



**EVALUATION OF THE EFFICACY OF ENZYMATIC PRODUCT
« ENDOFEED DC» (ENDO-1, 3 (4)-BETA-GLUCANASE AND ENDO-1, 4-BETA-XYLANASE
PRODUCED BY *ASPERGILLUS NIGER*)**

(Adopted on 4 December 2002)

1. BACKGROUND

The product “Endofeed DC” (endo-1, 3 (4)-beta-glucanase and endo-1, 4-beta-xylanase produced by *Aspergillus niger*), is already provisionally authorised for the use as feed additive for the animal category “chickens for fattening” (first authorisation: Commission Regulation EC No 1436/98) until 30 June 2004.

The Commission received a request for a permanent Community authorisation for this animal category under the conditions set out in the following table:

Table 1

| No. | Additive | Chemical formula, description | Species or category of animal | Minimum content | Other provisions |
|----------------|--|--|-------------------------------|--------------------------------------|---|
| | | | | IU/kg of complete feedingstuff | |
| Enzymes | | | | | |
| 25 | Endo-1,3(4)-beta-glucanase EC 3.2.1.6 | Preparation of Endo-1,3(4)-beta-glucanase and Endo-1,4-beta-xylanase produced by <i>Aspergillus niger (phoenicis)</i> (NRRL 25541) having a minimum activity of: Endo-1,3(4)-beta-glucanase: 1100 IU ¹ /g Endo-1,4-beta-xylanase: 1600 IU ² /g | Chickens for fattening | Endo-1,3(4)-beta-glucanase 138 IU | Recommended dose per kilogram of complete feedingstuff: • Endo-1,3(4)-beta-glucanase: 138 IU • Endo-1,4-beta-xylanase: 200 IU For use in compound feeds rich in non-starch polysaccharides |
| | Endo-1,4-beta-xylanase EC 3.2.1.8 | | | Endo-1,4-beta-xylanase 200 IU | |

¹ 1 IU is the amount of enzyme which liberates 1 micromole of reducing sugars (glucose equivalents) from oat beta-glucan per minute at pH 4.0 and 30°C

² 1 IU is the amount of enzyme which liberates 1 micromole of reducing sugars (xylose equivalents) from oat xylan per minute at pH 4.0 and 30°C

2. TERMS OF REFERENCE

The Scientific Committee for Animal Nutrition (SCAN) is requested to advise the Commission on the efficacy of the product " Endofeed DC" when used as a feed additive in feedingstuffs, under the conditions proposed by the Company (see table 1).

3. OPINION OF THE COMMITTEE

3.1. History

Endofeed DC[®] is an enzymatic product with two declared enzymatic activities. This product proposed for use in chickens for fattening and laying hens was already the subject of SCAN evaluations. The outcome of these evaluations was satisfactory and led to the addition of the product in the "*SCAN report on the use of certain enzymes in animal feedingstuffs*" on 25 January 1999.

The SCAN is not aware of any more recent information that would require a re-evaluation of its opinion on the safety of the product. Consequently the present opinion deals only with issues related to efficacy.

3.2. Studies concerning the efficacy of the product "Endofeed DC"

3.2.1. *Efficacy tests in chickens for fattening*

Four experiments were presented in the efficacy dossier.

Three experiments were conducted to determine the efficacy of Endofeed DC[®] in chickens for fattening at three different locations in 2001.

The fourth experiment was not included. Because the statistical analyses of the data were performed among treatments:

Enzyme addition: Without (0 ppm) vs. with enzymes (75 + 125 + 175 ppm)

Dose of the enzyme complex: 75 ppm vs. 125 + 175 ppm

125 ppm vs. 175 ppm

There were not statistical analyses of the data among treatment: without enzyme addition (Control Group) vs. with 125 ppm Endofeed DC[®] Group (Recommended dosage).

3.2.2. First experiment

A total of 1920 day-old, sexed broiler chicks (Ross 308 strain) were used. Chickens were fed diets based on barley and soybean and allocated by sex to one of 3 treatments: Group 1: negative control (0), Group 2 with 100 and Group 3 with 125. The Group 2 was not further considered as the level of inclusion is below the minimum recommended dose (Table 2).

Table 2: Experimental design

| Group | Treatment Endofeed DC®/kg | Replicates of each group | | Broilers per replicate | Age of broilers, Day |
|-------|------------------------------|--------------------------|---------|------------------------|-------------------------|
| | | Males | Females | | |
| 1 | 0 | 8 | 8 | 40 | 1 – 42 |
| 3 | 125 | 8 | 8 | 40 | 1 - 42 |

Concentrations of the active substances in the pelleted feeds were analyzed on 9 samples of diets in two laboratories. Laboratories found respectively a recovery rate of 83 % and 119 % for β -glucanase and of 73 % and 104 % for xylanase.

Performance of the broilers was determined on the basis of feed intake, body mass (Day 42) and feed conversion per period (Table. 3). Over the total period (Day 1-42) supplementing the broiler diets with Endofeed DC® at 125 ppm had no effect on feed intake. Supplementing the diets with 125 ppm Endofeed DC® significantly improved the final body mass and the feed conversion compared with the control group 1.

Table 3: Growth performance (P<0.05)

| Age of broilers, day | Feed intake, g/broiler/day | Body mass, g/broiler | Feed conversion, kg/kg | |
|----------------------|----------------------------|----------------------|------------------------|---------|
| | 1 - 42 | 1-42 | 1 - 21 | 1 - 42 |
| Group 1 | 100.2 | 2166 a | 1.610 a | 1.991 a |
| Group 3 | 99.7 | 2280 b | 1.546 b | 1.882 b |

a,b – Values in columns with different superscripts differ significantly

Incidence of vent pasting was determined at 11 days on trial. There were a significantly higher proportion of chickens with some incidence of sticky droppings in the control group 1 compared to the Endofeed DC® group 3.

Intestinal viscosity was determined on one chicken per pen at the end of the experiment and was not significant between groups.

Litter quality was visually assessed during the last week. Results indicated that supplementing Endofeed DC® improved litter quality, and that this effect increased with the level of Endofeed DC.

3.2.3. Second experiment

A total of 1440 day-old, male broiler chicks (Hybro G strain) were used. Chickens were fed diets based on barley and soybean and allocated to one of three treatments Group 1: negative control (0), Group 2 with 100 and Group 3 with 125. The Group 2 was not further considered as the level of inclusion is below the minimum recommended dose (Table 4).

Table 4: Experimental design

| Group | Treatment ppm Endofeed DC/kg | Replicates of each group | Broilers per replicate | Age of broilers, Day |
|-------|---------------------------------|-----------------------------|---------------------------|-------------------------|
| 1 | 0 | 12 | 40 | 1 – 42 |
| 3 | 125 | 12 | 40 | 1 - 42 |

Concentrations of the active substances in the pelleted feeds were analyzed on 7 samples of diets in two laboratories. Laboratories found respectively a recovery rate of 80 % and 89 % for β -glucanase and of 62% and 102 % for xylanase.

Performance of the broilers was determined on the basis of feed intake, body mass (Day 42) and feed conversion per period (Table 5). For the overall period, from 0 to 42 days of age, supplementing the broiler diets with Endofeed DC[®] at 125 ppm had no effect on daily feed intake, body mass or mortality. Supplementing the diets with 125 ppm Endofeed DC[®] improved significantly feed conversion compared with the control group 1.

Table 5: Growth performance (P<0.05)

| Age of broilers, day | Feed intake, g/broiler/day | Body mass, g/broiler | Feed conversion, kg/kg | |
|----------------------|----------------------------|----------------------|------------------------|---------|
| | 1 - 42 | 1-42 | 1 - 21 | 1 – 42 |
| Group 1 | 95. | 2.420 | 1.403 a | 1.683 a |
| Group 3 | 93.3 | 2.452 | 1.354 b | 1.627 b |

a,b – Values in columns with different superscripts differ significantly

Vent pasting was monitored at 7 days of age and scored for every pen. The use of enzymes significantly reduced the incidence of vent pasting.

In vivo viscosity was measured at 36 days of age. Twelve samples per treatment were taken. Feeding the broilers diets supplemented with 125 ppm Endofeed DC[®] significantly decreased viscosity values as compared with the control group 1.

Litter quality was visually assessed at 21 and 42 days of age. At 21 days, no differences in wet litter were observed among the treatments. At 42 days of age, feeding the broilers diets supplemented with 125 ppm Endofeed DC[®] significantly decreased the appearance of wet litter values as compared with the control group 1.

3.2.4. Third experiment

A total of 2400 day-old, male broiler chicks (Ross 308 strain) were used. Chickens were fed diets based on barley, wheat, rye and soybean and allocated to one of three treatments Group 1: negative control (0), Group 2 with 100 and Group 3 with 125. The Group 2 was not further considered as the level of inclusion is below the minimum recommended dose (Table 6).

Table 6: Experimental design

| Group | Treatment ppm Endofeed DC/kg | Replicates of each group | Broilers per replicate | Age of broilers, Day |
|-------|---------------------------------|-----------------------------|---------------------------|-------------------------|
| 1 | 0 | 20 | 40 | 1 – 42 |
| 3 | 125 | 20 | 40 | 1 - 42 |

Concentrations of the active substances in the pelleted feeds were analyzed on 6 samples of diets in two laboratories. Laboratories found respectively a recovery rate of 39 % and 67 % (Group 3) for β -glucanase and of 10 % and 69 % for xylanase.

Performance of the broilers was determined on the basis of feed intake, body mass (Day 42) and feed conversion per period (Table 7). For the overall period, from 0 to 42 days of age, supplementing the broiler diets with Endofeed DC[®] at 125 ppm had no effect on daily feed intake. Supplementing the diets with 125 ppm Endofeed DC[®] improved significantly body weight gain and feed conversion compared with the control group 1.

Table 7: Growth performance (P<0.05)

| Group | Feed intake, g/broiler | Body mass gain, g/broiler | Feed conversion, kg/kg | |
|----------------------|------------------------|---------------------------|------------------------|---------|
| Age of broilers, day | 1 – 42 | 1 – 42 | 1 – 21 | 1 – 42 |
| 1 | 5272.0 | 2342.0 a | 2.247 a | 2.255 a |
| 3 | 5166.0 | 2.572.0 b | 1.949 b | 2.012 b |

a,b – Values in columns with different superscripts differ significantly

The incidence and severity of vent pasting/sticky droppings were assessed when the chickens were 10, 21 and 42 days of age. At 10 days of age of the chickens from control group were significantly more severely pasted than those of chickens fed Endofeed DC[®] supplemented diet. At 42 days no differences were observed between the broilers on any of the dietary treatments.

In vivo viscosity was measured at 21 days of age. Feeding the broilers diets supplemented with 125 ppm Endofeed DC[®] significantly decreased viscosity values as compared with the control group 1.

Litter quality was visually assessed at 10, 21 and 42 days of age. No significant differences in wet litter were observed between treatments.

3.2.5. *Comment*

In all experiments problems with recovery of enzymes after pelleting process were experienced. But in these studies, the efficacy results showed that enzymes were present in sufficient quantities.

3.3. Conclusion

The efficacy trials presented by the company were performed according to the SCAN guidelines for the assessment of enzyme additives. On the basis of the data submitted, it can be concluded that Endofeed DC[®] was efficacious in fattening chickens, after inclusion of the recommended dose per kilogram of complete feeding stuff (Endo-1,3(4)-beta-glucanase: 138 IU, Endo-1,4-beta-xylanase: 200 IU).