S. Afr. J. Anim. Sci. 6, 79–85 (1976)

EVALUATION OF THE PROTEIN QUALITY OF FISH MEALS BY MEANS OF THE NPU METHOD

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Receipt of M.S. 6.1.76

OPSOMMING: EVALUERING VAN VISMEEL OP GROND VAN PROTEÏENBENUTTING DEUR KUIKENS

Vier vismele is met behulp van Netto Proteïenbenuttingbepaling (NPB) waarby kuikens gebruik is, ondersoek en daarna is twee van die vismele in braaikuikenrantsoene getoets. Die NPB-waardes het betekenisvolle verskille tussen die vismele uitgewys, maar ten spyte hiervan was die invloed van die vismele op die groei van braaikuikens nie betekenisvol nie. 'n Metode waarin gebruik gemaak word van fluorodinitrobenseen, is aangewend om die beskikbaarheid van lisien in die vismele te bepaal en dié resultate sowel van aminosuurontledings van die vismele word gerapporteer. Die waarde van NPB-bepalings om vismeel se proteïenkwaliteit te toets, word bespreek.

SUMMARY:

Four fish meals were subjected to a Nett Protein Utilization (NPU) study of which two were then tested in broiler rations. The NPU values revealed significant differences between fish meals but the broiler growth rate failed to indicate differences in protein quality of the fish meals. Values for available lysine (fluorodinitrobenzene method) are also presented for the four fish meals as well as amino acid analyses. The relative merit of NPU for determining the protein quality of a fish meal is discussed.

Being one of the most important protein sources in formulating poultry diets the assessment of fish meal quality concerns nutritionists and feed manufacturers. Different approaches exist for evaluation. e.g. chick bioassay in which fish meal is the sole source of protein (Miller & Kifer, 1970) or growth test with cereal-based diets (Woodham & McDonald, 1968) or nitrogen retention method, usually NPU (Wessels 1972). The latter method was employed in the work reported here. Four fish meals from different factories on the South African West Coast were obtained and, as they differed in appearance and odour, differences with regard to their ability to support growth in chickens were expected. Our aim was to use the NPU method to test this hypothesis. Subsequent inclusion of two of the fish meals in broiler rations were planned.

Procedure

Experiment 1 – NPU determinations on fish meals

Housing and experimental animals

Eight hundred day-old Black Australop x White Leghorn cockerels were obtained from a local breeder. The chickens' masses were determined and only those within three grams of the batch mean were kept in experimental multi-cage units. Each unit consists of 48 cages arranged in 4 tiers with 12 cages per tier. An electrical tube heater divides each 12 cages in 2 sets of 6 cages facing away from one another. Four chickens were placed in each cage and received a conventional chicken starter mash for one week. At this stage the chickens' masses were again determined after an overnight fast and groups were rearranged so that the least difference in mass between groups existed.

During the entire period of the trial, light was continuously supplied.

Chemical analyses

The fish meals were analysed for protein (N x 6,25) by the conventional Kjeldahl method. Total amino acids were determined by iron-exchange chromatography using a Beckman amino acid analyser after hydrolysis of the fish meal sample in 6 M HCl under reflux and continuous oxygen-free nitrogen bubbling. It is basically the technique of Weidner & Eggum (1966) but a major alteration was made for determination of cystine; oxidation was carried out at 0°C and oxidant was removed by lyopolisation. These values are presented in Table 1 along with other characteristics of the test materials. The available lysine values were determined by the method of Carpenter (1960) as modified by Booth (1971) using fluorodinitrobenzene (FDNB).

Diets

The composition of the nitrogen-free basal diet as shown in Table 2 was similar to that described by Wessels (1972) with minor changes to sucrose and vitamin levels, while calcium, phosphorus and potassium sources were not included in the mineral premix. These were supplied as KH_2PO_4, $Ca(H_2PO_4)_2.H_2O$ and $CaCO_3$. Variable amounts of sucrose and fine cellulose powder were added to balance each experimental diet depending on the level of fish meal needed to provide 1.9% nitrogen.

In the nitrogen-free diet sucrose was added in the place of the protein source.

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Table 1

Chemical analyses and other characteristics of fish meals used in experiments 1 and 2 (amino acids in g per 16 g N)

Fish meals	A		B		C		D	
Crude protein % ≠	65.0		69.19		63.6		62.51	
Lysine % ≠	5.43		5.61		4.83		4.79	
Available lysine % ≠	3.89		5.19		3.98		4.66	
Lysine	8.35	(145)*	8.11	(141)	7.59	(132)	7.66	(133)
Histidine	2.66	(125)	2.20	(104)	2.36	(111)	2.10	(99)
Arginine	6.45	(100)	5.63	(88)	5.96	(93)	5.71	(88)
Threonine	4.85	(132)	5.81	(158)	4.80	(130)	5.95	(162)
Serine**	4.73	(90)	4.89	(93)	4.10	(78)	5.02	(95)
Glycine	6.80	(128)	7.24	(137)	4.64	(88)	7.20	(136)
Cystein***	0.94		0.76		0.96		0.78	
Valine	7.17	(156)	6.34	(138)	5.77	(125)	6.62	(144)
Methionine	3.72	(175)	3.48	(164)	3.66	(172)	3.73	(176)
Isoleucine	5.08	(129)	5.14	(130)	5.61	(142)	5.39	(136)
Leucine	8.95	(122)	9.84	(134)	7.09	(97)	9.94	(135)
Tyrosine	3.47	(108)	4.17	(129)	3.94	(183)	4.46	(138)
Phenylalanine	4.88	(133)	5.12	(139)	6.24	(169)	5.33	(145)
Colour:	Brown (normal)		Very dark brown		Brown with tint of green		Brown (normal)	
Odour:	Normal		Slightly burnt		Highly putrefied		Normal	

≠ Air dry basis

* Figures in brackets indicate extent of amino acid adequacy i.e. amino acid provided by the fish meals in this NPU study expressed as a percentage of the requirement suggested by the National Research Council (Bird *et al.*, 1971) scaled down to a 12% protein diet (14.76 MJ/kg) such as normal occurs in NPU rations.

** Inadequacy can be substituted by glycine and visa versa.

*** Oxidised with performic acid and determined as cysteic acid.

Table 2

Composition of basal diet for NPU

Item	%
Maize Starch	72.0
Sucrose	24.0
Refine vegetable oil	3.0
Mineral mixture	**
Vitamine mixture	*
Choline chloride	0.15
Ethoxyquin	0.013

** Provides (g per kg diet):
NaCl 6; $MgSO_4$, 3; Fe Citrate, 0.5; $MnSO_4 \cdot H_2O$, 0.35; $ZnCO_3$ 3 0.2; $CuSO_4 \cdot 5H_2O$, 0.3; KI, 0.01; $Na_2MoO_4 \cdot 2H_2O$, 0.01; $NaSeO_4$, 0.002.

* Provides (mg per kg diet):
Niacin, 50; Thiamine·HCl, 25; Riboflavin, 16; Ca pantothenate, 20; Pyridoxine, 6; Folic acid, 4; Biotin, 0.6; Vitamin B_{12}, 0.02; Vitamin K_1, 5; Vitamin C, 250; and in I.U. per kg diet: Vitamin A, 5000; Vitamin D_3, 800; Vitamin E, 12.

DL-methionine and L-isoleucine were added to raise the level of these amino acids in the protein source by 0,15% and 0,2% respectively. To obtain a factorial arrangement of the treatments the amino acids were added separately and in combination at the levels mentioned above to all 4 fish meals to give a total of 16 treatments. Each group consisted of 4 chickens and each treatment was replicated 5 times.

General procedure

The dietary treatments were allocated randomly to the cages and the chickens were given free access to the experimental diets for 8 days. Spilled food was recovered from the front of the tray and used for making corrections to feed consumed during the 8 day period. Chickens were then starved for 12 hours, killed with chloroform and their mass determined. Thereafter carcass moisture was determined in a forced draught oven run at 85°C initial temperature slowly being increased to 95°C until the last (usually the 5th) day when constant mass was observed. Nitrogen (N) content of the chickens were estimated from the equation by Wessels (1972):

$$y = 121,6 + 33,1\ x$$

where y represents carcass N in mg and x carcass moisture in g NPU values were calculated as described by Bender & Doell (1957):

$$NPU = \frac{Bf - Bk + Ik}{If} \times 100$$

where Bf and If represent the body N and feed N intake of a group of chickens which received a particular experimental diet and Bk and Ik the average body N and feed N intake of the groups which received the 'N-free' diet.

Experiment 2 – Growth tests on fish meals

Housing and Experimental animals

From 500 day-old unsexed Euribrid broiler chickens, 288 were selected for uniform bodymass. These chickens were divided into 24 groups and housed, 12 chickens per pen, in 4-tier electrically heated battery brooders up to 3 weeks of age whereafter they were transferred to indoor rearing platforms. Twentyfour-hour lighting was provided.

Diets: Two of the fish meals used in Experiment 1, viz. types A and C (described in Table 1) were used for the broiler trial.

The composition of the diets are shown in Table 3. The chickens thus received a 3-phase feeding regime whilst the 6 treatments were maintained. These consisted of 2 fish meals incorporated in least cost formulations and each fish meal treatment was supplemented with lysine monohydrochloride in order to have 3 lysine levels, viz. as formulated, and 0,05% as well as 0,1% addition of lysine. Diets were provided as mash. Chicken- and feedmass were determined bi-weekly until 8 weeks of age when all chickens were slaughtered. Gizzards were inspected for erosion lesions.

Results

Experiment 1

In Table 4 the average NPU values obtained for the different fish meals are shown. Analysis of variance on the results showed a highly significant difference between the means of the 4 fish meals as well as a significant fish meal-methionine interaction. It is clear that fish meal A which was selected as control in these experiments had the lowest mean NPU value which is significantly lower than the NPU values of both the fish meals C and D. According to its NPU value in this experiment, fish meal B could not be proved to be different from any other fish meal in the trial, as can be seen from Table 4. The significant methionine-fish meal interaction reflects a negative response of fish meal C and a positive response of all the other fish meals. A possible explanation for the effect of methionine in the case of fish meal C is that addition of methionine aggravated the decreased nitrogen retention cause by the first-limiting amino acid which could not be indicated in this experiment. Certain trends can be seen in Table 4 although they are not statistically significant, for instance it can be noticed that fish meal A benefitted from supplementation with methionine and with isoleucine.

Experiment 2

The results of the second experiment appear in Table 5. Statistical analysis showed that none of the treatments had a significant effect in comparison with the results obtained from the control. Inclusion of the fish meals at the same level in experimental rations was done to utilize only one least cost formulation in the trial. Final mean live body mass was practically the same for both the fish meals. At 4 weeks of age the same pattern can be observed. Feed conversion figures were also more or less the same. Lysine supplementation did not have a consistent effect on growth of broilers at the protein level and fish meal inclusion level used in this trial. The effect of the 6 treatments on incidence of gizzard erosion in the experimental birds at 8 weeks of age is shown in Table 5.

Table 3

Experimental diets# for broiler trial, 0–8 weeks

Ingredients	Weeks		
	0–3	3–6	6–8
	%	%	%
Yellow maize meal	71,3	77,0	79,0
Sunflower oil cake meal	3,2	5,6	7,0
Groundnut oil cake meal	7,8	–	–
Fish meal	16,2	13,4	9,9
Gluten (60% protein)	–	2,2	2,2
Monocalciumphosphate	0,18	0,6	0,6
Limestone powder	1,1	1,28	1,06
Lysine	–	0,055	0,055
Vitamin and Mineral Mix	***	***	***
Coccidiostat	**	**	**
Virginiamycin	*	*	*

Least cost formulations by local feed manufacturer

*** Provides mg/kg diet: Vitamin K, 9,92; Thiamine, 3,0; Riboflavin, 3,5; Pantothenic acid, 9,2; Niacin, 20,0; Pyridoxine, 4,5; Folic acid, 2,2; Choline, 450,0; Vitamin B_{12}, 0,004; Manganese, 50,0; Zinc, 25,0; Iodine, 1,5; I.U./kg diet; Vitamin A, 4000, Vitamin D_3, 600; Vitamin E, 8,0.

** 0,5 g/kg diet

* 5 mg/kg diet.

Table 4

Mean NPU values obtained in experiment 1

Fish meals		A	B	C	D
No methionine added	No isoleucine added	55,15	55,93	63,56	61,34
	Isoleucine added 0,2 per cent	57,49	56,25	62,67	60,51
Methionine added 0,15 per cent	No isoleucine added	57,29	60,15	57,31	62,21
	Isoleucine added 0,2 per cent	58,52	60,49	62,28	63,21
Mean NPU values over all treatments		57,11 (c)*	58,36 (abc)	61,46 (ab)	61,82 (a)

Coefficient of Variation: 6,65%

* Means followed by the same alphabetic letters in brackets do not differ highly significantly

Table 5

Live body mass and feed conversion of chickens reared on rations with two different fish meals and two lysine supplements

Age	Fish meal	Lysine supplemented	Average live body mass bird (gram)		Mean feed conversion	Gizzard errosion
			Group means	Overall means		
28 days	A	–	569,6	597,5	1,547*	
		0,05%	618,5			
		0,10%	604,4			
	C	–	602,8			
		0,05%	534,2	596,5	1,499	
		0,10%	655,0			
56 days	A	–	1647,0			23%
		0,05%	1670,0	1658,0	2,157	23%
		0,10%	1657,0			21%
	C	–	1600,0			35%
		0,05%	1559,0	1594,0	2,163	19%
		0,10%	1624,0			23%

* (g feed consumed per g live body mass)
Coefficient of variation: 6.7% (group body mass at 56 days)

Discussion

Recently Gous & du Preez (1975) published a NPU value of 0,64 (assumably 64%) for a South African fish meal. This value is in close agreement with the highest NPU values reported in experiment 1 (Table 4).

Generally the NPU technique is a worthwhile method to determine the amino acids which are first-limiting in fish meals. In this experiment it could not be proved that the fish meals used lacked methionine or isoleucine. Methionine could be the first-limiting amino acid in the 2 fish meals B and D judged by non-significant trends, and added isoleucine also gave a slight but non-significant response in diets which contained one of the fish meals. Moodie & Wessels (1972) showed that methionine was the first-limiting amino acid in some South African fish meals especially after storage for a long period. They also found isoleucine addition to depress NPU values obtained with chickens while we found 2 fish meals to respond positively to isoleucine supplementation, a result similar to that obtained by Wessels (1971) and Saunders (1973).

Comparison of the NPU values in our experiments (see Table 4) with the descriptions and chemical analysis of these fish meals in Table 1 indicates that a NPU test is superior for judging the quality of fish meal to appearance alone or chemical analysis alone. The growth trial also seems to indicate that conventional judging of fish meal on chemical analys (total amino acids) and appearance etc. can lead to wrong conclusions. Our results in this regard are substantiated by work done by Bunyan & Woodham (1964). These workers compared methods for evaluating protein in fish meals. Close agreement was found between different chemical and biological methods to detect an inferior fish meal. The inferior fish meal was clearly distinguished by Protein Efficiency Ratio (whith chickens), NPU (with rats), FDNB available lysine, Orange G absorption but not by chemical analysis for total amino acids, true protein or crude protein.

Apart from the early methods including the classic biological value (BV) there are methods that have been developed more recently to evaluate protein quality by growth assay e.g. Total Protein Efficiency (TPE) described by Woodham & McDonald (1968) and employed to evaluate a great number of fish meals. In these diets the test protein is not the sole source of protein and it is rightfully claimed that the test can be employed in such a way that it simulates conditions in practical rations with regard to the main ingredients of the ration.

Some workers prefer not to use a combination of protein sources as it may camouflage characteristics of the test protein. Miller and Kifer (1970) state that any evaluation of protein quality necessitates the feeding of the test material as the sole source of protein. These opposing points reflect differences in aim. In certain circumstances it may be helpful to know which amino acids a protein source lacks and which is the first-limiting amino acid. It could also be of value to know that one protein source is inferior to another in which case it will rely upon quality and quantity of other amino acid containing ingredients in the ration. Despite the advantages of protein evaluation by biological tests and despite the good agreement of the methods to rank protein sources in order of quality such as we have been able to show in our work reported here, there remains one very important disadvantage to values obtained in such a manner, and that is that they are not additive. It means that these values, although they give some good information, cannot be used directly in ration formulations. This is also true for other bioassays excluding bioassays in which an isolated amino acid is studied. Values that can be utilized for formulation of rations are for instance those of chemically determined total amino acids, FDBN available lysine, dye binding such as Orange G absorption, true protein and crude protein and probably the most superior of all, availability of individual amino acids by digestion trials. The latter techniques are reviewed and comparison of some of the methods are reported on by Elwell & Soares (1975).

The fact that chemically determined total amino acid content is by far the most widely used criterion protein quality in ration formulation and that it forms the key stone of protein evaluation in computor formulation of poultry diets, invites comment. Firstly one should consider whether the requirement for different classes of poultry and livestock for amino acid is expressed in terms of available or total amino acid? We use total amino acid to calculate ration formulae and our data reported here show that total amino acid content does not necessarily show up limiting amino acids. In Table 1 figures are given in brackets to show to what extent amino acids in a fish meal meet the requirements for chickens in a NPU type diet. In none of the cases was methionine inadequate and yet 2 fish meals responded to methionine supplementation. Methione could thus be present in adequate quantity according to chemical analysis but a large proportion of it not be available to the bird. This explanation is supported by Moodie & Wessels (1972) who found that methionine could be the limiting amino acid in some fish meals. The discrepancy between total lysine and available lysine (FDNB-method) also shown in Table 1, is a further example of the shortcoming of chemical analysis for total amino acids.

In conclusion we can say that the results of the experiments reported here support previous findings that total amino acid analyses are not always reliable estimates of available amino acids. NPU values on the other hand are not additive and could therefore not be directly employed in ration formulation. In view of this dilemma we are forced to determine digestability and availability of amino acids *in vivo*.

Acknowledgements

We are endebted to the Department of Agricultural Technical Services for funds to support this project. We also thank Mr. B. Benade of the Department of Biochemistry and our technical staff for co-operation in amino acid analyses. For supplying ingredients for the experimental diets we would like to thank the Meadow Feeds Co. We are grateful for the supply of Virginiamycin by A.S. Ruffel & Co.

References

BENDER, A.E. & DOELL, B.H. 1957. Biological evaluation of proteins: a new aspect *Br. J. Nutr.* 11, 140.

BIRD, H.R., CLADININ, D.R., COUCH, J.R., EDWARDS, H.M., HILL, F.W., McGINNIS, J., NABER, E.L., 1971. *Nutrient requirements of Poultry*. Sixth Revised Edition. No. 1.

BOOTH, 1971. Problems in the determination of FDNB-available lysine. *J. Sci. Fd Agric.* 22, 658.

BUNYAN, J. & WOODHAM, A.A. 1964. Protein quality of feeding-stuffs. *Br. J. Nutr.* 18, 537.

CARPENTER, K.J. 1960. The estimation of the available lysine in animal protein foods. *Biochem. J.* 77, 604.

ELLWELL, DIANNE & SOARES, J.H. JR. 1975. Amino acid bio-availability: A comparative evaluation of several assay techniques. *Poultry Sci.* 54, 78.

GOUS, R.M. & DU PREEZ, J.J. 1975. The sequential feeding of growing chickens. *Br. J. Nutr.* 34, 113.

MILLER, D. & KIFER, R.R. 1970. Factors affecting protein evaluation of fish meal by chick bio-assay. *Poultry Sci.* 49, 999.

MOODIE, I.M. & WESSELS, J.P.H. 1972. Fishing Industries Research Institute 26th annual report. pp 42.

WEIDNER, K. & EGGUM, B.O. 1966. Proteinhydrolysis: A discription of the method used at the department of animal physiology in Copenhagen Acta Agr. Scand. 16, 115.

WESSELS, J.P.H. 1971. The effect of curing treatment and storage on protein quality of fish meal. *Agroanimalia* 3, 125.

SAUNDERS, A.J. 1973. Nitrogen and amino acid retension with sunflower and fish meal by means of carcass analysis. M.Sc. thesis, University of Natal.

WESSELS, J.P.H. 1972. A study of the protein quality of different feather meals. *Poultry Sci.* 51, 537.

WOODHAM, A.A. & McDONALD, I. 1968. A chich growth test for the evaluation of protein quality in cereal-based diets. *Br. Poultry Sci.* 9, 65.