

Determination of Protein in Fish Meal

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A comparison of the Kjeldahl and Lowry methods for the determination of protein in fish meal has been made. The values obtained with the rapid colorimetric Lowry method, using extracts of total fish meal proteins, were lower than those obtained by the Kjeldahl method. For fish meal from different species the general factor 1.19 ± 0.03 (confidence limit for $P < 0.01$) may be used to convert protein values obtained by the Lowry method into protein values obtained by the Kjeldahl method.

1. Introduction

The Kjeldahl method is widely used to determine protein nitrogen contents in biological material.^{1, 2} When applied to fishery products, protein amounts are calculated employing the general factor 6.25 to convert nitrogen to protein, since the factor for all fish proteins has not yet been determined.^{1, 2} For the determination of total protein in fish meal, we have evaluated the Lowry method, since it is more specific, sensitive and rapid than the Kjeldahl method. The Lowry method has also been evaluated for the determination of myofibrillar proteins extracted from several fishes.³

2. Experimental

2.1. Materials

2.1.1. Folin-Ciocalteu reagent

This was obtained commercially from UCB (Brussels, Belgium). All other chemicals were analytical grade reagents.

2.1.2. Meal samples

Meal samples of squid (*Illex illicebrous argentinus*), hake (*Merluccius merluccius hubbsi*) and fish mixture (*Galeorhinus* spp., *Pampus* spp. and fish processing waste) were from commercial sources. Anchovy (*Engraulis anchoita*) meal was prepared in this laboratory.

2.2. Methods

To extract the whole protein, samples of fish meal (0.20 g) were digested in 25.0 ml of 1M sodium hydroxide solution in a boiling water-bath for 30 min. The digest was diluted ten-fold (v/v) with water and protein estimated according to the standard Lowry method.⁴ Working standards were prepared from normal human serum diluted 100-fold on 0.1M NaOH. Heating conditions and recovery assays in the presence of meal extracts showed that the variation of human serum was lower than the error of the colorimetric determination. Absorbance readings were obtained at 500 nm.

Kjeldahl nitrogen determinations were carried out as described by Pearson¹ using 0.20 g of solid samples or 2.00 ml of alkaline extract.

3. Results and discussion

As the Lowry method evaluates peptides in solution it was necessary to demonstrate that all of the protein of fish meal was solubilised by the digestion method employed. As is shown in Table 1, this was corroborated by Kjeldahl determinations of solid and digested samples.

Table 2 shows results given by both methods when applied to four different fish meals, including those of mollusc, white fish and fatty fish. Though results are different, there is a nearly constant ratio between the values obtained by the two methods.

Among components of fish meal that would interfere in the Lowry method,^{4, 6, 7} only lipids are present in significant quantities. It was found that their interference was less than 0.5% (as % protein). However, it is possible that other substances present in fish meal also interfere in the Lowry method.

Table 1. Kjeldahl determinations of fish meal protein ($N \times 6.25$) in original solid samples (s) and in alkaline extracts (a)

Protein % (w/w) ^a							
Hake		Squid		Anchovy		Fish-mixture	
s	a	s	a	s	a	s	a
61.5	61.2	55.3	57.3	57.8	57.6	56.6	58.5
58.4	52.4	60.5	60.8	59.2	60.4	58.1	59.6
61.0	60.5			61.4	58.9	60.5	56.1

^a Each value is the mean of three determinations. Average relative standard deviation less than $\pm 3\%$.

Table 2. Comparison of Kjeldahl and Lowry determinations of fish meal proteins

Meal	Protein % (w/w) ^a		
	Kjeldahl ^b	Lowry	Kjeldahl:Lowry
Anchovy	61.2 \pm 1.2 (24)	52.6 \pm 2.0 (24)	1.16 \pm 0.07
Fish-mixture	62.0 \pm 1.6 (20)	52.1 \pm 1.2 (59)	1.20 \pm 0.05
Hake	59.2 \pm 1.5 (20)	50.3 \pm 2.2 (25)	1.18 \pm 0.05
Squid	54.8 \pm 1.0 (16)	44.3 \pm 1.0 (48)	1.24 \pm 0.14

^a Mean values and confidence limits ($P < 0.01$) are shown and the number of samples are given in parentheses.

^b $N \times 6.25$.

The discrepancy between Kjeldahl and Lowry determinations is partially explained by the fact that Lowry determinations depend on the protein used as standard,^{2, 4, 5} and partially due to the use of the conventional factor (6.25) for the conversion of Kjeldahl nitrogen to protein. It has been reported¹ that for the major protein in fish (myosin) the factor is closer to 6.0, while for herring proteins⁸ it is 5.72. To obtain absolute values in protein determinations for either Lowry or Kjeldahl methods it would be necessary to use the endogenous protein from each species analysed in order to obtain a reliable standard for the Lowry method or a fish value of the Kjeldahl factor.

For fish meal proteins, experimental results show that the Lowry method gives lower values than the Kjeldahl method. However, both methods can be related by using the factor 1.19 ± 0.03 (confidence limit for $P < 0.01$), for the ratio of Kjeldahl:Lowry protein determinations. This has been shown to be statistically valid for the fish meals used in this study.

In the Lowry method described it takes 30 min to solubilise the proteins of 40 or more samples of fish meal. This time is less than the 45 min elapsed in the Kjeldahl digestion, even using the faster modern digestion systems along with efficient catalysts.⁹ Colour development and manual spectrophotometric measurement using the Lowry method requires only 60 min so that it is possible to analyse the protein content of 40 samples of fish meal with the Lowry method in 90 min. This time is appreciably shorter than that reported for the same number of samples analysed using an automated Kjeldahl analyser.²

Since the Kjeldahl method uses a conventional factor to convert nitrogen to protein it does not measure the true protein value of fish meals to which non-protein nitrogen (e.g. urea) has been added as adulterant.

This problem is avoided with the Lowry method which is suitable for determination of protein in fish meal because it evaluates only true peptides; the method is very sensitive, and it is possible to process a large number of samples simultaneously.

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