

The Stability of Vitamin A from Different Sources in Vitamin Premixes and Vitamin-Trace Mineral Premixes

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Abstract: This study was conducted to investigate the stability of five commercial vitamin A products in vitamin premixes and vitamin-trace mineral premixes. The five commercial products used were: Xinhecheng, Zhejiang medicine, Kingdomway, DSM, and BASF. The vitamin A products were stored in three vitamin premixes (for suckling, weanling, and finishing pigs) or in vitamin-trace mineral (VTM) premixes (for suckling, weanling, and finishing pigs). Vitamin premixes were stored in an environmentally controlled chamber set at 25 °C and 60% humidity. The VTM premixes were stored at room temperature (approximately 22 °C). Sampling was performed on d 0, 90, 180, 270, and 360. Stability was reported as the residual vitamin A activity (% of initial) at each sampling point. For the stability of vitamin premixes, all interactive and main effects of storage time and vitamin A product were not significant. For the stability of VTM premixes, there was no significant interaction effects between storage time, vitamin A product and main effect of vitamin A product, but the main effect of storage time was significant ($p < 0.01$). In conclusion, a longer storage time reduced vitamin A activity in VTM premixes but there was no difference in the stability of commercially available vitamin A.

Keywords: stability; storage; vitamin A; vitamin premix; vitamin-trace mineral premix



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1. Introduction

Vitamin A is essential for vision, reproduction, growth, and development [1–3]. Vitamin A deficiency can result in blindness, incoordination, reproductive disorders, and reduced growth performance [4,5]. In animal production, vitamin A is usually added to a complete diet to avoid vitamin A deficiency and promote the growth of farm animals. In addition, vitamin A is in the largest proportion in premixes and is the highest cost component of premixes, but vitamin A is the most sensitive to degradation by chemical and physical factors [6–8]. Therefore, the improvement of vitamin A product stability is the main challenge faced by vitamin manufactures. Several commercial vitamin A products are currently available in the Chinese market. These vitamin A products are produced from various vitamin manufactures that may have varying stabilities.

Currently, little published research exists evaluating the stability of commercial vitamin A products during storage. Therefore, the objectives of this study were to determine the storage stability of five commercial sources of vitamin A in premix, and provide a guideline for product formulation and application.

2. Materials and Methods

This study was conducted at the State Key Laboratory of Animal Nutrition at the China Agricultural University (Beijing, China). The Ethics Committee of Animal Care and Use was not consulted for this study because no animals were used.

2.1. Vitamin A Sources

This experiment used five commercially available vitamin A products: Xinhecheng Co., Ltd., Zhejiang, China; Zhejiang medicine Co., Ltd., Zhejiang, China; Kingdomway Group, Xiamen, China; DSM Nutritional Products, Heerlen, Netherlands; BASF, Ludwigshafen, Germany. Additionally, the specific vitamin A manufacturers are not disclosed in the results to protect proprietary information; thus, each vitamin A source in results is presented as manufacturer A to E. The manufacturing dates of all products were obtained from the original suppliers to ensure that the vitamin A products were within 6 months of manufacture and were not expired. These vitamin A products declared a potency of 500,000 IU/g. One IU vitamin A is equivalent to 0.30 µg retinol or 0.344 µg retinyl acetate [1].

2.2. Premix, Sampling, and Analysis

Each vitamin A product was added and mixed with either three vitamin premixes or three vitamin-trace mineral (VTM) premixes (Table 1). The amount added for each vitamin A product was determined such that including 0.30% vitamin premix or 1% VTM premix in the diet would provide the activity of vitamin A commonly used in pig diets (15,000 IU/kg for suckling pig, 12,000 IU/kg for weanling pig, and 8,000 IU/kg for finishing pig). Vitamin or VTM premixes were mixed at the designed levels with each vitamin A source, which were mixed using a paddle mixer for 5 min. The preparations of vitamin and VTM mineral premixes are described previously [9]. This process was repeated to yield six replicates per treatment. Vitamin premixes were stored in a controlled environment chamber setting at 25 °C and 60% relative humidity. The VTM premixes were stored in a storage room (approximately 22 °C). At sampling, each sample bag was pulled out at d 0, 90, 180, 270, and 360, and samples were sent immediately after collection to the Ministry of Agriculture and Rural Affairs Feed Efficacy and Safety Evaluation Center (Beijing, China) for vitamin A analysis using a slight modification of the AOAC official method (AOAC 2012.10) [10]. The stability of vitamin A in vitamin premixes and VTM premixes during storage was reported as the residual vitamin activity (% of initial) at each sampling point.

Table 1. The composition of vitamin and vitamin-trace mineral (VTM) premixes.

Item ¹	Vitamin Premixes			VTM Premixes		
	Suckling	Weanling	Finishing	Suckling	Weanling	Finishing
Vitamin A, 10 ⁴ IU/kg	500	400	267	150	120	80
Vitamin D ₃ , 10 ⁴ IU/kg	67	67	50	20	20	15
Vitamin E, IU/kg	50,000	33,333	25,000	10,000	3000	7000
Vitamin K ₃ , mg/kg	3333	1000	1333	1000	300	400
Vitamin B ₁ , mg/kg	167	133	67	50	40	20
Vitamin B ₂ , mg/kg	667	333	333	200	100	100
Vitamin B ₃ , mg/kg	23,333	15,000	10,000	7000	3000	4000
Vitamin B ₅ , mg/kg	13,333	11,667	10,000	4000	2000	4500
Vitamin B ₆ , mg/kg	1667	1000	667	500	300	200
Folic acid, mg/kg	5000	3333	3333	1500	1000	1000
Biotin, mg/kg	2333	2333	1000	700	500	300
Vitamin B ₁₂ , mg/kg	20	13	17	6	4	5
Choline, mg/kg	-	-	-	80,000	40,000	20,000
Copper, mg/kg	-	-	-	1500	1500	500
Iodine, mg/kg	-	-	-	12,000	10,000	4000
Iron, mg/kg	-	-	-	3500	3000	1500
Manganese, mg/kg	-	-	-	12,000	10,000	5000

Table 1. Cont.

Item ¹	Vitamin Premixes			VTM Premixes		
	Suckling	Weanling	Finishing	Suckling	Weanling	Finishing
Selenium, mg/kg	-	-	-	100	100	100
Znic, mg/kg	-	-	-	30	30	30

¹ Vitamin sources: Vitamin A, retinyl acetate; Vitamin D₃, cholecalciferol; Vitamin E, D,L- α -tocopherol acetate; Vitamin K₃, menadione sodium bisulfite; Vitamin B₁, thiamine mononitrate; Vitamin B₂, riboflavin; Vitamin B₃, nicotinic acid; Vitamin B₅, D-calcium pantothenate; Vitamin B₆, pyridoxine hydrochloride; Vitamin B₇, biotin; Vitamin B₉, folic acid; Vitamin B₁₂, cyanocobalamin; Choline, choline chloride. Trace mineral source: Copper, CuSO₄; Iodine, Ca(IO₃)₂; Iron, FeSO₄; Manganese, MnO; Selenium, NaSeO₂; Znic, ZnSO₄.

2.3. Data Analysis

Normality of the data was verified using the UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC, USA). The BOXPLOT procedure of SAS was used to check for outliers. Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA) to determine the interactive and main effects of storage time and vitamin A source on the activity of vitamin A in vitamin and VTM premixes. The LSMEANS statement was used to calculate treatment means, and the means were separated using the Tukey test. Results were considered significant at $p \leq 0.05$ and a tendency at $p \leq 0.10$. Residual vitamin A activity data are presented as means \pm SD, which was generated by Office 2019 (Microsoft Corporation, Redmond, WA, USA) and Adobe Illustrator 2020 (Adobe Inc., San Jose, CA, USA).

3. Results

There was no significant interaction or main effects of storage and vitamin A source on the stability of vitamin A in the vitamin premixes (Table 2; Figures 1–3). There was no significant interaction between storage time and vitamin A source on the stability of vitamin A in the VTM premixes (Table 2). The main effect of vitamin A source was not significant, but the main effect of storage time significantly affected the activity of vitamin A in the VTM premixes ($p \leq 0.01$). The loss of vitamin A activity in VTM premixes was increased during prolonged storage periods (Figures 4–6).

Table 2. The interaction effect and main effect probability of storage time and vitamin A source on the stability of vitamin A in vitamin and vitamin-trace mineral (VTM) premixes.

Item	Time \times Source	Time	Source
Vitamin premix (Suckling)	0.999	0.961	0.936
Vitamin premix (Weanling)	0.999	0.844	0.972
Vitamin premix (Finishing)	0.907	0.903	0.802
VTM premix (Suckling)	0.988	0.010	0.969
VTM premix (Weanling)	0.943	<0.01	0.877
VTM premix (Finishing)	0.984	<0.01	0.925

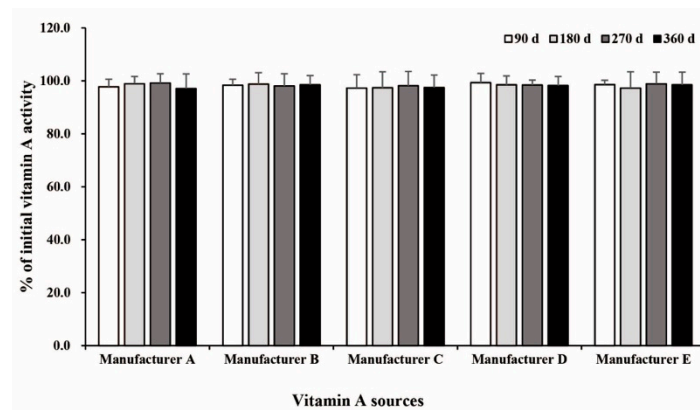


Figure 1. Mean (\pm standard deviation), $n = 6$ per treatment group. Effects of storage time and vitamin A source on residual vitamin A activity (% of initial) in vitamin premixes for suckling pigs. The p values relate to vitamin premix (time \times source, NS; time, NS; source, NS). NS represents not significant.

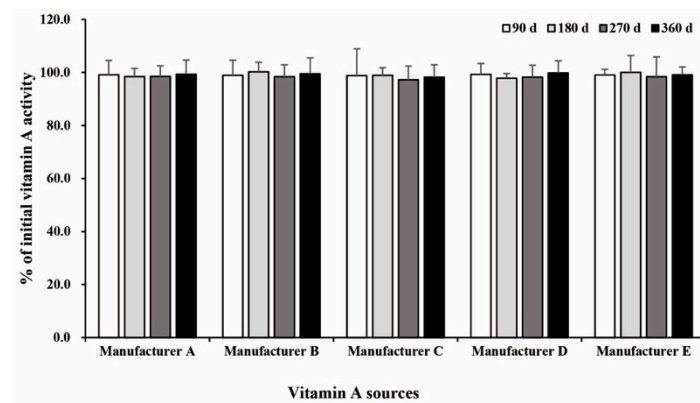


Figure 2. Mean (\pm standard deviation), $n = 6$ per treatment group. Effects of storage time and vitamin A source on residual vitamin A activity (% of initial) in vitamin premixes for weanling pigs. The p values relate to vitamin premix (time \times source, NS; time, NS; source, NS). NS represents not significant.

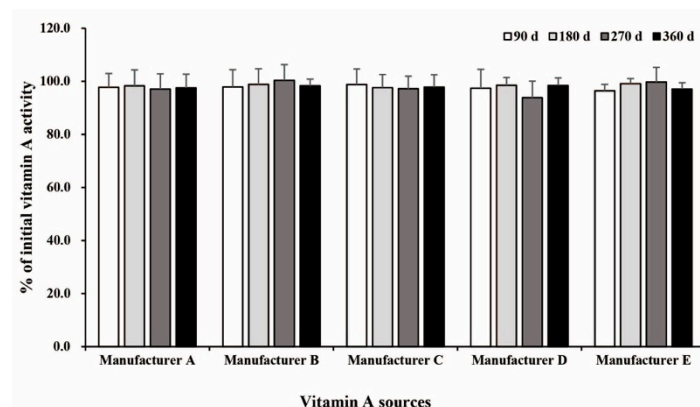


Figure 3. Mean (\pm standard deviation), $n = 6$ per treatment group. Effects of storage time and vitamin A source on residual vitamin A activity (% of initial) in vitamin premixes for finishing pigs. The p values relate to vitamin premix (time \times source, NS; time, NS; source, NS). NS represents not significant.

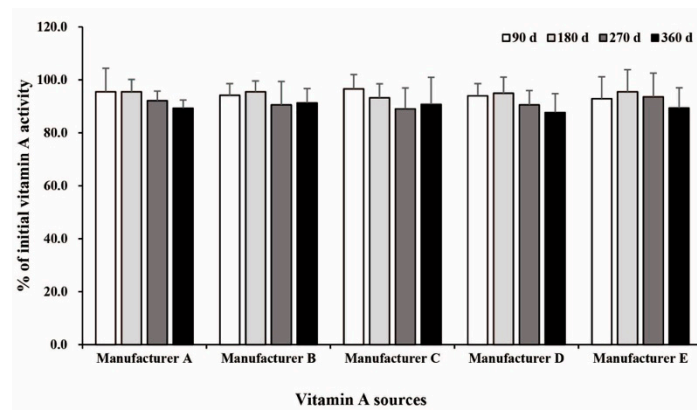


Figure 4. Mean (\pm standard deviation), $n = 6$ per treatment group. Effects of storage time and vitamin A source on residual vitamin A activity (% of initial) in vitamin-trace mineral premixes for suckling pigs. The p values relate to vitamin premix (time \times source, NS; time, $p = 0.01$, source, NS). NS represents not significant.

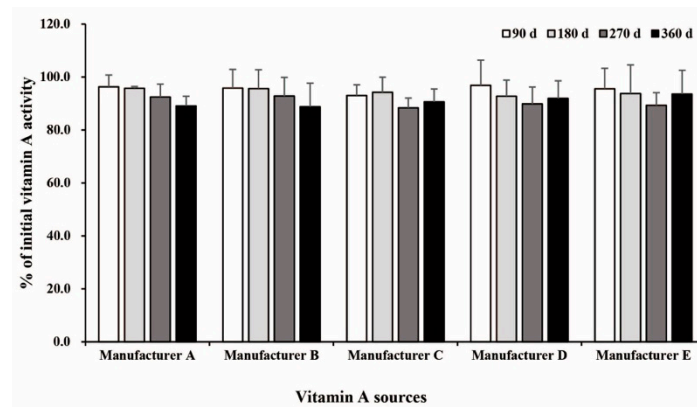


Figure 5. Mean (\pm standard deviation), $n = 6$ per treatment group. Effects of storage time and vitamin A source on residual vitamin A activity (% of initial) in vitamin-trace mineral premixes for weanling pigs. The p values relate to vitamin premix (time \times source, NS; time, $p < 0.01$ source, NS). NS represents not significant.

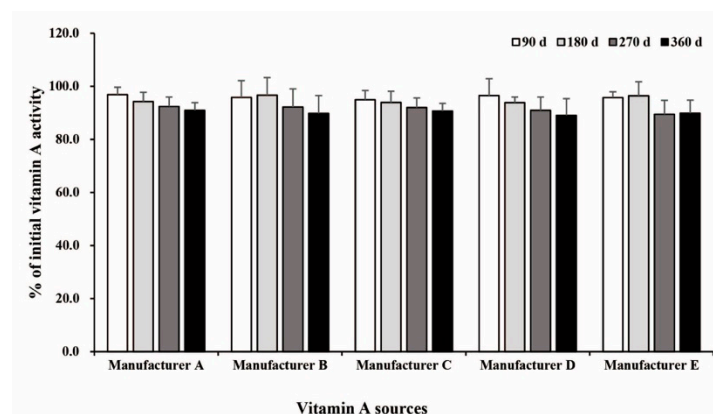


Figure 6. Mean (\pm standard deviation), $n = 6$ per treatment group. Effects of storage time and vitamin A source on residual vitamin A activity (% of initial) in vitamin-trace mineral premixes for finishing pigs. The p values relate to vitamin premix (time \times source, NS; time, $p < 0.01$; source, NS). NS represents not significant.

4. Discussion

Zhuge and Klopfenstein conducted a study on vitamin stability under room temperature storage and they reported that 56–57% of vitamin A was lost after one month of storage [11]. The BASF company (BASF 1994, cited by Whitehead) reported that the retention of vitamin A in VTM premix was 85% and 58% after one month and six months of storage, respectively [12]. Mooney and Aldrich investigated the stability of vitamin premixes under the conditions of 20 °C and 50% humidity [13], and they observed that after six months of storage, the retention of vitamin A was 76%. The loss activity of vitamin A during storage reported in previous studies is higher than the results from the present experiment. The high loss of vitamin A after stability testing reported in the previous study may be easily explained by a limitation in production processes decades ago that affected vitamin A stability. In the present study, it was observed that the loss of vitamin A in the premixes did not exceed 10% after 12 months of storage, which indicates that the commercially available vitamin A products have good stability. Shurson et al. [14] reported that vitamin A activity in vitamin and VTM premixes decreased with prolonged storage time. Additionally, the vitamin A stability in vitamin premix was 86% after 120 days of storage, while vitamin A activity in VTM premix decreased to 64.12% [14]. In the current study, the retention of vitamin A in vitamins and VTM premixes is higher than the results of Shurson's study [14]. This may be caused by the vitamin A production process, but on the other hand, these differences may be due to the composition of the premix in the study by Shurson, as well as the use of vitamin A in the free alcohol form. The current study indicated that extended storage time reduces the stability of vitamin A in the VTM premix, which is in agreement with the previous study [8,9].

The pure product of retinol has poor stability and cannot be used directly in feed production [15]. Feed-grade vitamin A mostly uses vitamin A acetate with better stability, but oily vitamin A acetate is usually processed into dry powder for feed processing. The commercially available vitamin A (dry powder) in the Chinese feed additive market is 500,000 IU/g. The dry powdered vitamin A sources vary in their susceptibility to degradation by chemical and physical factors, such as light, oxygen, temperature, and humidity [6]. The degradation of vitamin A is accelerated by trace minerals in the presence of a premix [6,9,12]. However, the present study observed that there was no significant effect of vitamin source on the stability of vitamin A in vitamins and VTM premixes and the retention of vitamin A was over 90% after one year of storage. This may be due to commercially available vitamin A products providing stability far superior to the raw vitamin product. On the other hand, this may be that different manufacturers are homogeneous in their technological development, reducing the difference in vitamin A stability. Coatings (e.g., carbohydrate, protein, ethyl cellulose) or encapsulation gives vitamins such as vitamin A greater protection against moisture, heat, and pressure during storage and processing [16]. Spray drying is a technique where a liquid product is atomized in a hot gas which instantaneously leads to the formation of powders. With the advantages of low cost, high productivity, good continuity, and rapid processing, this spray drying method is often used to prepare microencapsulation to protect vitamin A. Modified starch and gelatin are usually used as coating materials [17]. Currently, Xinhecheng, Zhejiang medicine, and Kingdomway have applied a spray drying approach for vitamin A production. DSM uses cross-linking coating technology to create a matrix of a cross-linked beadlet generally composed of gelatin, sugar, gum, starch or some similar type of hydrocolloid. The inclusion of antioxidants such as ethoxyquin, beta hydroxy acid, or butylated hydroxytoluene offers additional protection against oxidizing agents [16,18]. The BASF company also introduces the microencapsulation of vitamin A to produce microencapsulated vitamin A with sucrose, gelatin, modified starch, butylated hydroxytoluene, and sodium aluminosilicate. The vitamin manufacturer has developed vitamin A products with high quality and stability. Today's vitamin A product forms have marked advantages when they are used in feed manufacturing.

5. Conclusions

The stability of commercially available vitamin A during storage is affected by storage time regardless of vitamin A source. Vitamin A stored in a vitamin premix was the most stable. In VTM premixes, longer storage times reduced vitamin A activity, but vitamin A sources from different manufacturers have the same stability during storage.

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Data Availability Statement: The data presented in this study are available on request.

Conflicts of Interest: The authors declare no conflict of interest.

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