This is provisional English translation of an excerpt from the original full report.

Risk Assessment Report

Phytase produced using *Trichoderma reesei* RF8694 strain

(Feed Additive)

Food Safety Commission of Japan (FSCJ)
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ABSTRACT

The FSCJ conducted a risk assessment of a feed additive, phytase produced using *Trichoderma reesei* RF8694 strain (hereinafter referred to as "QPT2 phytase"), based on the evaluation documents for feed additive designation.

There are three kinds of formulations in a feed additive containing QPT2 phytase: liquid, powder and granular. The recommended additive amount is 150-500 phytic acid decomposition power (phytase units: FTU) per kg of feed for pigs, chickens, and quails; and 500 FTU per kg of feed for aquatic animals.

In the genotoxicity study, an *in vitro* reverse mutation test, an abnormal chromosome test and an *in vivo* micronucleus test were implemented. The results were negative for all. Consequently, the FSCJ determined that there is no genotoxicity on QPT2 phytase.

In a 13-week subacute toxicity study in rats, no toxicity was observed due to the administration. Accordingly, the FSCJ determined that the no-observed-adverse-effect level (NOAEL) should be the highest dose of 1,000 mg/kg bw per day (227,000 FTU/kg bw per day for phytase). Although no residue test was conducted, *in silico* digestion analysis suggested that QPT2 phytase was dissolved into short peptide chains by various protein enzymes. Therefore, it was thought that QPT2 phytase would not remain *in vivo* of the target animals; and that the health effects on humans would be negligible as long as it is used appropriately as a feed additive.

The FSCJ presumed that the diluting agent, etc. included in this feed additive would have negligible effects on health when consuming this substance, considering its usage, existing toxicity assessments, directions and dosage.

From the results of a safety test for the target animals, the FSCJ assumed that there would be no safety issues for these animals when applied at the recommended additive amount.

Given the above, the FSCJ concluded that the probability of this feed additive affecting human health through food would be negligible as long as it is used appropriately.

Further, the FSCJ received an application from the Ministry of Agriculture, Forestry and Fisheries

(MAAF) on February 27, 2024 for a risk assessment regarding the safety of genetically modified feed additives, in accordance with the provisions of Appendix 2-2 of *the Ministerial Ordinance on the Specifications and Standards of Feeds and Feed Additives* (Ordinance No. 35 of July 24th, 1976 of the Ministry of Agriculture, Forestry and Fisheries) and is currently processing this request. Therefore, the MAAF should respect the results of this assessment when processing the designation of QPT2 phytase as a feed additive.