

STABILITY REPORT FOR **AG 175™**

Robert Gauthier, DVM. ACPV
Jefo Vice-President Research and Development

The protease **AG 175™** has been used extensively worldwide for many years, under all sorts of manufacturing conditions (mash feeds, pelleted feeds, expanded feeds, premix etc.) without any stability issue.

AG 175™ is very stable, assays on various lot numbers have shown no loss of activity over a 2 year period, at room temperature and at accelerated aging process.

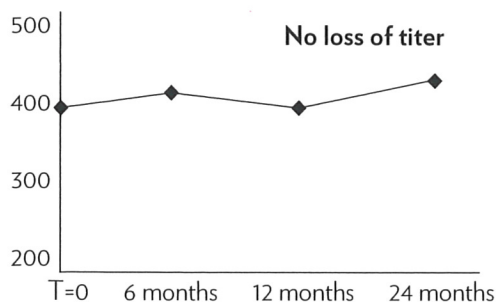
Part of the quality control is the determination of enzyme activity from the finished feeds after manufacturing, usually after pelleting or expansion or extrusion. Assays were realized at "l'Institut Européen de l'Environnement de Bordeaux, in France".

The normal pelleting conditions are:

- Steam injection in the conditioning chamber.
- Added moisture up to 16-18% relative humidity.
- Pressure: 4135 to 4880 kPa.
- Duration: 60 to 90 seconds.
- Temperature: 70 to 90° C. (note, some tests with an expander/ extruder up to 121° C did not damage the product).
- Normal cooling after pelleting.

A long term stability was conducted at various temperature over a two year period and showed no loss of activity:

STABILITY OVER TIME



TRIAL DESIGN - Stability of **AG 175™** over time

Trial done over a 2 year period by the DGCCRF research laboratory in Rennes in 1991. Samples of **AG 175™** were kept during 2 years and the enzyme activity measured at 6 months intervals after heating for 10 minutes at 25, 70 and 80°C. The Anson titration method was used. There was no loss of titer over time.

STABILITY AT HIGH TEMPERATURES DURING COMMERCIAL FEED PROCESSING

Description of the assay: The protease enzyme in **AG 175™** was mixed with ground soybeans and also with a commercial poultry feed in order to evaluate its stability at 120-121°C. during extrusion and expansion of the material.

The analysis were performed by l'Institut Européen de l'Environnement de Bordeaux, in France.

The results are reported as a reading of the optical density of the solutions of the extracted enzymatic complex, after incubation with an azocasein substrate.

Sample	Description	Optical density reading	Loss of titer
A-1	Ground soybeans + protease in AG 175™	0.242	-
A-2	Same as A-1, extruded at 121°C	0.169	(-30.1%)
B-1	Poultryfeed + protease in AG 175™	0.248	-
B-2	Same as B-1, expanded at 120°C	0.179	(-27.8%)
B-3	Same as B-2, after pelleting	0.169	(-31.8%)

Summary of assay: at extremely high temperature (40°C above normal pelleting temperature) the loss of enzyme activity was $\pm 30\%$.