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#### Short communication

## Stability of vitamins in premixes

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#### Abstract

The stability of Vitamins A, E, and K<sub>3</sub> in premixes during storage in controlled conditions was studied over a period of one year. Analysis on vitamins content was performed at the beginning of the study and after 3, 6, and 12 months. The effect of the added choline chloride on the vitamin stability was also examined. All vitamins were more stable in a premix containing no choline chloride than in a premix containing choline chloride. During storage for 12 months, the concentrations of Vitamins A, E, and K<sub>3</sub> in the sample without choline chloride decreased to 53%, 59%, and 80% of their initial values, respectively. In the sample containing choline chloride, the concentrations of these vitamins decreased to 39%, 50%, and 9% of their initial values, respectively.

Keywords: Vitamins; Stability; Premixes; Choline chloride

#### 1. Introduction

Some of vitamins, contained in premixes are labile and lose their biological activity by formation of stereoisomers and by oxidation. This is initiated or catalysed by air, light, heat, moisture, mineral acids, metal ions, unsaturated fats, and oxidants. Reports on the study of the stability of vitamins in feeds under different processing conditions (Gadient

Abbreviations: BHT, butylhydroxytoluene; HPLC, high performance liquid chromatography; P, probability; S.E.M., standard error of the mean

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and Fenster, 1994; Dozier, 2002; Anderson and Sunderland, 2002) as well as reports on the study of the stability of vitamins in different matrices (Schröder et al., 1985; De Man et al., 1986; Dahl et al., 1994; Frias and Vidal-Valverde, 2001) including feeds and premixes (Leerbeck and Hjarde, 1975; Parrish and Patterson, 1983; Zhuge and Klopfenstein, 1986; Dove and Ewan, 1990, 1991; Gadient and Fenster, 1994; Shurson et al., 1996; Matyka et al., 1996; Jaśkiewicz et al., 1998) during storage period are available. Mainly the effect of the presence of minerals on the stability of vitamins during storage period of some weeks or months was studied. But a manufacturer should guarantee the stability of vitamins during storage as long as possible. In this view, the study of the stability of Vitamins A, E, and K<sub>3</sub> in a premix containing no minerals during storage over the period of one year under selected conditions and its dependence on the presence of choline chloride was performed in our laboratory.

#### 2. Materials and methods

#### 2.1. Apparatus

Water bath SW 22 (Julabo Labortechnik, Seelbach, Germany) and the linear shaker HS 501 digital (IKA Labortechnik, Staufen, Germany) were used for the preparation of samples and standards. Measurements were performed with an HPLC system Waters Alliance 2690 (Waters, Milford, MA, USA) equipped with a computer with a Millennium program for the system control and data processing, a column LiChrosorb Si 60, 5  $\mu$ m, 250 mm × 4 mm (Merck, Darmstadt, Germany), and Waters 2487 Dual  $\lambda$  Absorbance Detector (Waters). The column was kept at the ambient temperature.

#### 2.2. Samples

Two samples of a vitamin premix (not containing minerals) were examined. One kilogram of each sample contained  $16,000,000\,\mathrm{IU}$  of Vitamin A,  $2500\,\mathrm{mg}$  of Vitamin D<sub>3</sub>,  $12,000\,\mathrm{mg}$  of Vitamin E,  $1000\,\mathrm{mg}$  of Vitamin K<sub>3</sub>,  $2000\,\mathrm{mg}$  of Vitamin B<sub>1</sub>,  $4000\,\mathrm{mg}$  of Vitamin B<sub>2</sub>,  $2000\,\mathrm{mg}$  of Vitamin B<sub>6</sub>,  $15\,\mathrm{mg}$  of Vitamin B<sub>12</sub>,  $18,000\,\mathrm{mg}$  of nicotinamide,  $9000\,\mathrm{mg}$  of calcium pantothenate,  $40\,\mathrm{mg}$  of biotin,  $600\,\mathrm{mg}$  of folic acid,  $8000\,\mathrm{mg}$  of lysine and methionine,  $5000\,\mathrm{mg}$  of threonine,  $1200\,\mathrm{mg}$  of tryptophan,  $40,000\,\mathrm{mg}$  of the antioxidant Sanox,  $2000\,\mathrm{mg}$  of the aroma Sweet Vanilla, and the rest was organic carrier. One of the samples contained also  $140,000\,\mathrm{mg}$  of choline chloride. Vitamin A contained in the samples was in the form of coated retinyl acetate, Vitamin E was in the form of DL- $\alpha$ -tocopheryl acetate, and Vitamin K<sub>3</sub> was in the form of menadione sodium bisulphite. Samples were stored in paper bags in the dark at the temperature of  $25\,^{\circ}\mathrm{C}$  and at the relative humidity of 60%, and analysed at the beginning of the study and after 3, 6, and  $12\,\mathrm{months}$ .

#### 2.3. Reagents

Ethanol, potassium hydroxide, petroleum ether (boiling range 40–60 °C), hexane, *iso*-propanol, 1,2-dichloroethane, ammonia solution (analytical or chromatography grade

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