EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
C2 - Management of scientific committees; scientific co-operation and networks

REPORT OF THE SCIENTIFIC COMMITTEE ON ANIMAL NUTRITION ON THE SAFETY OF THE ENZYMATIC PRODUCT ROXAZYME G2® FOR USE AS FEED ADDITIVE IN LAYING HENS AND PIGLETS

(adopted on 19 June 2002)

1. BACKGROUND

The product Roxazyme G2®, preparation of endo-1,4-beta-glucanase EC 3.2.1.4, endo-1,3(4)-beta-glucanase EC 3.2.1.6, endo-1,4-beta-xylanase EC 3.2.1.8 produced by *Trichoderma longibrachiatum* (ATCC 74 252), identified under E.C. number 11, is provisionally authorised for the use as feed additive for chickens for fattening.

The Commission received a request for a provisional Community authorisation for two other animal categories: laying hens and piglets, under the conditions set out in the following table.

The product is intended for use in compound feed rich in non-starch polysaccharides (mainly arabinoxylans and beta glucans) e.g. minimum 40% wheat, triticale or barley.

The company producing Roxazyme G2®, prepared a dossier that has been submitted through the national rapporteur (Belgium) to the Commission. The dossier was checked by the Member States for its compliance with the requirements of Council Directive 87/153/EEC fixing the guidelines for the assessment of additives in animal nutrition. The Member States concluded in the Standing Committee for Animal Nutrition on 7th of June 2001 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 7th of June 2001.

2. TERMS OF REFERENCE

The Scientific Committee for Animal Nutrition (SCAN) is requested to give an opinion on the safety of Roxazyme G2®, preparation of endo-1,4-beta-glucanase EC 3.2.1.4, endo-1,3 (4)-beta-glucanase EC 3.2.1.6, endo-1,4-beta-xylanase EC 3.2.1.8

produced by *Trichoderma longibrachiatum* (ATCC 74 252), for laying hens and piglets.

Table 1:

No	Additive	Chemical formula, description	Species or category of animal	Minimum content Units of activity per Kg of complete feedingstuff	Other provisions
ENZYMES					
11	Endo-1,4-beta-glucanase EC 3.2.1.4 Endo-1,3(4)-beta-glucanase EC 3.2.1.6 Endo-1,4-beta-xylanase EC 3.2.1.8	Preparation of: endo-1,4-beta-glucanase EC 3.2.1.4, endo-1,3(4)-beta-glucanase EC 3.2.1.6, endo-1,4-beta-xylanase EC 3.2.1.8 produced by *Trichoderma longibrachiatum* (ATCC 74 252) having a minimum activity of: Endo-1,4-beta-glucanase: 8'000 U/g or ml ([13]) Endo-1,3(4)-beta-glucanase: 18'000 U/g or ml ([14]) Endo-1,4-beta-xylanase: 26'000 U/g or ml ([15])	Laying hens	Endo-1,4-beta-glucanase: 400 U Endo-1,3(4)-beta-glucanase: 900U Endo-1,4-beta-xylanase: 1'300 U	Recommended dose per kilogram of complete feedingstuff: Endo-1,4-beta-glucanase: 400 – 1'280 U Endo-1,3(4)-beta-glucanase: 900 – 2'880 U Endo-1,4-beta-xylanase: 1'300 – 4'160 U
			Piglets		Recommended dose per kilogram of complete feedingstuff: Endo-1,4-beta-glucanase: 400 – 1'600 U Endo-1,3(4)-beta-glucanase: 900 – 3'600 U Endo-1,4-beta-xylanase: 1'300 – 5'200 U

3. OPINION OF SCAN

3.1. General introduction

The product has already been assessed by SCAN and was considered satisfactory for chickens for fattening and turkeys for fattening.

Such assessment implies compliance with safety assessement requirements; these requirements include absence of toxic effects in a bacterial mutagenicity assay and an in vitro test for chromosome aberrations, absence of skin and eye irritation and acute inhalation toxicity, and absence of adverse effects in a 90day rodent repeated dose toxicity study.

The present request for approval concerns the same enzyme preparation derived from the same micro-organism to be used in two different animal categories: laying hens and piglets.

For these specific target animals a tolerance study is required. The levels of inclusion in the feed recommended by the company range from 50 to 160 mg product / kg feed for laying hens and from 50 to 200 mg product / kg feed for piglets.

3.2. Safety for laying hens

3.2.1. Tolerance test

The company performed a tolerance study using laying hens. In this study 160 hy-line brown hens were allocated in one room provided with 80 cages (2 hens/cage), and subdivided into 20 blocks of 4 adjacent cages each. The Roxazyme G2® experiment included 10 days of a pre-experimental period and lasted 6 weeks (from 52 to 57 weeks of age). The total laying hens was divided in four treatment groups (respectively not supplemented, supplemented with 0.6, 6.3 and 31.3 times the maximum recommended level of inclusion in the feed):

- control diet (based on barley),
- control diet + 100 mg Roxazyme G2® /kg,
- control diet + 1000 mg Roxazyme G2® /kg and
- control diet + 5000 mg Roxazyme G2® /kg.

Samples of feed (500 g) were analysed. β–glucanase and xylanase activities present in feeds were measured by a method using a chromogenic substrate.

The following performance characteristics were recorded:

– body weight: at the beginning and at the end of the experiment,

– egg production, egg weight and percentage of broken eggs: daily,

– feed intake and feed conversion (g feed / g eggs): every 3 weeks,

– general health examination: daily.

At the end of the experiment, blood samples were taken from 10 laying hens/treatment for haematology analysis. After blood collection, laying hens were euthanatized by use of sodium pentobarbital intravenously. Observations at necropsy were performed. At this time the following weight measurements were determined: digestive tract (including proventriculus, gizzard, intestine and caeca), and liver weights. Moreover, the appearance size of the abdominal fat depot was evaluated by a score (from 1 to 4).

3.2.2. Results

The enzyme activities measured in the different diets were considered to be acceptable.

The results have shown that the administration during 6 weeks of 100, 1000 and 5000 mg Roxazyme G2® /kg in the feed significantly increased feed intake in birds at 100 mg compared to the other test groups and controls (see table 2). No other statistically significant events were seen.

No significant differences between treatments on initial and final body weight and weight gain were observed.

Table 2: Laying hens performance (from 52 to 57 weeks of age)

Level of inclusion of Roxazyme G2® (mg/kg feed)	Feed intake (g/d)	Laying rate (%)	Egg weight (g)	Egg mass (g/d)	Feed conversion
Control	104.3[a]	83.2	69.2	57.7	1.83
100	108.6[b]	87.3	69.1	60.3	1.80
1000	106.1[ab]	87.5	68.5	59.9	1.78
5000	105.2[ab]	87.6	69.1	60.5	1.74
Probability	P<0.05	n.s.	n.s.	n.s.	n.s.

n.s.: not significant
a, b: The values in the same column followed by different letters are significantly different at p<0.05

There were no level-related changes in the haematological parameters measured.

The necropsy did not reveal any significant gross pathology changes. No significant differences in digestive tract or liver weights. The appearance of visceral fat depot was not modified.

3.2.3. Conclusion

This product has already been assessed for general safety and found safe by SCAN for animals of the same species (chickens for fattening), for workers and for consumers. On the basis of the tests provided for the extension of use to another animal category of the same species: laying hens, the product appears to be well tolerated, even after inclusion at 5000 mg/kg and is considered safe for the laying hens when used at the levels claimed by the petitioner in table 1.

3.3. Safety for piglets

3.3.1. Tolerance test in piglets

The company performed a tolerance study in piglets. In this study, 96 crossbred piglets (German Landrace pig x Piétrain) with an average of body weight at weaning of 7.2 ± 1 kg (approx. 4 weeks of age) were distributed over 48 floor pens (2 piglets/pen) and subdivided into four groups of 24 piglets each (respectively not supplemented, supplemented with 0.5, 5 and 50 times the maximum recommended level of inclusion in the feed)::

- control diet (based on wheat),
- control diet + 100 mg Roxazyme G2®/kg,
- control diet + 1000 mg Roxazyme G2®/kg and
- control diet + 10 000 mg Roxazyme G2®/kg.

The Roxazyme G2® tolerance experiment lasted 35 days.

Concentrations of β–glucanase and xylanase activities of Roxazyme G2® in feed samples were analysed.

The following performance characteristics were recorded:

– body weight gain, feed intake and feed conversion : on days 1-14, 15-21, 22-28 and 29-35 and total period (from weaning during 35 days)

– general health examination: at least once a day.

After the termination of the study, observations at necropsy and organ weights (i.e. liver, heart, kidney and spleen) were recorded. In addition haematology and blood chemistry was performed on 24 piglets (6 piglets per dose level).

3.3.2. Results

At the beginning of the trial (1-2 weeks), most of the piglets had diarrhoea. After this period, the general health status appeared normal. All diets used during the length of this experiment were accepted and consumed by the piglets.

With respect to average body weight gain, there were no significant differences whatever the level used for the overall period between dietary treatment in comparison to the control diet (see table 3).

For feed intake and feed conversion , evaluating the whole trial period, there was no significant difference in the average between the different dietary treatments with Roxazyme G2® compared to the control diet as shown in table 3.

Table 3: Piglet performance (during 35 days after weaning)

Level of inclusion Roxazyme G2® (mg/kg)	Weight gain (g/d)	Feed intake (g/d)	Feed conversion
Control	308.1 ± 55.8	549.3 ± 87.4	1.77 ± 0.17
100	317.8 ± 41.4	552.8 ± 44.9	1.75 ± 0.13
1000	310.4 ± 58.2	539.2 ± 79.6	1.75 ± 0.11
10 000	305.6 ± 50.9	534.2 ± 26.7	1.77 ± 0.23
Probability	n.s.	n.s.	n.s.

Gross pathology (including weights of liver, heart, kidneys and spleen), and haematology and blood chemistry did not show any differences between the treatment groups.

3.3.3. Conclusion

The results of the tolerance test presented by the company are satisfactory and it can be considered that the product is well tolerated in piglets.

Diarrhoea was noticed at the beginning of the experiment. This is however expected in piglets in the post weaning period because of the change of feeding system. It indeed disappeared during the test.

3.4. General conclusion

On the basis of the data presented, SCAN can conclude that the product Roxazyme G2® is safe when used under the conditions prescribed in table 1 for the laying hens and for piglets.