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PROCESSING LOSS AND STORAGE STABILITY OF ASCORBIC ACID IN DRY FISH FEED

By
KJARTAN SANDNES and FINN UTNE
Institute of Vitamin Research
Directorate of Fisheries
N–5013 Bergen, Norway

ABSTRACT

The processing losses of ascorbic acid (AsA) in two commercial dry pelleted fish feeds and a laboratory cold pelleted diet have been determined. One of the commercial feeds was investigated on AsA losses during storage for 24 weeks at two different temperatures, 4°C and 20° C. Values for processing and storage losses are given showing that substantial amounts of supplemented AsA were destroyed. Possible detrimental factors affecting AsA stability in feeds are discussed.

INTRODUCTION

Knowledge of the ascorbic acid (AsA) requirement of fish has accumulated since KITAMURA et al. (1965) first showed that AsA was needed for salmonids. This need has later been confirmed by other workers (POSTON, 1967; HALVER et al., 1969; SATO et al., 1978a) and a specific need for this vitamin has also been found for channel catfish, *Ictalurus punctatus* (WILSON and POE, 1973); yellowtail, *Seriola quinqueradiata* (SAKAGUCHI et al., 1969), and Japanese eel, *Anguilla japonica* (ARAI et al., 1972). The only species so far known to synthesize AsA sufficient to cover its nutritional needs is the carp, *Cyprinus carpio* (SATO et al., 1978b).

Deficiency symptoms of fish scurvy were described by HALVER et al. (1975) and includes lordosis, scoliosis and severe distortion of support cartilage. They indicate that a level of 100 mg AsA/kg dry feed covered the requirement for normal growth in rainbow trout, *Salmo gairdneri*, whereas a supplement of 8–10 times this amount was necessary to promote rapid collagen synthesis and wound repair.

These findings raised the problem of the stability of AsA in dry pelleted fish feed, extensively used in modern fish farming. Thus HILTON et al. (1977a) reported that laboratory processed trout diets with graded levels of up to 1280 mg AsA/kg lost all supplemented AsA within 6 weeks when stored at room temperature. In a steam pelleted guinea pig diet for laboratory use nearly 20% of the added vitamin was lost during processing and about 35% of the

remaining AsA was detected after 6 weeks storage at room temperature (EVA et al., 1976). SLINGER et al. (1979) reported nearly total loss (94–97%) of added AsA after steam pelleting and storage for 6 months (20°C) of a salmonid diet.

The present communication reports on the stability of AsA in a commercial dry pelleted trout feed produced in Norway. The processing losses are compared with feed from a Danish feed producer and a cold pelleted diet made in our laboratory.

MATERIALS AND METHODS

Feed

Commercial trout feed in meal form, 2.4 mm pellet (steam pelleted) and starter feed (crushed pellet) were obtained from a Norwegian mill. All three were taken from the same batch supplemented with 440 mg AsA/kg (Type EC[1], 97,5% AsA, Hoffmann la Roche). The feed was steam treated to 60°C before processing. The temperature of the emergent pellets reached 70–75°C, decreasing to room temperature after approx. 70 min. The meal contained 12% moisture plus an additional 1½–2% through steaming before pelleting. A crumb loss of about 15% during pelleting was returned to the production line.

The feed samples were packed in the company's own multiwall paper sacks containing 25 kg. On the arrival at the laboratory one sack each of unprocessed meal blend, pellet and crushed pellet were placed at two different storage temperatures, 4°C and 20°C. Samples were analyzed immediately for its AsA content and later at intervals every four weeks for a total of 24 weeks.

A commercial pelleted Danish feed[2] (3.5 mm) supplemented with 600, 1200 and 2400 mg AsA/kg (Type EC, 97.5% AsA, Hoffmann la Roche) were received frozen and analysed for its AsA content immediately after thawing. Processing conditions resembled those of the Norwegian feed exept for a somewhat lower crumb loss (~10% returned to the production line) and a faster temperature decrease, the pellets reaching room temperature within 12–20 min.

The laboratory processed feed was cold pelleted (2.5 mm) (i.e. without steaming). Coated AsA of the same type as in the commercial diets was used for supplementation. The dry ingredients were mixed in a batch of 8.5 kg with the addition of 600 ml water. The feed was dried overnight at 25°C, after which fat was added. Fish oil with antioxidant addition was used as fat source in all feed blends.

[1] coated with ethyl cellulose.

[2] generously produced us for by Dansk Ørredfoder A/S, DK-7330 Brande.

Analysis

AsA was determined by an automated fluorometric method described by ROY et al. (1976). Dry feed samples of 5 g were ground and extracted in 100 ml 0.5% oxalic acid and filtered. The filtrate was transferred to 2 ml plastic cups and placed in an automatic sampler for analysis.

In a fully automated flow-through system AsA was oxidized to dehydroascorbic acid (DHA) using N-bromosuccinimide. DHA further underwent a condensation reaction with o-phenylenediamine and a fluorescent quinoxaline was formed, proportional to the total amount of AsA (AsA + DHA) in the sample. The net fluorescence was measured on a recording Kontron SFM-23 spectrofluorometer and compared with standard solutions of AsA (Fluka, p.a.). All reported values refer to total AsA.

RESULTS AND DISCUSSION

The coated AsA, type EC, used for feed supplementation, was analyzed for its ascorbic acid content by the method outlined. Three replicates gave an average of 98.0% AsA, as compared with the producer's value of min. 97.5% AsA. A recovery experiment using additions of 0, 200, 400 and 800 mg AsA type EC to a mixed feed gave the following analytical values (mg/kg): 60 ±12, 221±10, 443±17 and 949±104. These values correspond to recoveries of 82%, 98% and 113%, averaging 98%±16 (S.D.).

Values of AsA contents and calculated processing losses in the feeds are given in Table 1. A substantial part of the added vitamin was lost during production, ranging from 44% to 61% in the samples of commercial pelleted feeds. The cold pelleted laboratory produced feed showed a loss of 23% upon processing, as compared to virtually no loss after simple mixing and immediate extaction as in the recovery experiment. Surprisingly, the non-pelleted meal retained only 50% of the added 440 mg AsA/kg, indicating an immediate onset of the processes contributing to the destruction of the vitamin. The proximate chemical analyses of the diets are given in Table 2, analyzed by conventional methods. The values show a lower fat content in the Danish feed than in the Norwegian feed.

Fig. 1 shows the AsA contents of the meal blend, pellets and crushed pellets during storage for 24 weeks at two different temperatures (4°C and 20°C). Stored at room temperature the feeds had lost nearly all the AsA-content after 16 weeks. Calculated on the vitamin contents after processing the storage losses after 24 weeks at 4°C were for meal form, pellets and crushed pellets resp. 35%, 27% and 45%. Taking the initial supplementation into consideration the total losses after 24 weeks at 4°C were almost 70% both in meal form and in pelleted diet, and about 80% for the crumbled feed.

Table 1. Loss of ascorbic acid after processing.

Feed	Supplemented Ascorbic acid (mg/kg)	Value found by analysis*	% loss
Norwegian, meal form	440	220±14	50
» , 2.4 mm pellet	440	186± 8	58
» , crumbled	440	171±13	61
Danish, 3.5 mm pellet	600	313±12	48
» , » » »	1200	651±21	46
» , » » »	2400	1334±21	44
Lab. processed, 2.5 mm pellet	400	308± 8	23

* 3 samples ± standard deviation.

Table 2. Proximate chemical analyses (% of total).

Feed	Protein[1)]	Fat[2)]	Dry matter	Ash[3)]	Crude fiber[4)]
Norwegian	45.1	18.3	93.9	10.6	3.3
Danish	52.8	11.3	90.6	12.1	1.8
Lab. processed	50.3	12.2	94.8	11.6	3.5

[1)] Kjeldahl, Directorate of Fisheries, Centr. Lab., Methods 1979.
[2)] Ethyl-ether, Soxhlet extraction.
[3)] Ignition at 550°C for 16 hours.
[4)] Statens Landbrukskjemiske Kontrollstasjon (1959) (mod. from AOAC, 1945).

According to information given by Hoffmann la Roche, this type of coated AsA is fairly stable to air if protected from humidity. The tough treatment in a modern pelleting mill – including addition of steam, high pressure and temperature – obviously has a deleterious effect on the vitamin. Presumably a considerable amount of the coating breaks during this process exposing the pure AsA to the environment. This initiates the destruction of the vitamin which continues until virtually no supplemental AsA remains in the diet (HILTON, 1977a).

AsA is by far the most labile of the known vitamins. Among factors contributing to the oxidation of AsA are air, heat and oxidizing enzymes (BENDER, 1978). The reaction is strongly catalyzed by multivalent cations, especially iron and copper.

Usually a mineral mixture is added to practical fish diets, and this could be a possible contribution to the destruction of AsA, but further information is needed in this field.

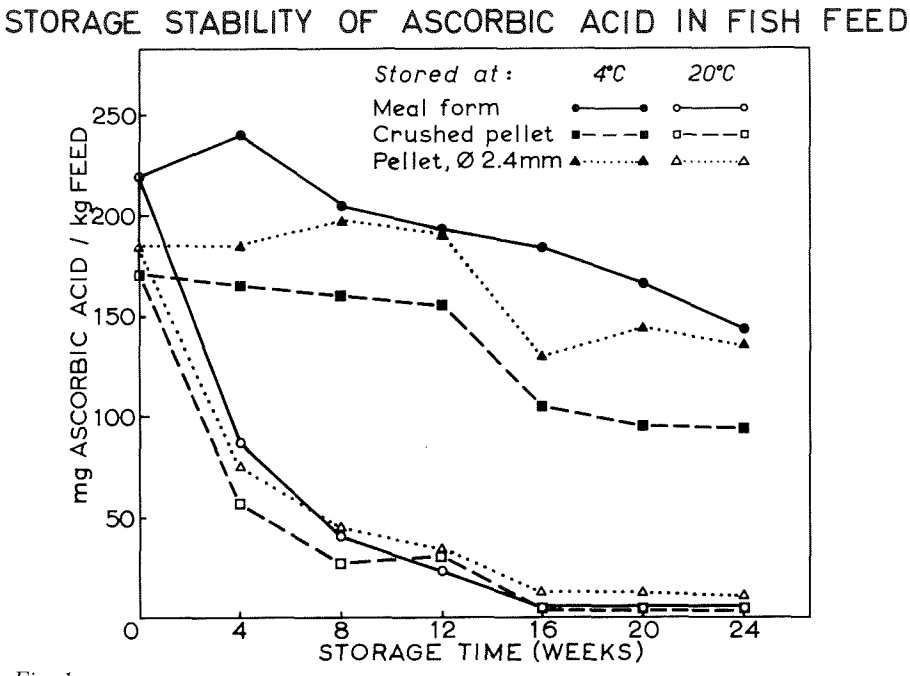

Fig. 1.

AsA acts as an antioxidant due to its strong reducing properties. Use of high levels of polyunsaturated oils in the feed may therefore necessitate an increased level of supplementation and/or requirement of this vitamin for the fish (HUNG and SLINGER, 1980).

Probably enzymatic destruction plays a minor role as the activity of oxidative enzymes should be greatly diminished due to unfavourable processing conditions.

In addition to processing and storage losses of AsA, a further considerable amount is lost through leaching. HILTON (1977a) reported that crumbled pellets lost about 10% of the added vitamin (400 mg/kg) in 10 seconds in water.

100 mg AsA/kg dry feed has been found to keep the fish healthy (EIFAC, 1971). HILTON et al. (1977b) indicated that the liver AsA concentration may be used as an index of the AsA status of rainbow trout and that levels of 20 μg/g (wet weight) or lower are marginal requiring immediate supplementation. A high correlation was stated between dietary AsA and liver AsA. These findings correspond well with work at our institute (SANDNES, 1982), indicating that a level of 100 mg AsA/kg dry feed at the time of feeding meets the demands of the fish. Clearly the supplementation of AsA in a dry pelleted feed for salmonids must exceed the net requirement of the fish.

We find it impossible to suggest a general level of addition of AsA in a salmonid diet because of the various parameters involved, among them feed composition and form, processing conditions, time and condition of storage and leaching. But with the present processing procedures of commercial dry fish feeds a supplementation in the range of 400–800 mg/kg seems reasonable if the feed is fairly well stored and used within 6 months from the date of production.

However, according to the vitamin losses shown in the present paper we find it appropriate to stress the unfavourable conditions for ascorbic acid, and possibly other nutritional factors as well, during processing in the modern feed industry. Technical improvements seem warranted to protect unstable essential nutrients from degradation.

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