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Determination of the antioxidant efficacies in fish meal of two oxidation products of ethoxyquin

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DETERMINATION OF THE ANTIOXIDANT EFFICACIES IN FISH MEAL OF TWO OXIDATION PRODUCTS OF ETHOXYQUIN

Executive Summary

Two oxidation products of ethoxyquin, a quinolone and a dimer, were tested for their antioxidant efficacy in fish meal at 25°C compared with ethoxyquin. It was found that the quinolone had approximately 85% and the dimer about 63% of the antioxidant efficacy of the ethoxyquin. To allow for variation in efficacy from one meal to another, a safety margin was calculated from the statistical variation in these results (90% lower confidence limit for an individual meal as used, giving relative efficacies of 63% for quinolone and 44% for the dimer).

To correctly evaluate the stability of a fish meal treated with ethoxyquin it is necessary to measure not only the presence of residual ethoxyquin but also the levels of residual quinolone and dimer which are formed during the oxidation of ethoxyquin. Applying the efficacy products to these two breakdown products can be used to calculate the total antioxidant capability in a fish meal using the formula:

Total EQ equivalence = EQ + (0.63 x Q) + (0.44 x EQ-d)

where: EQ = ethoxyquin
Q = quinolone
EQ-d = dimer

INTRODUCTION

Oxidation of the antioxidant ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethyl-quinoline, EQ) has been shown by Thorisson (1987) to give rise to at least two products (i) a quinolone (2,6-dihydro-2,2,4-trimethyl-6-quinolone) and (ii) a dimer $1,8^1$-di (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline). Both compounds could be detected in a stored anchovy meal that had been treated with EQ. It was found by Thorisson (1987) that the EQ-dimer had no antioxidant activity in fish oil while the quinolone was a powerful antioxidant, similar to EQ. Subsequently it was found at FIRI that in fish oil the dimer had about one-third and the quinolone about three-quarters the antioxidant efficacy of EQ (de Koning and van der Merwe 1992). In view of the uncertainty of the antioxidant efficacy of especially the EQ-dimer it was decided to determine the efficacy of both the quinolone and the dimer in fish meal relative to EQ.

To this end a reactive fish meal was treated with either 400 mg kg^{-1} EQ, 400 mg kg^{-1} quinolone or 400 mg kg^{-1} dimer and stored at 25°C for about one year. At day 0 and at approximately bi-monthly intervals the polyunsaturated fatty acid (PUFA) contents of the residual meal lipids were determined. The efficacies of the three antioxidants were then assessed by comparing the decrease in PUFA contents of their respective lipids. The experiment was carried out in duplicate on meals collected on two separate occasions. This series of tests are described as Part I.

While the experiment was in progress Dr Stuart Barlow visited FIRI. He was shown the results of Part I after 60 days' storage, which showed an almost 90% efficacy for both the quinolone and the dimer relative to EQ. After further discussion IFOMA agreed to provide funding for a supplementary test (Part II) lasting six months and using smaller amounts of antioxidants in the hope of obtaining more precise information on the efficacy of these antioxidants. The reasoning behind this additional work was as follows:

So far comparative efficacies were determined by treating meals with identical levels of antioxidants and obtaining varying degrees of protection of PUFA. These different levels of protection were then equated with the varying efficacies of the different antioxidants. However, if things were turned around and different levels of antioxidants were added so as to hopefully produce identical protection we have another method of grading antioxidants. Briefly, it amounts to the following: in the first instance we have identical inputs and varying outputs and in the second instance we have varying inputs and (hopefully) identical outputs.

The second approach is obviously a hit-and-miss affair but nevertheless some useful information might be gained from it. It was therefore decided to carry out a test lasting half a year with the following amounts:

> Meal 1 to be treated with 100 mg kg^{-1} EQ, 200 mg kg^{-1} EQ-dimer and 150 mg kg^{-1} quinolone. Meal 2 to be treated with 200 mg kg^{-1} EQ, 400 mg kg^{-1} EQ-dimer and 300 mg kg^{-1} quinolone, i.e. double the amount of antioxidant of meal 1 but identical relative amounts. These quantities were chosen in the expectation that the dimer would have half the efficacy and the quinolone two-thirds the efficacy of EQ.

The experimental procedures and results of Part I are presented first by kind permission of the S.A. Fish Meal Marketing Company (PTY) Ltd. Part II follows.

PART I

EXPERIMENTAL

EQ, EQ-dimer and quinolone

Pure EQ was obtained by vacuum distillation of a commercial product as described (de Koning 1987). It was obtained as a yellow fluorescent oil of n_D^{20} = 1,5734. EQ-dimer and quinolone were both prepared by oxidation of EQ with tert-butylhydroperoxide according to the method of Thorisson (1987). The EQ-dimer was a khaki-coloured solid of m.p. 108-109°C and the quinolone a yellow-coloured solid of m.p. 108-109°C.

Fish meals

Untreated fresh pilchard *(Sardinops ocellata)* meal was collected from a local fish meal factory and quickly transported to FIRI in closed polypropylene buckets. The meal was divided into four portions. Three portions were treated individually with 400 mg kg^{-1} EQ, 400 mg kg^{-1} EQ-dimer or 400 mg kg^{-1} quinolone while the fourth portion remained untreated. The meal was then stored at 25°C and samples taken at approximately bi-monthly intervals starting on day 0. An anchovy *(Engraulis capensis)* meal was used in the second test and treated identically.

Extraction of the lipids and determination of the fatty acid composition

The residual lipids of the meals were extracted according to a modified Bligh and Dyer method (de Koning *et al.* 1985). The fatty acid compositions were determined by gas chromatography of the methyl esters (de Koning *et* al. 1985).

Determination of EQ, EQ-dimer and quinolone

These compounds were also determined in the meals at about bi-monthly intervals by gas chromatography according to the method developed at FIRI (de Koning and van der Merwe 1992).

RESULTS AND DISCUSSION

The complete fatty acid compositions of the lipids extracted from the pairs of fish meal (meal 1 and meal 2) are recorded in Table I. The PUFA contents only of these meals at the different stages of maturity are recorded in Table II and the figures clearly indicate that EQ is the best antioxidant. In meal 1 after storage of 350 days at 25°C the PUFA content in the meal treated with 400 mg kg^{-1} EQ had decreased from 47.1 % to 36.0% while in the meals treated with quinolone and EQ-dimer it had decreased to 29.0% and 27.0% respectively. It should be noted that an untreated control had after 350 days storage a PUFA content of only 6.0%, which gives an indication of the protective action of the various antioxidants. Results for meal 2 were essentially similar and again EQ was the best antioxidant while the quinolone was the second best followed by the EQ-dimer. In order to quantify the efficacies of the quinolone and EQ-dimer relative to EQ the decrease in PUFA content of the EQ-treated meal (i.e. efficacy 100%) was subtracted from the decrease in PUFA content of the control meal (i.e. efficacy 0%). From this value together with the decrease in PUFA content of the quinolone and EQ-dimer treated meals efficacy values for these two antioxidants were calculated. For instance on day 350 control meal 1 showed a decrease in PUFA content of 41.1% while the corresponding decrease for the EQ-treated meal was only 11.1%, in other words 30.0% PUFA was the protection given to the meal by EQ. The quinolone-treated meal had a decrease in PUFA content of 18.1% and the EQ-dimer 20.1%, therefore the protection afforded by the quinolone and dimer were 23.0% and 21.0% respectively. This yields efficacy values for the quinolone and EQ-dimer of 76.7% and 70.0% respectively. In this way five efficacy values were calculated for the quinolone and the dimer and a mean was obtained. These calculated values are tabulated in Table III. Somewhat differing average values were found for the quinolone while the average value for the EQ-dimer was identical in the two meals. It seems prudent to adopt the lower value for the quinolone, so in future work efficacy values for the quinolone and EQ-dimer of 75% and 70% respectively will be quoted. The values previously determined in fish oil were 75% for the quinolone and 33% for EQ-dimer. *If a new antioxidant is therefore to be used in fish meal it should be tested in fish meal. It is not satisfactory to carry out a quick accelerated test at elevated temperatures in fish oil.* The high efficacy value for the EQ-dimer is to some extent good news for EQ producers as EQ is converted largely into EQ-dimer in fish meal.

The residual EQ, quinolone and EQ-dimer found in the meals after storage are recorded in Table IV. In meal 1 both EQ and quinolone were not detectable any more from day 238 onwards in contrast to EQ-dimer which was still present at 84 mg kg^{-1} after 238 days and 55 mg kg^{-1} after 350 days. This feature was also evident in meal 2 which after 350 days still had 253 mg kg^{-1} EQ-dimer, while the EQ content had decreased to 48 mg kg^{-1} and the quinolone to 10 mg kg^{-1} · The fact that the EQ-dimer has a longer "half-life" than EQ can be used to establish whether an aged

meal has originally been treated with EQ, even when EQ itself is no longer detectable. It is also reassuring to know that in the absence of EQ there is still EQ-dimer present which has about 70% of the efficacy of EQ.

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PART II

EXPERIMENTAL

EQ, EQ-dimer and quinolone

Pure EQ was obtained by vacuum distillation of a commercial product (de Koning 1987).

EQ-dimer and quinolone were both prepared by oxidation of EQ with tert-butylhydroperoxide according to Thorisson (1987).

Fish meals

Untreated pilchard *(Sardinops ocellata)* meal was collected from a local fish meal factory and transported to FIRI in polypropylene buckets. The meal was divided into four portions. Three portions were treated with either 100 mg kg^{-1} EQ, 200 mg kg^{-1} EQ-dimer or 150 mg kg^{-1} quinolone while the fourth portion remained untreated. The meal was then stored at 25°C for about 180 days and samples taken at approximately bi-monthly intervals for determination of fatty acid composition. The second meal, an anchovy *(Engraulis capensis)* meal was also divided into four portions. Three portions were treated with either 200 mg kg^{-1} EQ, 400 mg kg^{-1} EQ-dimer or 300 mg kg^{-1} quinolone while the fourth portion remained untreated as control. These meals were also stored at 25°C for about 180 days.

Extraction of the lipids and determination of the fatty acid composition

The residual lipids of the meals were extracted according to a modified Bligh and Dyer method (de Koning et al. 1985). The fatty acid compositions were determined by gas chromatography of the methyl esters (de Koning *et al.* 1985).

Determination of EQ, quinolone and EQ-dimer

These compounds were determined in the fish meal samples after 180 days storage according to the method developed at FIRI (de Koning and van der Merwe 1992).

RESULTS AND DISCUSSION

The complete fatty acid compositions of the lipids extracted from meal 1 and meal 2 are recorded in Table 1. The PUFA contents only of these meals at the different stages of maturity are recorded in Table 2. The results indicate that within the timespan of 180 days the amount of antioxidant added had little or no effect on the protection rendered to the meals. For instance meal 1 with 100 mg kg^{-1} EQ showed a decline in PUFA content from 36.0% to 29.0% i.e. 7 percentage points while meal 2 with 200 mg kg^{-1} EQ showed a decline in PUFA from 39.9% to 27.2% i.e. 12.7 percentage points. The same can be said of the meals treated with the quinolone and EQ-dimer.

Both meals were reactive as can be seen from the decline in PUFA content of the controls. Meal 1 was a pilchard meal obtained from Gansbaai Marine Products and meal 2 was an anchovy meal obtained from the Suid Oranje factory in Saldanha Bay.

If we ignore the different amounts of antioxidant added, as this seems irrelevant, we obtain efficacies for the quinolone and the EQ-dimer similar to those obtained previously when all the meals were dosed with 400 mg kg^{-1} antioxidant.

In order to quantify the efficacies of the quinolone and the EQ-dimer relative to EQ the decrease in PUFA content of the EQ-treated meal (i.e. efficacy 100%) was subtracted from the decrease in PUFA content of the control meal (i.e. efficacy 0%). From this value together with the decrease in the PUFA content of the quinolone and EQ-dimer-treated meals efficacy values for these two antioxidants were calculated. For instance on day 180 the control meal 1 showed a decrease in PUFA content of 28.4% while the corresponding decrease for the EQ-treated meal was only 7.0%, in other words a protection of 21.4% PUFA was given to the meal by the EQ. The quinolone-treated meal had a decrease in PUFA content of 8.3 % and the EQ-dimer 15.3 %, therefore the protection afforded by the quinolone and dimer were 20.1% and 13.1% respectively. This yields efficacy values for the quinolone and EQ-dimer of 93.9% and 61.2% respectively. Three efficacy values for the quinolone and the dimer for each meal were calculated and a mean was obtained. The calculated efficacy values are tabulated in Table 3. Inspection of the mean efficacies shows a reasonable agreement between the two meals. The efficacy of the quinolone in meal 1 was as high as 94.8% that of EQ while the EQ-dimer scored 63.9%. In meal 2 the values were 81.1% and 51.9% respectively. The conclusion to be drawn from this experiment is that the efficacy values for the quinolone and the dimer are about 88% and 58% the value of EQ respectively.

These are surprisingly high values since in fish oil the antioxidant efficacy of the quinolone was about 75% and the efficacy of the EQ-dimer only about 33% that of EQ. *If a new antioxidant is therefore to be used in fish meal it should be tested in fish meal. It is not satisfactory to carry out a quick accelerated test at elevated temperatures in fish oil*.

The residual EQ, quinolone and EQ-dimer found in the meals at the end of the storage period are recorded in Table 4. In both meals the amounts of EQ and quinolone have declined to negligible levels but the amount of EQ-dimer was still substantial namely 25 mg kg^{-1} in meal 1 and 188 mg kg^{-1} in meal 2. This is again evidence that the EQ-dimer has a much longer "half-life" than EQ and this together with the newly found high efficacy value of the dimer is good news for EQ producers as EQ is converted largely into EQ-dimer in fish meals. It is certainly reassuring to know that in the absence of EQ there is still EQ-dimer present which has about 60% of the antioxidant activity of EQ.

REFERENCES

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