

Effect of lysine and milk-protein supplements on the protein efficiency ratio of wheat macaroni

By G. S. BAINS, S. VENKATRAO, D. S. BHATIA
AND V. SUBRAHMANYAN

Central Food Technological Research Institute, Mysore, India

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The milling of wheat into flour and semolina is known to diminish the quality of the protein. For example, Hove, Carpenter & Harrel (1945) found that the protein efficiency ratio (PER) of wheat, about 1.4, was decreased to 0.84 for patent flour at dietary levels of protein of 10.2 and 9.8 respectively. Others reported a PER of 0.25 for 70% extraction flour determined at an 8% level of protein in the diet (Harris & Burrell, 1959) and of 1.68 for whole wheat (Morgan, 1931). Increased consumption of such milled products in processed forms such as bread and macaroni in countries where people consume largely vegetarian diets would necessitate fortification of such products to improve both quality and quantity of protein. The main limiting essential amino acid of cereals and their milled products is lysine (Osborne & Mendel, 1914, 1919; Mitchell & Smuts, 1932; Block & Mitchell, 1946-7). The possibility of improving the quality of bread proteins has been studied by several workers, including Carlson, Hafner & Hayward (1946), Rosenberg & Rohdenburg (1951, 1952), Rosenberg, Rohdenburg & Baldini (1954), Hutchinson, Moran & Pace (1956*a, b*), Jahnke & Schuck (1957), Sabiston & Kennedy (1957), Howard, Monson, Bauer & Block (1958), Brown, Flodin, Gray & Paynter (1959), McLaughlan & Morrison (1960) and Morrison & Campbell (1960), but macaroni products have not received similar attention. Our investigation was therefore undertaken with a view to improving the protein content and the nutritive value of macaroni protein by supplements such as lysine and milk protein, as determined by measurement of PER and of liver composition with weanling rats.

EXPERIMENTAL

Preparation of diets

Macaroni samples were prepared in the form of ringlets from semolina obtained from indigenous durum wheat. Enriched macaroni was produced from a blend of semolina, casein and skim-milk solids (90:7:3) with added calcium carbonate and tricalcium phosphate (0.5 parts each). Vitamin additions per 45.4 kg of the blend were: thiamine hydrochloride 125 mg, riboflavine 250 mg, calcium pantothenate 250 mg, vitamin A acetate 0.75×10^6 i.u., vitamin D₂ (ergocalciferol) 0.05×10^6 i.u. The product was made in 125 kg batches in the pilot plant at this Institute as described earlier by Subrahmanyam, Bhatia, Bains & Rajasekharan (1958). After extrusion and cutting, the ringlets were passed through an oscillating pre-drier and steamed for

1½ min before entering the final drier (TTHA-Buhler Brothers, Uzwil, Switzerland). The dry- and wet-bulb temperatures were maintained at 55° and 47° respectively in the first zone and at 55° and 50° in the third zone of the drier. There was an atmosphere of saturated humidity in the middle zone maintained at 55°. Under these conditions, it took nearly 4 h to dry the product to a moisture content of about 10%.

L-Lysine monohydrochloride, at the rate of 0.25 g/10 g protein, was intimately mixed with the powdered samples of control and enriched macaroni before preparation of the diet (Table 1). Addition of lysine to macaroni at this level does not modify the characteristic flavour of wheat macaroni.

Table 1. *Percentage composition of experimental diets*

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4
Wheat macaroni (control)	75.8	—	—	—
Wheat macaroni + lysine monohydrochloride	—	75.8	—	—
Wheat macaroni enriched with casein and skim-milk solids	—	—	53.4	—
Enriched wheat macaroni + lysine monohydrochloride	—	—	—	53.4
Starch	9.7	9.7	32.1	32.1
Miscellaneous*	14.5	14.5	14.5	14.5

* Arachis oil 10, salt mixture 2, vitaminized starch 1, cellulose powder 1, and shark-liver oil 0.5 parts.

Measurement of PER

The rat growth method described by Campbell (1961) was used for determining the PER of the products at a 10% level of protein in the diets, which were adequate for the vitamin (Chapman, Castillo & Campbell, 1959) and mineral (Hubbell, Mendel & Wakeman, 1937) requirements of the animals. Four randomized groups of ten weanling male albino rats each 28 days old were fed *ad lib.* on diets moistened with warm water (60°), for a period of 4 weeks, during which weekly increases in weight and total food consumption were recorded. The initial mean group weights ranged from 44.2 to 44.4 g and the weights of individual rats from 40 to 48 g. The animals were housed in individual cages with wire-screen bottoms. At the end of the experiment, the animals were killed under Amytal (amylobarbitone; Eli Lilly and Co. Ltd, Basingstoke) anaesthesia and the livers were removed for immediate weighing and drying to constant weight at 90–95°.

Analytical methods

Nitrogen contents of liver and macaroni samples were determined by the micro-Kjeldahl method (Association of Official Agricultural Chemists, 1960). The lipid contents of the livers were determined by extraction with diethyl ether in a Soxhlet apparatus. Total lysine was estimated microbiologically (Barton-Wright, 1952) with *Leuconostoc mesenteroides* P 60 on Difco medium. The available lysine was determined by the 2,4-dinitrofluorobenzene method of Carpenter (1960).

RESULTS

Values for PER, liver weight, and liver composition were examined statistically, and the results are shown in Table 2.

The PER of wheat macaroni was significantly lower than that of macaroni fortified with either L-lysine monohydrochloride or casein and skim-milk solids. Incorporation of lysine in the enriched macaroni appeared to enhance the PER slightly, but the difference was not statistically significant.

Table 2. *Effect of supplements of lysine, or milk solids with casein, or both on the protein efficiency ratio of wheat macaroni and composition of the livers of experimental groups of rats fed on the various macaroni diets for a 4-week period*

(Mean values for groups of ten rats)										
Diet no.	Source of protein in the diet	Food intake (g)	Protein intake (g)	Gain in weight (g)	PER*	Fresh liver wt (g)	Liver fat content (dry basis) (%)	Liver nitrogen content (dry, fat-free basis) (%)	Protein content of macaroni (%)	Total lysine monohydrochloride in protein (%)
1	Wheat macaroni	193	19.59	21.6	1.10	3.49	18.17	8.60	12.0	3.16
2	Wheat macaroni + lysine monohydrochloride	260.2	26.95	56.8	2.11	4.99	13.24	8.96	12.0	5.40
3	Enriched wheat macaroni	277.4	28.98	71.8	2.48†	4.22	15.37	9.15	17.0	6.80
4	Enriched wheat macaroni + lysine monohydrochloride	276.9	29.08	76.5	2.63	4.72	15.03	9.43	17.0	9.60
	Standard error of the difference	—	—	—	±0.098	±0.24	±0.89	±0.17	—	—

* g gain in weight ÷ g protein eaten.

† Mean of nine values.

The mean PER values appeared to be rectilinearly related to the lysine contents of the products up to a level of 6.8% lysine monohydrochloride, whereas at 9.6% lysine monohydrochloride (product no. 4), the PER was not rectilinearly increased further. There also appeared to be a good correlation between the PER values and the mean gain in weight of the groups fed on different macaroni diets.

The available lysine content of enriched macaroni was approximately the same as its total lysine content determined microbiologically. There was thus almost no loss of availability during processing.

The mean values for liver nitrogen contents and fresh liver weights of animals fed on the enriched macaroni diet were significantly higher than those of the control group. The livers of the control group, however, were significantly richer in fat than those of groups fed on enriched macaroni diets.

DISCUSSION

The investigation has shown the possibility of improving the nutritive value of macaroni with either lysine or milk protein without affecting the taste and acceptability of the product. Processing conditions used in the preparation of enriched macaroni caused almost no loss of available lysine as determined chemically. The higher concentration of fat in the livers of rats on the plain macaroni diet appears to have been associated with the deficiency of lysine in the diet (Singal, Hazan, Sydenstricker & Littlejohn, 1953). Fortification of macaroni with lysine alone significantly improved the quality of protein as measured by PER, overall gain in weight and percentage of fat

in the livers of the rats. However, the advantages of fortification with milk protein were enhanced protein content (17%) of the product, still higher PER (2.48) and better overall gain in weight of the rats.

SUMMARY

1. Tests with rats showed that the mean protein efficiency ratio of plain wheat macaroni made from semolina at a 10% level of protein intake was 1.11 compared with 2.48 for enriched macaroni prepared from a blend of semolina 90, casein 7 and skim-milk solids 3%.

2. Supplementation of both macaroni products with lysine monohydrochloride (0.25 g/10 g protein) increased the PER values to 2.11 and 2.63 respectively.

3. Rats given plain macaroni fortified with lysine had a significantly lower percentage of fat in their livers than the control group.

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