



**REPORT OF THE SCIENTIFIC COMMITTEE ON ANIMAL NUTRITION ON  
THE SAFETY OF THE ENZYMATIC PRODUCT QUATRAZYME HP®  
FOR USE AS FEED ADDITIVE IN LAYING HENS**

(adopted on 19 June 2002)

**1. BACKGROUND**

The product « Quatrazyme HP® » preparation of endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase produced by *Aspergillus niger* (CNCM I-1517), (E.C. No. 24), is already provisionally authorised for the use as feed additive for the animal category chickens for fattening. The Commission received a request for a provisional Community authorisation for the animal category “Laying hens” under the conditions set out in the following table 1:

Table 1: Annex entry proposed by the Company.

Additive	Chemical formula, description	Species or category of animal	Minimum content	Maximum content	Other provisions
			Units of activity/kg of complete feedingstuff		
<b>Enzymes</b>					
Endo-1,4-beta-xylanase EC 3.2.1.8	Preparation of endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase produced by <i>Aspergillus niger</i> (CNCM I-1517) having a minimum activity of: 28 000 QXU <sup>1</sup> /g 140 000 QGU <sup>2</sup> /g	Laying hens	420 QXU	840 QXU	1. In the directions for use of the additive and premixture, indicate the storage temperature, storage life, and stability to pelleting.  2. Recommended dose per kilogram of complete feedingstuff: 560 QXU 2 800 QGU.  Containing xylenes and/or glucanes, for example diets containing a minimum of 20% wheat and/or barley.
Endo-1,3(4)-beta-glucanase EC 3.2.1.6			2 100 QGU	4200 QGU	

<sup>1</sup> 1 QXU is the amount of enzyme which liberates 1 micromole of reducing sugars (xylose equivalents) from oat xylan per minute at pH 5.1 and 50°C

<sup>2</sup> 1 QGU is the amount of enzyme which liberates 1 micromole of reducing sugars (glucose equivalents) from barley beta-glucan per minute at pH 4.8 and 50°C

The company producing Quatrazyme HP<sup>®</sup> prepared a dossier that has been submitted through the national rapporteur (France) to the Commission. The dossier was checked by the Member States for its compliance with the requirements of Council Directive 87/153/EEC fixing guidelines for the assessment of additives in animal nutrition. The Member States concluded in the Standing Committee for Animal Nutrition on 29 February 2000 that the dossier fulfilled these requirements.

The authorisation procedure laid down in article 4 of Council Directive 70/524/EEC as last amended by Council Directive 96/51/EC includes a period of 320 days for the evaluation of the dossier submitted to the Commission. The Standing Committee for Animal Nutrition started the evaluation of the product on 29 February 2000.

## **2. TERMS OF REFERENCE**

The Scientific Committee for Animal Nutrition (SCAN) is requested to assess the safety of “Quatrazyme HP<sup>®</sup>” preparation of endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase produced by *Aspergillus niger* (CNCM I-1517) for the laying hens.

## **3. OPINION OF SCAN**

### **3.1. History**

Quatrazyme HP<sup>®</sup> is an enzymatic product with two declared enzymatic activities. The same product proposed for use in chickens for fattening has already been the subject of SCAN evaluation. The outcome of that evaluation was satisfactory and led to the addition of the product Quatrazyme HP<sup>®</sup> for chickens for fattening in the "*SCAN report on the use of certain enzymes in animal feedingstuffs*" on 25 January 1999.

The safety assessment of that product included check of the absence of toxic effects in a bacterial mutagenicity study and *in vitro* test for chromosomal aberrations, absence of skin and eye irritations in rabbits and acute inhalation toxicity in rats, and absence of adverse effects in 13-week toxicity study by oral route in rats.

The enzyme preparation has not been modified and the final presentation and carrier is the same. The Enzyme fermentation is presented in powder using dextrin as carrier.

Considering that the new claim covers an extension of use to an other animal category of the same species, laying hens, only impact on the new target animal will be checked.

### **3.2. Tolerance test in laying hen.**

Quatrazyme is proposed to be included between 15 mg /kg (corresponding to 420 IU of xylanase and 2100 IU of  $\beta$ -glucanase) as minimal dose and 30 mg/kg (corresponding 840 IU of xylanase and 4200 IU of  $\beta$ -glucanase) as maximum dose for laying hens diets. The company recommends to use Quatrazyme HP<sup>®</sup> at 20 mg /kg of feed as others provisions.

The company carried out two tolerance studies in laying hens.

3.2.1. *First experiment. (1997)*

A first test was carried out in 1997 with a total of 135 laying hens of 28 weeks of age, at the beginning of experiment. The experiment lasted 84 days. The performance of laying hens was measured over three periods of 28 days.

Egg production was recorded daily. Feed consumption and feed conversion were recorded and calculated for each 28 days period. Laying hens were weighed at the beginning and at the end of experiment.

The total of laying hens was divided in three experimental groups.

The aim of the experiment was to compare the laying hens performance fed with three different diets:

- control diet (50 % wheat , 20 % barley),
- control diet + 20 mg of Quatrazyme HP<sup>®</sup> /kg feed (recommended dose), and
- control diet + 200 mg of Quatrazyme HP<sup>®</sup> /kg feed (tolerance level).

Table 2 : Results.

Dose of Quatrazyme HP <sup>®</sup> (mg/kg feed)	Laying rate (%)	Egg weight (g)	Egg mass produced (g/day)	Feed conversion g feed / g egg	Live body weight change (g)
Control	93.4±0.7	59.7±0.8	55.7±0.7	1.97±0.02 <sup>a</sup>	-102.5±22.1 <sup>b</sup>
20 mg/kg	94.5±1.2	60.0±0.4	56.7±0.5	1.95±0.03 <sup>ab</sup>	-29.8±11.5 <sup>a</sup>
200 mg/kg	94.0±0.9	60.4±0.6	56.7±0.9	1.91±0.01 <sup>b</sup>	+60.5±15.0 <sup>c</sup>

The values in the same column followed by different letters are significantly different at P<0.05

During the whole tolerance test no mortality was recorded and no bird was found with signs of physiological disorder or illness. The hens had a normal feeding behaviour.

Laying hens fed with Quatrazyme HP<sup>®</sup> had a significant better feed conversion in relation to the layer hens fed with control diet. Also laying hens fed with enzyme supplementation has shown a significant effect on the live weight variation from the beginning to the end of experiment. That effect is attributed to the fact that wheat-barley diets without enzyme do not cover the energy requirements of laying hens.

Considering the above, the SCAN can conclude that an overdosing at 200 mg Quatrazyme HP<sup>®</sup> / kg feed was well tolerated by laying hens. However as the product is proposed for inclusion in the feed up to 30 mg/kg, this test does not cover the highest dose proposed by the company. For use at 30 mg/kg feed, an other tolerance test using ten times that dose should be carried out and results submitted in order to establish the safety of Quatrazyme HP<sup>®</sup> at 30 mg/kg inclusion in the feed.

### 3.2.2. *Second experiment (July 2001 to February 2002)*

A total of 90 laying hens (ISA-Brown), 26 weeks old were used.

The performance of laying hens was determined over eight periods of four weeks.

The study started when chickens were 26 weeks old.

Animals were located in individual cages. 30 laying hens were distributed for each experimental treatment.

Fifteen cages contiguous were monitored for feed intake and feed conversion rate. Egg production was recorded daily. Feed consumption and feed conversion were recorded and calculated for each 28 days period (estimation). The average and egg weight was only recorded during four days for each period. Therefore, the egg weight and egg daily mass was an estimation. The feed composition was 47% wheat, 30% barley, 15% soybean and some ingredients commonly used.

As for the previous test, three different diets were used:

- T-1 control ,
- T-2 Quatrazyme at 20 mg/kg (recommended) and
- T-3 Quatrazyme at 1000 mg/kg.

Different parameters were checked: daily egg production, egg weight (by estimation of one sample of four eggs by period), feed intake from fifteen individual cages (average of two groups per treatment) for each period of four weeks.

Table 2: Results

Level of inclusion of Quatrazyme (mg/kg)	Feed intake (g/d)	Laying rate (%)	Egg weight (g)	Egg mass (g/d)	Feed conversion
0	116.8	80.7	62.0	50.0	2.33
20	117.0	85.9	62.8	53.9	2.17
1000	117.5	85.8	62.7	53.8	2.18

Results of the test show no mortality among the animals, and no modification of the laying hens performance for any of the experimental treatments.

Even though it was found that laying hens fed with Quatrazyme HP<sup>®</sup> have shown a significant better-feed conversion rate than control as a consequence of greater daily egg mass production (estimation over four egg by period). Laying hens chosen and allotted in the Quatrazyme groups started with high egg production from the beginning. It must be noticed that no covariant analysis was performed.

From this experiment it can be concluded that Quatrazyme HP<sup>®</sup> at 1000 mg/kg of feed (33 times, treatment T-3) the maximum dose recommended (30 mg/kg) does not reduce the laying performance and does not affect negatively the feed intake and the behaviour of laying hens. In conclusion laying hens are able to tolerate the Quatrazyme HP<sup>®</sup> supplementation at 33 times the maximum recommended level.

### **3.3. Conclusion**

This product has already been assessed for general safety and found safe by SCAN for chickens for fattening (initial application), for workers and for consumers.

On the basis of the tolerance tests provided for the extension of use to another animal category of the same species: laying hens, the product appears to be well tolerated. Consequently, SCAN concludes that the product is safe for laying hens, when used at the levels claimed by the petitioner and presented in table 1.