very limited.

Discussion

Because of the variability of vitamin D content of fish meal it will be necessary to continue to supplement fish feeds with this vitamin. For example, taking the requirement of salmon fry for vitamin D of 3,000 iu/kg (see report), it can be assumed that fish meal and fish oil will be the main natural sources. If it is assumed fish oil contains 2,000 iu/kg[1] and 20% is used in salmon feed, along with 60% fish meal, a fish meal with less than around 4,000 iu/kg would result in a diet requiring supplementation; that is, virtually all of the fish meals tested (see earlier) would require supplementation. If the full requirement is added, in all cases the present EC limit for vitamin D would be exceeded.

There is therefore a need to get the EC maximum limit on vitamin D content of fish feeds raised.

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1 also variable see earlier report on "Feedstuff Regulations"

Table 1

Vitamins A and D in Fish Meals[1]

IAFMM Ref No.	Lipid[2] %	Vitamin D iu/kg[3]		Vitamin A[4] iu/kg Chemical
		Chemical	Chick	
1	12.7	397	355	6,710
2	8.3	2,803	2,182	243,710
4	11.4	7,154	10,409[5]	13,400
5	10.5	2,054	3,705	2,240
6	12.0	1,327	1,662	34,900
7	10.7	221	182	33,460
8	12.1	1,763	2,295	13,330
9	13.3	465	556 309	12,930
10	12.6	1,295	2,309 2,318	24,050
11	12.2	1,511	1,410	22,190
12	10.6	683	710	28,160
13	9.7	922	716	32,050
14	9.9	900	575	13,590
15	13.6	2,253	3,419	14,310
16	11.2	697	838	2,820

[1]Commercial samples from Chile, Denmark, Faeroes, Ireland, Norway, Peru, Scotland, South Africa, USA.

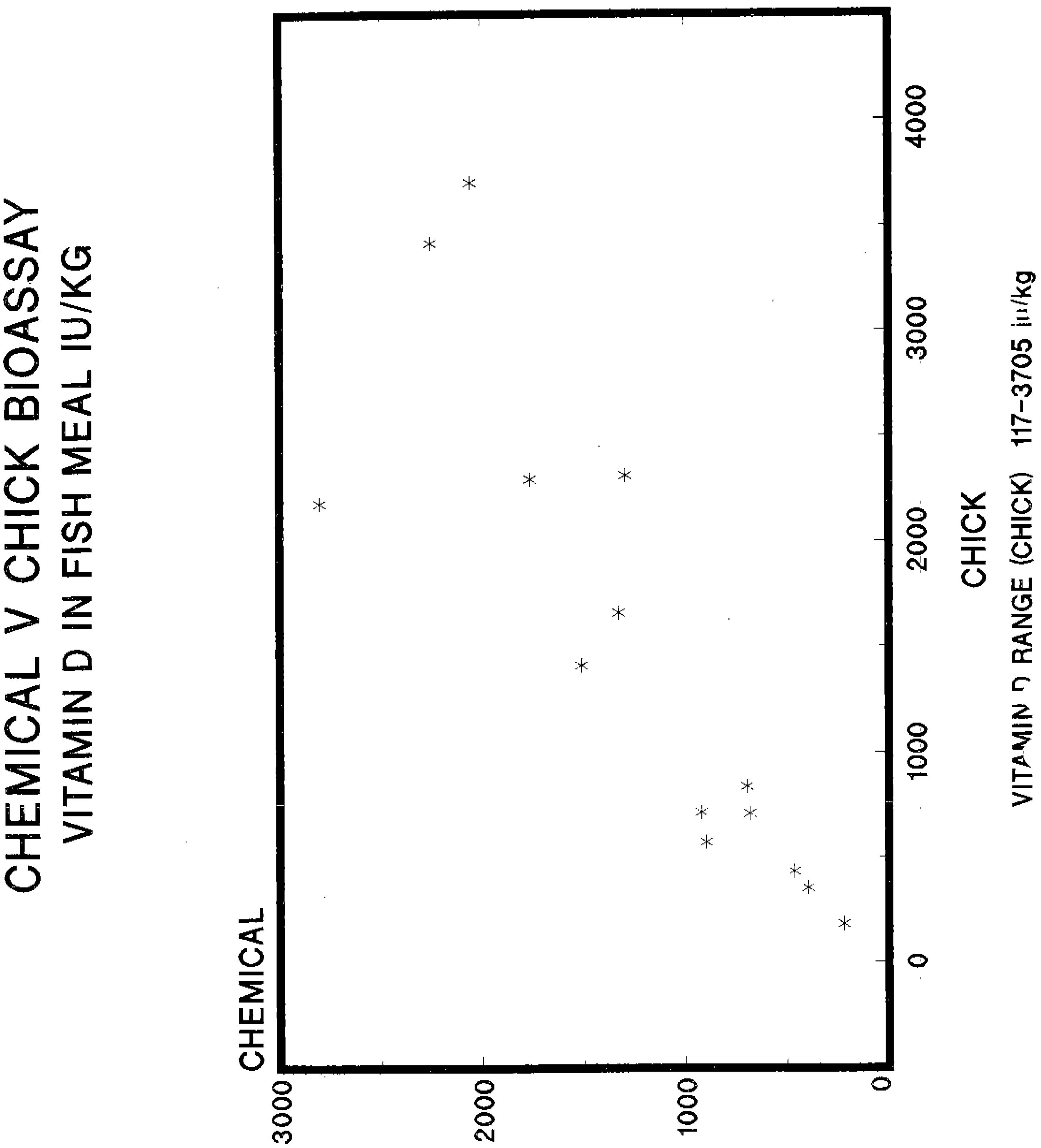
[2]By chloroform/methanol extraction.

[3]Determined by Institute of Nutrition, Bergen, Norway.

[4]Determined by Roche, Welwyn, Herts, UK

[5]Unexpectedly high vitamin D determination. Three batches of sand eel oil measured for vitamin D by Steins Laboratory, Copenhagen, show low values (below 100 iu/kg). Consequently another sand eel meal sample is to be obtained for repeat vitamin D analysis.

FIGURE 1



CHEMICAL V CHICK BIOASSAY
VITAMIN D IN FISH MEAL IU/KG
CHEMICAL
3000
2000
1000
0
0
1000
2000
3000
4000
CHICK
VITAMIN D RANGE (CHICK) 117–3705 iu/kg

THE FEEDING STUFFS REGULATIONS 1988 -

MAXIMUM CONTENT OF VITAMIN D

PERMITTED IN FISH FEEDS

1. The Need to Raise the Maximum Amount of Vitamin D_3 Permitted in Fish Feeds

According to Chapter A of Part IV, Schedule 4, of the above Feeding Stuffs Regulations the maximum amount of Vitamin D_3 permitted in a fish feed is 2,000 iu per kg (1). The major fish species farmed in Europe are the salmonids. Their requirement for vitamin D_3 has been estimated to be in the range of 1,600 to 3,000 iu per kg feed.

It would appear that maximum limits have been set for land animals, which generally require 200 to 1,200 iu per kg of feed, at 2,000 to 4,000 iu per kg. Fish have been put in the 'other' kind of animal category with a 2,000 iu per kg limit. This does not reflect their higher requirement for this vitamin.

Curiously, this maximum limit is below the vitamin D occurring in the natural diet of the salmon. The salmon has been shown to be an opportunist feeder, capturing most types of fish with which it can cope physically. In practice, the herring family form the main species consumed. Other species would include capelin, sprat, mackerel, sand eel, etc. (2, 3, 4). Oily fish in Northern European waters contain on average 6,400 iu vitamin D per kg (5).

There is a need for a special provision for a higher maximum vitamin D_3 content in fish feeds. I would propose that this should be 10,000 iu per kg feed.

2. The Efficacy of Vitamin D_3

The two major natural forms of vitamin D are vitamin D_2 (ergocalciferol, which occurs predominantly in plants) and vitamin D_3 (cholecalciferol, which occurs in animals).

Vitamin D is essential for normal bone formation in animals. The fat soluble D vitamin (D_2 and D_3) is converted to 1,25-dihydroxy vitamin D, the active metabolite (6). Functioning with the parathyroid hormone, it regulates plasma calcium and phosphorus concentrations, mediates the resorption of bone with the release of calcium and phosphorus, participating in mineral and skeletal homeostasis. The relative potency of vitamin D has been measured in animals subject to vitamin D withdrawal in terms of their antirachitic potencies. A value of 1.0 was found. For trout, vitamin D_3 has been found to be at least three times as effective as vitamin D_2 in satisfying the requirement for vitamin D (7).

3. Safety of Vitamin D_3

Excessive levels of vitamin D are toxic. Clinical signs of toxicity are well documented (6). The US National Research Council have reviewed vitamin tolerance of animals - a copy of the chapter dealing with vitamin D is attached (6). For most land animal species the presumed maximum safe level of vitamin D_3 for long-term feeding conditions (more than 60 days) is 4 to 10 times the recognized dietary requirement. For short term feeding (less than 60 days) most land animal species can tolerate 100 times their apparent dietary requirements.

Fish are more tolerant of high vitamin D_3 levels in their feed. Rainbow trout tolerate 300 times their requirement (one million iu per kg feed) (7). Trout fingerlings fed 3.75 million iu vitamin D_3 per kg of diet for 40 weeks had hypercalcemia and increased packed cell volume but there was no difference in their rate of growth or survival (8).

The proposed maximum limit for vitamin D_3 content of fish feeds (10,000 iu per kg feed) is only marginally higher than the amount in the natural diet of the salmon (see earlier). This should lead to amounts in the edible parts of farmed salmon no higher than those in wild salmon. Furthermore, there is no reason to expect that raising the permitted maximum level of vitamin D_3 in fish feeds would pose any threat to the environment.

4. Vitamin D_3 in Fish Feeds

Salmonid feeds contain high levels of fish meal and fish oil. A salmon feed, for example, contains typically 50% fish meal and 12% fish oil. Not surprisingly, vitamin D contents in both reflect those in the oily fish (see ref. 5).

Samples of fish meal and fish oil from different species of fish were sent to a commercial laboratory in the U.K. and a laboratory in Iceland specialising in fish oil analysis. Both are experienced in vitamin D_3 analysis. The conclusions reached from this work are as follows:-

1. The content of vitamin D_3 in the samples was found to be very variable between fish meal and oil made from different species of fish.

2. The results of the analysis of the fish oils from the two laboratories did not agree; in one case one laboratory found no vitamin D_3 in a sample, whereas the other found 3,000 iu/kg; on another sample one laboratory found double the level of vitamin D_3 found by the other laboratory.

3. The amounts of vitamin D_3 in most fish meals and fish oil (though not all) would make a strong case for raising the EC limit on the maximum content allowed in fish feeds. The highest levels of vitamin D_3 found in fish meals and fish oils are in line with values for the content in fresh whole fish.

5. Recommendations

The existing chemical methods for determining vitamin D_3, when applied to fish meals and oils, do not appear to work satisfactorily. In feeds for farmed fish, especially salmonids, fish meal and oil are major components. The chemical method of vitamin D_3 determination may not work satisfactorily with these fish feeds.

It is recommended that a chemical method should be compared with bioassay to determine vitamin D_3 in fish meals, where these represent the main types (fish species) in world trade.

The current difficulties in enforcing EC legislation concerning maximum vitamin D_3 permitted in fish feeds should be brought to the attention of EC legislators.

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PROJECT:

DETERMINATION OF VITAMIN D IN FISH MEAL.

With few exceptions, vitamin D is found in extremely low concentrations in marine samples. However, vitamin D has high biological activity at low concentration. Vitamin D has therefore been determined by biological assays, but they are time consuming and expensive. Various chromatographic methods have been reported for determining the contents of vitamin D in foods and feeds.

Information regarding vitamin D content in fishmeal is scarce, mainly due to analytical problems with determination of vitamin D. This project will include determinations of vitamin D in fifteen fishmeals using HPLC and a biological assay, and an evalution of these methods.

I Chemical determination.

A modified HPLC method for the determination of vitamin D in feeds based on Egaas and Lambertsen (1979); Johnsson et al. (1989) has been developed at Institute of Nutrition. This method will be applied on the fish meal samples.

II Biological determination.

The chicken bioassay for the determination of vitamin D in the fishmeals will also be carried out. The biological test is carried out using a profylactic X-ray method. The chickens are divided into groups of 15 animals each, and each group is supplemented with reference standards or sample material in their feed. After 3 weeks of feeding the animals are X-rayed and the tarsal-metatarsal distance is measured, and the content of vitamin D of the samples is calculated.

Duration: May - December 1991.

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Naturally occurring vitamin D_3 in fish products analysed by HPLC, using vitamin D_2 as an international standard.

Internat. J. Vit. Nutr. Res. 49, No. 1, 35-42, (1979)

JOHNSSON, H., HALÉN, B., HESSEL, H., NYMAN, A. and THORZELL, K.

Determination of vitamin D_3 in margarines, oils and other supplemented food products using HPLC.

Internat. J. Vit. Nutr. Res. 59, 262-268, (1989)

VITAMIN D_3 IN FISHMEAL

Analysed by the chicken bioassay method and by HPLC.

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NUMBERING AND ORIGIN OF THE FISHMEALS:

IAFMM ref.No.	Source
1	Nordsildmel 1 Norway
2	Nordsildmel 2 Norway
4	Thyborøn Denmark
5	Pescal (1+2+6) Peru
6	Skagen Denmark
7	Havsbrun 3 Færoes
8	Iceland 1
9	Iceland 2
10	UFP Scotland
11	IAWS Ireland
12	Corpesca Chile
13	San Pedro Chile
14	Itada Chile
15	FIRI South Africa
16	Zapata USA

INTRODUCTION.

Traditionally, the analysis of vitamin D has been performed by biological assays, performed on either rats or chicken (British Standards Institution 1940; Braekkan 1948). In these bioassays, the antirachitic property of the vitamin is utilized. The bioassays have over years proven their accuracy and sensitivity, but have rather serious disantvantages in being laborious, time-consuming and expensive.

However, one important advantage of the chicken bioassay method, which has been applied in this analysis, is its ability to discriminate between the two major forms of vitamin D; vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). Vitamin D_3 is exclusively found in mammals, birds and fish, while vitamin D_2 are found in plants, and phyto- and zooplankton. In most animals, vitamin D_2 and vitamin D_3 has equal activity. Chicken however, cannot utilize vitamin D_2.

Several methods for the determination of vitamin D based on HPLC has been developed (Egaas and Lambertsen 1979; Pask-Hughes and Calam 1982; Takeuchi and al. 1984; Monard and al. 1986; Johnsson and Hessel 1987,1989; Hung 1988). They all include a step of saponification and extraction, together with one ore more purification steps before final analysis. Although improvements in both precision and time of analysis have been made, these methods are still quite laborious, especially when handling large amounts of samples. The methods also involve the use of considerable quantities of the hazardous, but requisite organic solvents in the extraction steps.

MATERIALS AND METHODS

15 fishmeals from producers throughout the world were

comparatively analyzed for the content of vitamin D_3 by the chicken bioassay and a newly developed HPLC method.

The chicken bioassay.

Principle:
The chicken bioassay is prophylactic. One day old chicken were given vitamin D-free feed for one week. The chicken were then divided into groups of 15 animals each, and given various doses of vitamin D_3-standard and fishmeal supplied in their vitamin D_3-free feed for a time span of three weeks. The chicken were then subjected to x-ray photography of their tibia, in where the cartilage-thickness between tarsus and metatarsus (t.m.t.-distance) was measured. The degree of calcification in the intertarsal joint is a measure of the amount of vitamin D_3 supplied in the feed, and the vitamin D_3-content in the supplied samples were determined by statistical means.

HPLC determination.

Principle:
The samples were saponified in an alkaline solution, and unsaponifiable matter was extracted in hexane. This fraction containing vitamin D was purified in a normal phased HPLC system, and the fraction containing vitamin D was analysed in a reversed phase HPLC system and detected by UV at 265 nm. The vitamin D_3-content is calculated on the basis of the internal standard (vitamin D_2) added to each sample.

RESULTS

Table 1 shows the results of the comparative analysis. A statistical t-test for dependent samples designed for such a comparative analysis has been performed in order to evaluate the validity of the HPLC-method compared to that of the chicken bioassay, and shows that there were no significant difference in the results between the two methods.

CONCLUSION

The vitamin D_3-content of the different fishmeals as seen from table 1, analyzed by the biological and chemical method, varies from about 200 IU (5 μg)/kg to about 10000 IU (250 μg) /kg of fishmeal.

ACKNOWLEDGEMENTS

The authors greatly appreciate the skilled technical assistance of Nils Skjerve, Aase Heltveit, Idun Kallestad and Kjersti Ask.

IAFMM ref.No.	HPLC (IU/kg)	Chicken (IU/kg)
1	397	355
2	2803	2182
4	7154	10409
5	2054	3705
6	1327	1662
7	221	182
8	1763	2295
9	465	309
10	1295	2314
11	1511	1410
12	683	710
13	922	716
14	900	575
15	2253	3419
16	697	838
t=	1.728	
p=	0.106	

Table 1:

The vitamin D_3-content of the fishmeals determined by the HPLC-method and the chick bioassay method. (1 IU = 0.025 μg.)

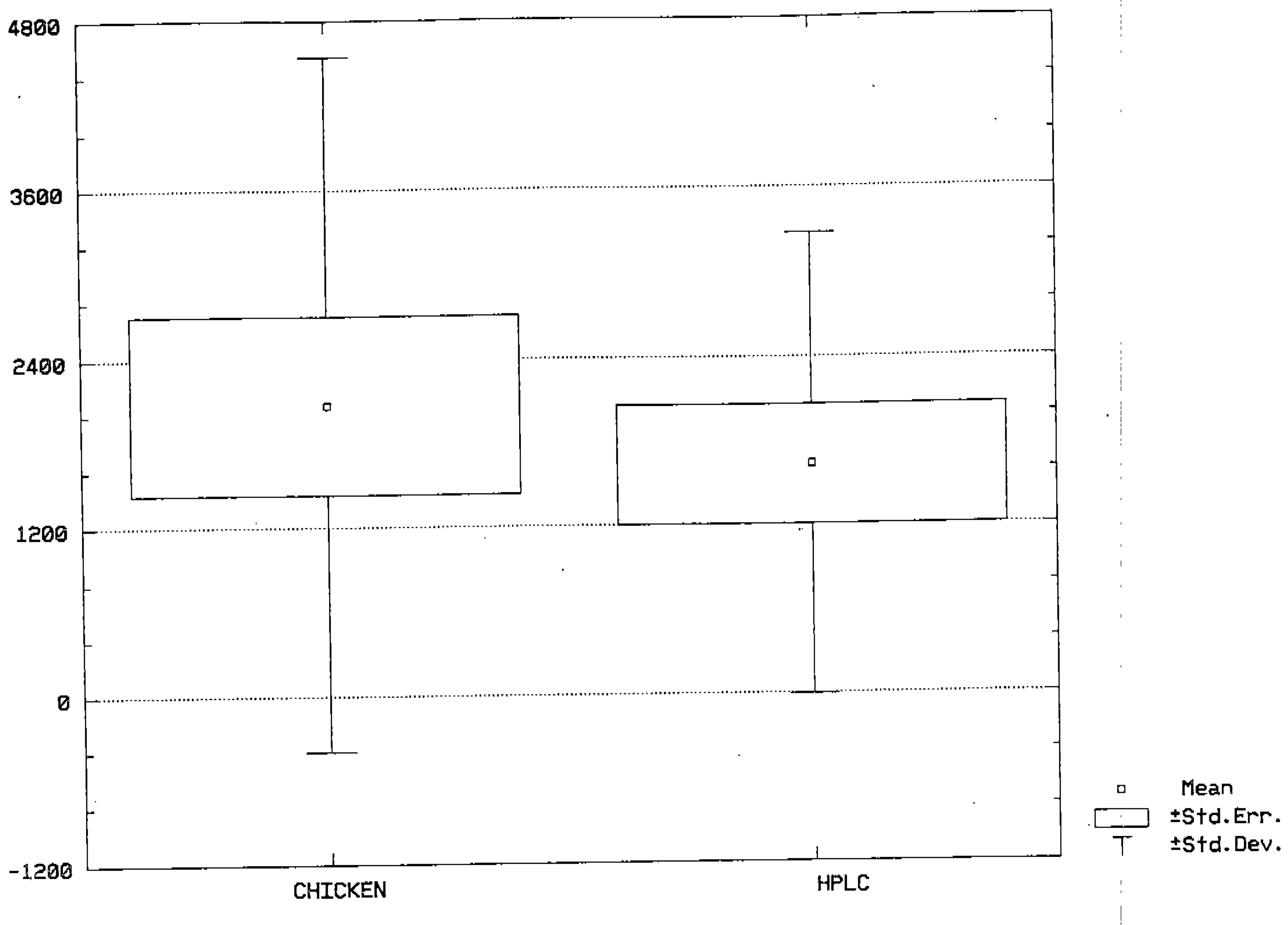

Figure 1:

An illustration showing the mean value of all 15 fishmeals by each of the two methods, together with the standard error and the standard deviation.

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