

Effect of Oxidation of Dietary Fish Lipids on the Quality of Proteins in Diet

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The paper reports results of a study on the effect of oxidised fish oil in the diet on the quality of dietary fish proteins. With increased oxidation of oil, digestibility and utilization of the protein registered a decrease. Oxidation of fish oil beyond a stage was found to affect the intake of diet itself and rats lost weight rapidly.

Effect of oxidation of dietary lipids on the quality of proteins is a much discussed problem. But a survey of literature on the topic reveals conflicting results. Devadasan (1981) in a recent review has attempted to summarise the divergent views expressed by different authors on the subject, with particular reference to the effect of oxidation of residual lipids on the quality of fish protein concentrates. Variations in the results reported by different authors can be attributed to different reasons. Many of these studies were based on *in vitro* chemical indices of protein quality. *In vivo* feeding trials reported also differ in the test animals used, conditions of the experiments etc. making it difficult to draw clear conclusions of a general nature. In many of these studies, the aim was to assess the effect of lipid oxidation on the protein quality when the protein is meant as feed for farm animals or chicken. However, it is known that there are clear differences in the capacity of different animals to utilise proteins damaged to varying extents by processing. Such species differences are possible in the utilisation of proteins affected by protein-oxidised lipid interactions also. Fish oils, due to their highly unsaturated nature, are easily oxidised. So a study on the effect of lipid oxidation on protein quality assumes greater significance in fish based diets. Since albino rats resemble man most in their nutritional requirements, an experiment using albino rats was taken up to ascertain the effect of oxidation of fish oils on the nutritional quality of fish proteins meant for human consumption.

Materials and Methods

Fish protein concentrate (FPC) used for the study was prepared from *Nemipterus japonicus*. For defatting, hexane was used. Last traces of the solvent were removed under vacuum. This FPC served as the sole source of protein in all cases. Fresh sardine oil, oxidised to different extents by bubbling air for varying periods, was used as the sole source of oil in the different diets. Minerals (Hebbel *et al.*, 1937) and Vitamins (Chapman *et al.*, 1959) were added to the diet to meet the requirements. For meeting the fibre requirements 5% cellulose was incorporated in the diet. The final diet in all cases had the composition, proteins 10% lipids 10%, cellulose 5%, salt mixture 4%, vitamin mixture 1%. The diets were balanced with corn starch. After mixing, diets were analysed for the exact composition (protein, fat, ash and moisture) for facilitating calculation of results.

3 weeks old albino rats were used for the study. The diets for the different groups of rats were identical in all respects except that the oil used, was oxidised to varying degrees. The extent of lipid oxidation was monitored by estimating the peroxide value (P.V.) as per the methods of Swern (1961).

Rats were housed in metabolism cages. Five rats were put on each diet. Each rat was put in a separate metabolism cage to facilitate collection of faecal matter and urine. After 5 days' adaptation period on the diet (rats were put on the respective diet,

feeding at the rate of 8 g diet/rat/day), collection of faecal matter and urine was started and the collection continued for 10 days. Urine was collected in bottles containing 5 ml dil. sulphuric acid to minimise loss of volatile ammoniacal nitrogen. Residual feed and faecal matter were collected daily and kept at 4°C pending analysis. At the end, the combined samples were weighed and analysed for their nitrogen content by the microkjeldahl procedure. Nitrogen content of urine samples were also analysed using an aliquot from the total measured volume at the end of the period. Digestibility, nitrogen utilised as % of intake, nitrogen utilised as % of absorbed nitrogen etc. were then calculated in each case (Table 1)

Results and Discussion

Results in Table 1 show that with increased lipid oxidation (as monitored by the peroxide value of oil in the diet), the digestibility and utilization of protein decreases. When the P.V. increased from 7.16 mg% peroxide O_2 to 44 mg% peroxide O_2 , digestibility decreased from 93.81% to 64.27%. Protein N_2 utilised as % of N_2 intake decreased from 86.68% to 50.65% and protein N_2 utilised as % of absorbed N_2 decreased from 95.96% to 78.97%. Results thus show that lipid oxidation clearly reduces protein digestibility in rats. But utilization of the absorbed N_2 was not affected to the same extent. The changes were not very pronounced when peroxide values increased from 7.16 to 17.5. But further increase in the peroxide value to 44 resulted in substantial reduction in the protein quality as can be seen from the data.

Weight gain/g of protein consumed also showed a steady decrease with increased lipid oxidation, as is evident from Table 1.

Beyond the 44 mg% peroxide O_2 mark, oxidation of oil affected the acceptability of the diet. Rats declined food and lost weight rapidly. Studies using these diets were abandoned after a few days.

Moorjani *et al.* (1965) have reported that air dried anchovy meal with normal fat content during storage at 28–33°C for 2 months did not show any loss in PER values, when tested on rats though the FDNB available lysine content, pepsin digestibility etc. were affected adversely. But Giesler *et al.* (1967) reported that digestibility of the protein was adversely affected by changes in dietary lipids. Devadasan *et al.* (1982) in a study using calves did not notice any pronounced changes in digestibility or utilisation of the dietary fish proteins when proteins were allowed to interact with fish lipids at elevated temperatures before defatting the FPC. Results of Njaa *et al.* (1966) also did not show any significant reduction in protein quality as a result of lipid oxidation, in herring meals, when tested using rats. However in fish meal samples prepared from certain species like North Sea Herring, protein quality was found to be affected by lipid oxidation in the same study. His studies further showed that addition of antioxidants to fish meals cannot arrest the deteriorative changes completely. These observations suggest that protein-oxidised lipid interactions show variations due to factors like fish species used etc. also. But March *et al.* (1966) have shown that fish meal prepared from different species like herring and menhaden did not

Table 1. Effect of oxidation of dietary fish lipids on the quality of fish proteins in diet (All values are mean of 5 determinations)

Peroxide value as mg% peroxide O_2 of the oil	Digestibility of protein % Mean $\pm$ SD	Protein utilised as % of intake Mean $\pm$ SD	Protein utilised as % of absorbed protein Mean $\pm$ SD	Weight gain per gm protein consumed Mean $\pm$ SD
7.16	93.81 $\pm$ 0.751	86.68 $\pm$ 1.275	95.96 $\pm$ 0.399	2.92 $\pm$ 0.311
17.5	90.49 $\pm$ 0.960	86.54 $\pm$ 1.190	95.63 $\pm$ 0.715	2.71 $\pm$ 0.075
44.0	64.27 $\pm$ 2.390	50.65 $\pm$ 7.800	78.97 $\pm$ 13.04	1.59 $\pm$ 0.483

SD = Standard deviation

differ in the deteriorative changes due to protein-oxidised lipid interactions during storage of fish meal. For this study, they had used chicken as the test animal. Opstvedt *et al.* (1970) observed that prevention of lipid oxidation in herring meals during storage by addition of antioxidants had a beneficial effect on the protein quality of the meal. March *et al.* (1965) also showed that lipid oxidation in fish meal caused 6-9% reduction in growth of chicken fed on those fish meals. According to some other authors, oxidation of fish oils in diet is not harmful for animals (Barlow & Pike, 1977) but in fish feeds, oxidised fish oils should be avoided (Murai & Andrews, 1974).

In our study, we have tried to see the effect of oxidised oils, on the utilization of proteins of FPC from *Nemipterus japonicus* meant for human consumption. *N. japonicus* proteins are rich in sulphur amino acids and lysine (unpublished results) which are more susceptible to oxidative damage as a result of protein-oxidised lipid interactions. Presence of oxidised sulphur amino acids in the protein chain is known to make it resistant to enzymic hydrolysis, reducing the digestibility of protein. But once absorbed, methionine sulphoxide can be utilised by the system to the same extent as DL methionine itself (Njaa, 1962; Slump & Schreuder, 1972). But Miller & Samuel (1970) is of the view that methionine sulphoxide has only 25% biological activity of DL methionine. Results of the present study also suggest that oxidised lipids reduce digestibility drastically but utilization of absorbed nitrogen is not affected to the same extent. This may be due to partial utilization of the absorbed oxidised methionine, cysteine etc. Oxidation of fish oils in the initial stage may be affecting only the sulphur amino acids without resulting in pronounced deterioration of protein quality. The relatively less pronounced reduction in digestibility and utilisation of proteins when peroxide value of the oil increased from 7.16 mg% peroxide O_2 to 17.5 mg% peroxide O_2 (Table 1) probably represents this stage. But further oxidation of oils seems to affect the other essential amino acids like lysine in the protein causing substantial reduction in the protein quality as seen from the data presented. Such a phenomenon

has been reported by Matoba *et al.* (1984) also. Oxidation of oils to give a P. V. beyond 44 mg% peroxide O_2 was found to affect the acceptability of diet itself. Rats declined the diet and lost weight rapidly and the studies using these groups were abandoned after a few days.

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