



## Update of the opinion of the Scientific Committee on Animal Nutrition on the use of astaxanthin-rich *Phaffia rhodozyma* in feedingstuffs for salmon and trout

(Adopted on 22 January 2003)

### 1. BACKGROUND

Astaxanthin-rich *Phaffia rhodozyma* (Product Ecotone™) has been evaluated by the Scientific Committee on Animal Nutrition. The Committee issued its opinion in February 2002. The company ADM, producer of Ecotone™, sent a dossier in reply to this SCAN opinion.

### 2. TERMS OF REFERENCE

The Scientific Committee for Animal Nutrition (SCAN) is requested to consider the dossier built by the company and to update its opinion of February in the light of the information provided.

EEC No.	Additive	Chemical formula, description	Species or category of animal	Maximum Age	Minimum Content	Maximum Content	Other provisions
					mg/kg complete feedingstuff		
12	Astaxanthin-rich  <i>Phaffia rhodozyma</i>  (ATCC 74219)	Concentrated biomass of the yeast <i>Phaffia rhodozyma</i> (ATCC 74219), killed, containing at least 4.0g astaxanthin per kilogram of additive and having a maximum ethoxyquin content of 2000 mg/kg.	Salmon	-	-	100	The maximum content is expressed as astaxanthin.  Use permitted from the age of 6 months onwards.  The mixture of the additive with canthaxanthin is allowed provided that the total concentration of astaxanthin and canthaxanthin does not exceed 100 mg/kg in the complete feedingstuff.  Ethoxyquin content to be declared.
			Trout	-	-	100	

### 3. OPINION OF THE COMMITTEE

SCAN reported on the use of astaxanthin-rich *Phaffia Rhodozyma* in feedingstuffs for salmon und trout on 06 February 2002. The company responded to the report by submitting new data (supplementary dossier III (originally dated January 2001) and a supplementary dossier (dated June 2002).

#### 3.1. Product identity and conformity

When formulating his scientific opinion on Ecotone™ SCAN was always aware that the total dossier deals with different products which was not only reflected by different product names but also by different production procedures.

By the most recent information, “ECOTONE™ *Phaffia Rhodozyma* Yeast” is now composed only by *Phaffia Rhodozyma* yeast, a consistent astaxanthin content is achieved by blending different fermentation batches with different astaxanthin levels. This information differs from all other previous statements in the dossiers. The product contains now 4,500 mg astaxanthin/kg – this level is also guaranteed as minimum level in the Specification and Information Sheet of the company – and as added 2,000 mg ethoxyquin/kg. However also a minimum content of 4,000 mg astaxanthin/kg Ecotone™ is listed in the dossier with the additional information, that the actual concentration of the marketed product is 4,500 mg/kg.

The chemical composition is within the range given for Red Star® *Phaffia* Natural Color.

For further considerations SCAN assumes that the product “Ecotone™ *Phaffia Rhodozyma* Yeast” contains 4,500 mg astaxanthin/kg (and 2,000 mg ethoxyquin/kg).

#### 3.2. Carotenoids in Ecotone®

According to the most recent information astaxanthin in Ecotone™ amounts in its 3R,3'R-isomeric form to about 73 % of the total carotenoid content of the product. Within the astaxanthin fraction, about 94.5 % are in the trans form and 5.5 % in the cis form.

Other carotenoids are β-carotene and related compounds (1.7-4.2 %), echinenone (1.0-1.6 %), 3-hydroxy-echinenone (6.6-8.4 %), 3-hydroxy-3'4'-didehydro-βΨ-carotene (2.9-4.1 %), phoenicoxanthin (2.9-4.1 %) and adonixanthin (3.4-3.9 %) as well as unknown compounds (1.8-4.5 %). By this Ecotone™ differs - primarily by lower astaxanthin content in the total pigment fraction - from the data of Andrewes, Phaff and Starr (1976).

#### 3.3. Stability in feed

SCAN referred in the February 2002 report on the fact that storage stability data for Ecotone™ were presented only for pelleted but not for extruded fish feed. The company gives now the additional information that the storage

stability study referred to in the SCAN report from February 2002 was conducted with extruded pellets. The original data sheet (Annex VII of supplementary dossier II) consists only of tables without any additional information on extruding or pelleting or how the pellets have been produced.

The data remain unchanged as given in the previous SCAN-report. Extrusion (extruded pellets) leads to a loss of astaxanthin between 4 and 12 %, after 4 weeks storage, about 40 % of the original astaxanthin content are lost. In a field trial with atlantic salmon, the storage loss of astaxanthin from Ecotone™ was also measured. 4 months after feed production more than 40 % of the original level were lost.

### **3.4. Efficacy trials**

#### *3.4.1. Information provided in the dossier*

3 trials are reported in supplementary dossier III, 2 field trials with atlantic salmon and one trial with trout, the latter one was already part of supplementary dossier II, appendix I and cited in the previous SCAN report (see table 3, page 7).

Therefore 2 new trials are to consider. Trial 1 (appendix 1) was performed with 1.45 million salmon in 19 sea cages from 200.300 up to 700-1,500 g body mass at 4 stations for about 5 months (with some variation between the stations). Astaxanthin from Ecotone™ was compared with synthetic astaxanthin (ca 56 mg/kg). The astaxanthin concentration in feed was analysed. Water temperature is not given. Flesh pigmentation was measured by Roche Colour Card/SalmoFan, astaxanthin by spectrophotometry at start of trial and after 2-3 and 5 months. There were no significant differences in flesh pigmentation between fish fed the two astaxanthin sources.

Trial II (appendix 2) was performed with 1,200 fish (2 replicates/treatment) in a commercial sea farm under practical conditions for 14 months. Body mass at start was 220 g, specific growth rate amounted to 0.65. Astaxanthin from Ecotone™ was compared with synthetic astaxanthin. Feed was freshly prepared every 2 months, the astaxanthin content analysed (target concentration: 60 mg/kg). Pigmentation of the flesh was studied visually (Roche Colour Card) and by astaxanthin analyses (HPLC). Ten fillets/treatment were subjected to a freezing trial (-20°C), analyses were made one and two months thereafter. At the end of the trial astaxanthin in the fillets was not significantly different between the two astaxanthin sources. The visual measurement at the end showed a significant difference in favour of astaxanthin from Ecotone™. About 11-12 % of the astaxanthin consumed were retained in the body.

No differences in the behaviour of astaxanthin from both sources became evident by analyses of frozen fillets after one and two months.

### 3.4.2. Conclusion

Both trials confirm generally the flesh pigmenting properties of astaxanthin and specifically of astaxanthin from Ecotone<sup>TM</sup>. But this was also the base of the previous SCAN opinion.

The two trials on atlantic salmon could be accepted although they show minor lacks in the study design as compared with the recommendations given for astaxanthin fish trials under “4. Conclusions” of the previous SCAN report<sup>1</sup>.

### 3.4.3. Is there a need for trials on trout and atlantic salmon?

In supplementary dossier III is an information given by an independent expert, which should in the opinion of the company obviously serve as a proof, a scientific conclusion, that trials on trout and atlantic salmon are not necessary for an approval for both species if the efficacy of astaxanthin is well demonstrated for trout or atlantic salmon because both fish share common properties of astaxanthin metabolism. The carotenoid pigmentation of all salmonid species is governed by the same principles, but the expert also states that “quantitative differences may be found in absorption capacity and utilization, and in metabolic transformation”.

SCAN does not accept the company’s conclusion, that positive efficacy trials on one salmonid fish species would be sufficient to authorize the compound for all other salmonid fish. It is a principle of EU feed additive authorisation and established in corresponding guidelines to require for efficacy trials in all animal categories for which an approval is requested.

## 3.5. Environmental aspects

In its previous report SCAN expressed the opinion that “there is no justification for the absence of any data, because not absorbed astaxanthin could amount up to 50 % of the ingested. Its fate as well as its influence on other water living organisms need not to be without any environmental impact, as long as no data are presented”. If the retention rate of astaxanthin amounts to about 11 %, about 89 % of the astaxanthin intake will be excreted, as such, as specific metabolites or as physiological metabolic products.

In the recent supplementary dossier the company argues that astaxanthin from Ecotone<sup>TM</sup> will not add to the loading of the environment at a higher extent compared to synthetic sources. This argument can be accepted, but it stresses the opinion of SCAN given in the previous report under “4. Conclusion”: “SCAN wishes to draw the attention of the Commission to the

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<sup>1</sup> SCAN report adopted on 6 / 2 / 2002 on the use of astaxanthin-rich *Phaffia rhodozyma* in feedingstuffs for salmon and trout, available at [http://europa.eu.int/comm/food/fs/sc/scan/outcome\\_en.html](http://europa.eu.int/comm/food/fs/sc/scan/outcome_en.html)

fact that ..... the active colouring substance, astaxanthin, should undergo a dedicated risk assessment.”

### **3.6. Conclusion**

“Ecotone™ Phaffia Rhodozyma Yeast” contains 4,500 mg astaxanthin/kg (and 2,000 mg ethoxyquin/kg). Ecotone consists only of the *Phaffia* yeast (various fermentation batches) and will not contain any other feed material. Ecotone™ is labelled according to this statement.

Astaxanthin is rather unstable during feed storage. There will be no principal difference between synthetic astaxanthin and astaxanthin derived from Ecotone™.

The efficacy of Ecotone™ as carrier of astaxanthin and therefore its pigmenting properties are shown in two trials on trout and two trials on atlantic salmon. These four experiments show some imperfections in the experimental design in regard to the opinion of SCAN how such trials should be performed, however there is no doubt that astaxanthin also from the *Phaffia* yeast colours flesh (see page 7 of the previous SCAN report).

The environmental impact of astaxanthin, as well as of Ecotone™ as source of astaxanthin, can not be evaluated in the absence of any data.

SCAN wishes to draw the attention of the Commission to the fact that all the toxicity data presented and previously accepted by the SCAN refer to an older product which is not identical to Ecotone™ as the processing of the yeast is different.