Impact of storage conditions and premix type on water-soluble vitamin stability

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ABSTRACT

Mitigation options to reduce the risk of foreign animal disease entry into the United States may lead to degradation of some vitamins. The objective of Exp. 1 was to determine the impact of 0, 30, 60, or 90 d storage time on water-soluble vitamin (riboflavin, niacin, pantothenic acid, and cobalamin) stability when vitamin premix (VP) and vitamin trace mineral premix (VTM) were blended with 1% inclusion of medium-chain fatty acid (MCFA) (1:1:1 blend of C6:C8:C10) or mineral oil (MO) with different environmental conditions. Samples stored at room temperature (RT) (approximately 22 °C) or in an environmentally controlled chamber set at 40 °C and 75% humidity, high temperature high humidity (HTHH). The objective of Exp. 2 was to determine the effect of heat pulse treatment and MCFA addition on water-soluble vitamin (riboflavin, niacin, pantothenic acid, and cobalamin) stability with two premix types. A sample from each treatment was heated at 60 °C and 20% humidity. Therefore, treatments were analyzed as a 2 × 2 factorial, with two premix types (VP vs. VTM) and two oil types (MO vs. MCFA). For Exp. 1, the following effects were significant for riboflavin: main effect of premix type (P < 0.0001), storage condition (P = 0.015), and storage time (P < 0.0001); for pantothenic acid: premix type × storage time × storage condition (P = 0.003) and premix type × oil type (P < 0.0001) interactions; and for cobalamin: premix type × storage condition (P < 0.0001) and storage time × storage condition (P < 0.0001) interactions and main effect of oil type (P = 0.018). The results of Exp. 2 demonstrated that there was an interaction between oil type and premix type for only pantothenic acid (P = 0.021). The oil type did not affect the stability of riboflavin, niacin, or cobalamin and pantothenic acid stability was not different within similar premixes. The only difference in water-soluble vitamin stability between VP and VTM was for pantothenic acid (P < 0.001). The results of this experiment demonstrated that the stability of water soluble vitamins are dependent on the vitamin of interest and the conditions at which it is stored.

Key words: environmental condition, heat treatment, medium chain fatty acid, vitamin stability

INTRODUCTION

In response to the increasing threat of foreign animal disease entry into the United States, the U.S. feed industry has taken active steps to reduce the risk of pathogen entry through ingredients. Some ingredients, such as vitamins, are not available via domestic production and must be imported. While it is unlikely that these products are contaminated due to their manufacturing process, there is still request to further reduce risk of pathogen contamination.

Mitigation options to reduce the risk of foreign animal disease entry may include storage of imported ingredients or inclusion of a chemical additive. Diel (2019) reported that storing Vitamin D for 39 d at 4 °C or 26 d at either 15 °C or 30 °C leads to 99.99% degradation of Senecavirus A, a domestic pathogen that is also a surrogate for foot and mouth disease virus. This report has led several feed manufacturers or ingredient suppliers to hold vitamin premix (VP) or vitamin and trace mineral premix (VTM) for at least 40 d prior to use. However, storage for this time and temperature may lead to degradation of some vitamins. A pulse of high temperature for a short duration may have the same efficacy for viral degradation, but there is limited research reporting its effect on vitamin stability.

Other research has suggested that a 1% inclusion of a medium-chain fatty acid blend (MCFA) made of equal parts C6:0, C8:0, and C10:0 can reduce the risk of porcine virus transmission through feed (Cochrane et al., 2016). At this time, however, the inclusion of MCFA for the purpose of mitigating viral risk is not approved in animal food (Title 21 Code of Federal Regulations Part 573); and the author is unaware of any current Food Additive Petition seeking its approval. In the interim, some feed manufacturers have chosen to add MCFA-containing ingredients or use them to partially replace their existing fat source because of studies reporting a benefit to pig growth performance (Thomson et al., 2018), and this nutritional use falls under the “Fat Product, Feed Grade” definition described by the Association of American Feed Control Officials (2022). One option may be to include MCFA in place of the mineral...
oil (MO) conventionally used to control dust in VP and VTM. However, additional research is needed to determine if the MCFA interact with nutrients in these premixes. It is hypothesized that altering storage conditions or adding MCFA to the premix to reduce viral survivability will negatively affect vitamin stability.

Therefore, the first objective (Exp. 1) of this experiment was to determine the impact of 0, 30, 60, or 90 d storage time on water-soluble vitamin (riboflavin, niacin, pantothenic acid, and cobalamin) stability when stored as a VP or VTM and blended with 1/1% inclusion of MCFA (1:1:1 blend of C6:C8:C10) or MO with different environmental conditions. The second objective (Exp. 2) of this experiment is to determine the effect of heat pulse treatment and MCFA addition on water-soluble vitamin (riboflavin, niacin, pantothenic acid, and cobalamin) stability with two premix types. This paper is published in conjunction with Saensukjaroenphon et al. (2020a, 2020b) which reported stability results are presented in Saensukjaroenphon et al. (2020a). Fat-soluble vitamin stability results from this experiment are presented in Saensukjaroenphon et al. (2020b). Both premixes did not contain choline. Masonry sand was added to the vitamin premix to keep the concentrations of the vitamins the same between the VP and VTM. The 25.9-kg vitamin premix, 4.1-kg phytase, and 2.1 xylanase were mixed in a 0.085 m³ paddle mixer (Davis model 2014197-SS-S1, Bonner Springs, KS, USA) then mixed with either 15.5-kg masonry sand or 15.5 kg trace mineral premix for 5 min. This yielded a 47.6-kg batch of 1) VP and 2) VTM. Each premix was equally discharged into three separate 15.9 kg aliquots. A 2.5-kg subsample of each aliquot was taken to create a 7.5-kg experimental premix treatment. The 7.5-kg premixes were mixed for 10 s using a mixer (Hobart model HL-200, Troy, OH, USA) equipped with an aluminum flat beater model HL-20 that had 3.69% coefficient of variation (CV) when it was validated for uniform liquid addition. Following the 10 s dry mix, either a 74.8-g of 1:1:1 commercial blend of C6:0, C8:0, and C10:0 MCFA (PMI Nutritional Additives, Arden Hills, MN, USA) or 74.8-g of MO were added using a pressurized hand-held sprayer with a fine hollow cone spray nozzle (UNIJET model TN-SS-2, Wheaton, IL, USA). The premixes were mixed for an additional 90 s post-oil application. The mixed samples were divided to obtain eight individual 900-g samples, which were placed in paper bags. These samples served as the experimental unit for all treatments. This process was repeated to yield three replicates per treatment. The mixing steps are illustrated in Figure 1.

**Materials and Methods**

**Ingredient Sources and Manufacturing**

A VP (DSM Nutritional Products, Inc., Parsippany, NJ, USA) and VTM were manufactured for both storage condition and heat pulse treatment experiments as outlined in Table 1. Both premixes contained phytase and xylanase and phytase stability results from this experiment are presented in Saensukjaroenphon et al. (2020a). Fat-soluble vitamin stability results from this experiment are presented in Saensukjaroenphon et al. (2020b). Both premixes did not contain choline. Masonry sand was added to the vitamin premix to keep the concentrations of

| Ingredients                  | Vitamin trace mineral premix | Vitamin premix |
|------------------------------|------------------------------|----------------|
|                             | % inclusion | Batch, kg | % inclusion | Batch, kg |
| KSU Swine Vitamin¹           | 25.89       | 57.07     | 54.35       | 25.89     |
| KSU Trace Mineral²           | 32.60       | 15.53     | 0.00        | 0.00      |
| Masonry Sand                 | 0.00        | 0.00      | 32.60       | 15.53     |
| HiPhos GT5000³               | 8.70        | 4.14      | 8.70        | 4.14      |
| Belfeed B 1100 MP⁴           | 4.35        | 2.07      | 4.35        | 2.07      |
| Total                        | 100.00      | 47.63     | 100.00      | 47.63     |
| Formulated concentration, ppm|             |           |             |           |
| Riboflavin                   | 1,797       |           | 1,797       |           |
| Niacin                       | 10,781      |           | 10,781      |           |
| Pantothenic Acid             | 5,989       |           | 5,989       |           |
| Cobalamin                    | 7           |           | 7           |           |

¹Composition per kilogram: 1,653,000 IU Vitamin A, 661,376 IU Vitamin D3, 17,637 IU Vitamin E, 13.3 mg Vitamin B12, 1,323 mg Menadione, 3,307 mg Riboflavin, 11,023 mg d-Pantothenic Acid, and 19,841 mg Niacin. Rice hulls and calcium carbonate are carriers in the premix.

²Composition per kilogram: 73 g Iron from ferrous sulfate, 73 g Zinc from zinc sulfate, 22 g Manganese from manganese sulfate, 11 g Copper from copper sulfate, 198 g Iodine from calcium iodate, and 198 g Selenium sodium selenite. Calcium carbonate is a carrier in the premix.

³Composition per kilogram: 5,000,000 FYT Phytase (Aspergillus oryzae)

⁴Composition per kilogram: 98,000 U Xylanase (Bacillus subtilis)
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Temperature reached 60 °C after 2 h and 21 min in the chamber. The samples were held at 60 °C for 9 h and 27 min. The individual premix samples were riffle divided twice to yield two 200-g sub-samples; then, they were sent to commercial laboratories for analyses as previously explained in Exp. 1. The results of vitamin after heat pulse treatment were reported in percent stability, which was calculated by dividing the vitamin concentration by the initial vitamin concentration and then multiplying by 100.

Statistical Analysis

Data were analyzed as two separate completely randomized experiments: Exp. 1) impact of MCFA vs. MO addition on vitamin concentration in VP or VTM over time in RT or HTHH, which used the day 0 samples as an initial concentration, with day 30, 60, and 90 values reported as a percent stability; and 2) impact of MCFA vs. MO addition on vitamin concentration in vitamin or VTM premixes subjected to a pulse of high temperature. Experiment 1 was arranged as a 2 × 2 factorial with two types of premixes (VP vs. VTM), two types of oil addition (MO vs. MCFA), two storage conditions (RT vs. HTHH), and three time points of vitamin retention (day 30, 60, and 90). Linear and quadratic contrast were used to for analysis of time. Experiment 2 was arranged as a 2 × 2 factorial with two types of premixes (VP vs. VTM), and two types of oil addition (MO vs. MCFA). Data were analyzed using the GLIMMIX procedure of SAS v9.4 (Cary, NC, USA), with all main effects and relevant interactions were included. Contrasts were used to compare the linear or quadratic effect of vitamin stability over time. Results were considered significant if \( P \leq 0.05 \).

RESULTS

Initial Vitamin Activity

The initial concentration of vitamins riboflavin, Niacin, pantothenic acid, and cobalamin are reported in Table 2 for VP with MO, VP with MCFA, VTM with MO and VTM with MCFA. The formulated vitamin concentration was 1,797, 10,781, 5,989, and 7 mg per kg for riboflavin, niacin, pantothenic acid, and cobalamin, respectively. The initial
concentration of niacin and pantothenic acid was more than 88% of formulated concentration for all four premixes. The vitamin source of the premixes was manufactured and stored for 172 d before it was used to make premixes. However, the cobalamin was 20% to 27% than the original expected concentration of the vitamin source. For initial riboflavin concentration, the percent of formulated vitamin concentration was 92%, 85%, 71%, and 70% for VP with MO, VP with MCFA, VTM with MO, and VTM with MCFA, respectively. The riboflavin concentration in VTM was low across all treatments in this study.

**Experiment 1: Storage Condition Experiment**

The stabilities of water-soluble vitamins are provided in Tables 3–6. The interactive means are reported within each table when significant and when there is no evidence of interaction the main effect means are reported. There were no four-way or three-way interactions among combinations of oil type, premix type, storage condition, and storage time ($P > 0.200$) for all four water-soluble vitamins except the premix type x storage condition x storage time interaction of pantothenic acid ($P = 0.002$). There was a difference for pantothenic acid stability between RT and HTHH for both VP and VTM after they were stored for 30 d. However, there was a difference for pantothenic acid stability between RT and HTHH for both VP and VTM as described by interactions. Additionally, the oil type only affected cobalamin ($P = 0.018$). Cobalamin was more stable in premixes with MO added compared to premixes with MCFA added regardless of storage condition and storage time. The storage condition affected the stability of riboflavin ($P = 0.015$) and niacin ($P = 0.031$). Both riboflavin and niacin were more stable when premixes were stored under RT versus HTHH regardless of storage time and oil type. There was a quadratic decrease on the riboflavin stability as the storage time increased ($P < 0.001$).

**Experiment 2: Heat Pulse Treatment Experiment**

There was no interaction between oil type and premix type for the stability of water-soluble vitamins ($P > 0.134$) except pantothenic acid ($P = 0.021$; Table 7). The VP with MCFA had a greater pantothenic acid stability compared to VTM with MCFA. However, there was no difference for pantothenic acid stability when MO was added in VP versus VTM. The oil type did not affect the stability of water-soluble vitamins ($P > 0.062$). There was also no significant difference for niacin, pantothenic acid, and cobalamin between VP and VTM ($P > 0.054$) but pantothenic acid ($P < 0.001$) decreased stability in VTM compared to VP. The VP had a greater pantothenic acid stability as compared to VTM after premixes were heated at 60 °C for 9 h and 27 min.

**DISCUSSION**

**Experiment 1: Storage Condition Experiment**

Frye (1994) reported that the lower assay tolerance of water-soluble vitamins was 88, 87, 86 and 86% for riboflavin, niacin, pantothenic acid and cobalamin, respectively. After accounting for the variation of water-soluble vitamin assays, the following effects remain significant for riboflavin: main effect of premix type, storage condition and storage time; for niacin: no effect of premix type, oil type, storage condition and storage time; for pantothenic acid: premix type x storage time x storage condition and premix type x oil type interactions; and for cobalamin: premix type x storage condition and storage time x storage condition interactions and main effect of oil type.

Riboflavin was more stable in VP (93.9%) compared to VTM (81.0%) regardless of oil type, storage condition and storage time. Marchetti et al (2000) reported that the riboflavin stability was 82.1% in VTM mixed with the sulphate salts of iron, zinc, manganese, copper and cobalt were stored at 37 °C for 90 d. In the experiment reported herein, the trace
mineral premix which is the mineral source of the premixes, consist of calcium carbonate, ferrous sulfate, zinc sulfate, copper sulfate, manganous oxide, sodium selenite, and calcium iodate. The percent loss of riboflavin was greater in VTM versus VP, which was probably caused by sulphate salts of metal ion in trace mineral. The riboflavin stability was similar between the premixes with 1% MO added and the premixes with 1% MCFA added regardless of storage condition and storage time. Moreover, the riboflavin stability was greater when premixes were stored under RT (89.3%) versus HTHH (84.9%) regardless of oil type and storage time. Moreover, increasing storage time from 30 to 90 d quadratically decreased the riboflavin stability from 89.3 to 80.3%. Therefore, riboflavin was stable in premixes up to 60 d under both conditions. However, the stability of riboflavin was greater in VP versus VTM. The MCFA did not impact

### Table 3. Effect of the premix type, oil type, storage temperature, and storage time on riboflavin stability for storage condition samples

| Item                              | Riboflavin stability, % |
|-----------------------------------|-------------------------|
| **Interaction**                   |                         |
| Vitamin premix                    | 90.4b                   |
| Vitamin premix                    | 96.2a                   |
| Vitamin trace mineral premix      | 82.5c                   |
| Vitamin trace mineral premix      | 79.4d                   |
| Pooled SEM                        | 1.7                     |
| **Main effect**                   |                         |
| Vitamin premix                    | 93.3s                   |
| Vitamin trace mineral premix      | 81.0i                   |
| Pooled SEM                        | 1.2                     |
| **Storage condition**             |                         |
| RT                                | 89.3s                   |
| HTHH                              | 84.9t                   |
| Pooled SEM                        | 1.2                     |
| **Storage time, days**            |                         |
| 30                                | 89.3s                   |
| 60                                | 91.8s                   |
| 90                                | 80.3y                   |
| Pooled SEM                        | 1.5                     |

1Included at 1% of the premixes.
2Mineral oil comprised of saturated aliphatic and alicyclic nonpolar hydrocarbons sourced as a byproduct of petroleum refining.
3Medium chain fatty acid, MCFA, comprised of a 1:1:1 blend of medium chain fatty acids (C6:0, C8:0, and C10:0).
4Room temperature, the average temperature and relative humidity were 22.1 °C and 28.4%, respectively.
5High heat and high humidity, the average temperature and relative humidity were 39.5 °C and 78.8%, respectively.
6Percent vitamin stability was calculated by dividing the vitamin activity at day 30, day 60, or day 90 by the analyzed initial vitamin activity and then multiplying by 100.

### Source of variation

| Source of variation | Probability, P < |
|---------------------|-----------------|
| Premix Type         | 0.0001          |
| Oil type            | 0.438           |
| Premix Type × Oil type | 0.013       |
| Storage condition   | 0.015           |
| Premix Type × Storage condition | 0.084   |
| Oil type × Storage condition | 0.391   |
| Premix Type × Oil type × Storage condition | 0.346   |
| Time                | 0.0001          |
| Linear              | 0.0001          |
| Quadratic           | 0.001           |
| Premix Type × Time  | 0.913           |
| Oil type × Time     | 0.770           |
| Premix Type × Oil type × Time | 0.775   |
| Storage condition × Time | 0.096   |
| Premix Type × Storage condition × Time | 0.335   |
| Oil type × Storage condition × Time | 0.523   |
| Premix Type × Oil type × Storage condition × Time | 0.173   |

*Means within premix type × oil type interaction followed by a different letter are significantly different (P ≤ 0.05).
**Means within a main effect of premix type followed by a different letter are significantly different (P ≤ 0.05).
***Means within a main effect of storage condition followed by a different letter are significantly different (P ≤ 0.05).
****Means within a main effect of storage time followed by a different letter are significantly different (P ≤ 0.05).
riboflavin stability. Gadient (1986) reported that riboflavin was slightly sensitive to both temperature and oxygen, and moderately sensitive to humidity during pelleting. The results of the current study demonstrated the combined impact of temperature, high humidity and exposed time that decreased the riboflavin stability by 4.4% when premixes were stored under HTHH versus RT regardless of oil type and storage time. This is in agreement with the research reported by Gadient (1986).

Niacin was stable when premixes were stored in RT and HTHH up to 90 d. The niacin stability was similar for both premix types that were mixed either MO or MCFA. Gadient (1986) reported that niacin was slightly sensitive to temperature, oxygen, and humidity during pelleting. The results of the current study demonstrated the combination of temperature, high humidity and exposed time did not affect the niacin stability. The niacin stability was above 93% for all treatments which agrees with data reported by Gadient (1986).

Pantothenic acid mixed in VP had similar stabilities on day 60, and 90 when stored at either RT or HTHH. The pantothenic acid stability was above 84.8% in the VTM stored under RT up to 60 d. However, the pantothenic acid stability was dramatically decreased in the VTM that was stored under HTHH longer than 30 d. The faster degradation rate in the VTM when stored under HTHH was probably caused by metal ion from the product and humidity from the environment. Additionally, the pantothenic acid stability was higher in VP mixed with MCFA (92.9%) versus MO (87.7%). However, VTM with MCFA added (76.7%) had a lower pantothenic acid stability compared to VTM with MO added (82.0%). The effect of MCFA on the degradation of pantothenic acid was not clear when it was added in the

### Table 4. Effect of the premix type, oil type, storage temperature, and storage time on niacin stability for storage condition samples

| Item | Niacin stability, % |
|------|---------------------|
| Premix type | Oil type | Storage condition | Storage time, days |
| Vitamin premix | Mineral oil | RT | 30 | 94.5 |
| Vitamin trace mineral premix | MCFA | HTHH | 30 | 93.1 |
| Pooled SEM | Pooled SEM | | | 8.5 |

| Source of variation | Probability, P< |
|---------------------|----------------|
| Premix Type | 0.482 |
| Oil type | 0.691 |
| Premix Type × Oil type | 0.038 |
| Storage condition | 0.031 |
| Premix Type × Storage condition | 0.666 |
| Oil type × Storage condition | 0.848 |
| Premix Type × Oil type × Storage condition | 0.577 |
| Time | 0.095 |
| Premix Type × Time | 0.247 |
| Oil type × Time | 0.174 |
| Premix Type × Oil type × Time | 0.750 |
| Storage condition × Time | 0.382 |
| Premix Type × Storage condition × Time | 0.865 |
| Oil type × Storage condition × Time | 0.320 |
| Premix Type × Oil type × Storage condition × Time | 0.418 |

1Included at 1% of the premixes.
2Mineral oil comprised of saturated aliphatic and alicyclic nonpolar hydrocarbons sourced as a byproduct of petroleum refining.
3Medium chain fatty acid, MCFA, comprised of a 1:1:1 blend of medium chain fatty acids (C6:0, C8:0, and C10:0).
4Room temperature, the average temperature and relative humidity were 22.1 °C and 28.4%, respectively.
5High heat and high humidity, the average temperature and relative humidity were 39.5 °C and 78.8%, respectively.
6Percent vitamin stability was calculated by dividing the vitamin activity at day 30, day 60, or day 90 by the analyzed initial vitamin activity and then multiplying by 100.
7Means within a main effect of storage condition followed by a different letter are significantly different (P ≤ 0.05).
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premixes. The laboratory variation might cause the uncertainty of MCFA effect on pantothenic acid stability. Gadient (1986) reported that pantothenic acid was slightly sensitive to oxygen, moderately sensitive to heat and highly sensitive to humidity during pelleting. The result of the current study demonstrated the combination of temperature, high humidity, and exposed time affected the pantothenic acid stability when VP were stored at HTHH for 90 d (80.2%) regardless of oil type which in agreement with Gadient’s report. However, the percent loss of pantothenic acid in VTM (43.9%) was greater than VP (19.8) when stored under HTHH for 90 d regardless of oil type.

Cobalamin was more stable when premixes were stored under RT versus HTHH. However, when premixes were stored under HTHH, the VP (37.7%) had a higher cobalamin stability compared to VTM (18.2%). The faster degradation rate of VTM was probably caused by metal ion from the product and humidity from environment. Regardless of oil type, increasing storage time from 30 to 90 d decreased the cobalamin stability from 42.2 to 17.6% when premixes were

### Table 5. Effect of the premix type, oil type, storage temperature, and storage time on pantothenic acid stability for storage condition samples

| Item | Oil type* | Storage condition | Storage time, days | Pantothenic acid stability6, % |
|------|-----------|-------------------|-------------------|--------------------------------|
| Interaction | | | | |
| Vitamin premix | RT1 | 30 | 104.6a |
| Vitamin premix | RT | 60 | 88.5c |
| Vitamin premix | RT | 90 | 82.3d,e,f |
| Vitamin premix | HTHH | 30 | 98.8b |
| Vitamin premix | HTHH | 60 | 87.3c,d |
| Vitamin premix | HTHH | 90 | 80.2fg |
| Vitamin trace mineral premix | RT | 30 | 94.1b |
| Vitamin trace mineral premix | RT | 60 | 84.8d,e |
| Vitamin trace mineral premix | RT | 90 | 77.9g |
| Vitamin trace mineral premix | HTHH | 30 | 86.7c,d |
| Vitamin trace mineral premix | HTHH | 60 | 76.5f |
| Vitamin trace mineral premix | HTHH | 90 | 56.1h |
| Pooled SEM | | | 1.8 |
| Vitamin premix | Mineral oil4 | | 87.7i |
| Vitamin premix | MCFA5 | RT | 92.9k |
| Vitamin trace mineral premix | Mineral oil | | 82.0m |
| Vitamin trace mineral premix | MCFA | | 76.7n |
| Pooled SEM | | | 1.1 |

Source of variation

| Source of variation | Probability, $P$< |
|---------------------|------------------|
| Premix Type | 0.0001 |
| Oil type | 0.986 |
| Premix Type × Oil type | 0.0001 |
| Storage condition | 0.0001 |
| Premix Type × Storage condition | 0.0001 |
| Oil type × Storage condition | 0.820 |
| Premix Type × Oil type × Storage condition | 0.235 |
| Time | 0.0001 |
| Premix Type × Time | 0.032 |
| Oil type × Time | 0.079 |
| Premix Type × Oil type × Time | 0.207 |
| Storage condition × Time | 0.022 |
| Premix Type × Storage condition × Time | 0.003 |
| Oil type × Storage condition × Time | 0.384 |
| Premix Type × Oil type × Storage condition × Time | 0.739 |

1Room temperature, the average temperature and relative humidity were 22.1 °C and 28.4%, respectively.
2High heat and high humidity, the average temperature and relative humidity were 39.5 °C and 78.8%, respectively.
3Included at 1% of the premixes.
4Mineral oil comprised of saturated aliphatic and alicyclic nonpolar hydrocarbons sourced as a byproduct of petroleum refining.
5Medium chain fatty acid, MCFA, comprised of a 1:1:1 blend of medium chain fatty acids (C6:0, C8:0, and C10:0).
6Percent vitamin stability was calculated by dividing the vitamin activity at day 30, day 60, or day 90 by the analyzed initial vitamin activity and then multiplying by 100.

*Means within premix type × storage condition × storage time interaction followed by a different letter are significantly different ($P \leq 0.05$).

*Means within premix type × oil type interaction followed by a different letter are significantly different ($P \leq 0.05$).
stored under HTHH. Whereas, there was no difference for cobalamin stability when premixes were stored for 30 to 90 d under RT. The HTHH was not the proper storage condition for both VP and VTM. The premixes with MO (64.2%) had a higher cobalamin stability compared to premixes with MCFA (58.8%) regardless of storage condition and storage time. The MCFA might increase the degradation of cobalamin when it was added in the premixes. Gadient (1986) reported that cobalamin was moderately sensitive to heat, oxygen, and humidity. The result of the current study demonstrated the combination of temperature, high humidity and exposed time affected the cobalamin stability when premixes were stored at HTHH for 90 d (17.6%) regardless of oil type and storage time which in contrast with Gadient’s report.

The heat, water, and oxygen molecules in the environment and the presence of metal ions in the premix may influence the oxidation rate of vitamins resulting in a decrease in water-soluble vitamin stability when premixes were stored under 39.5 °C and 78.8% relative humidity. This was supported by Tavcar–Kalcher and Vengust’s statement (2007) that the oxidation of some vitamins were catalyzed by air, light, heat, moisture, mineral acids, metal ions, unsaturated fats, and

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**Table 6.** Effect of the premix type, oil type, storage temperature, and storage time on cobalamin stability for storage condition samples

| Item                                      | Cobalamin stability, % |
|-------------------------------------------|------------------------|
| **Interaction**                           |                        |
| Vitamin premix                            | 93.0a                  |
| Vitamin premix                            | 37.7b                  |
| Vitamin trace mineral premix               | 97.1c                  |
| Vitamin trace mineral premix               | 18.2d                  |
| Pooled SEM                                | 2.2                    |
| **Main effect**                           |                        |
| Mineral oil                               | 64.2e                  |
| MCFA                                      | 58.8f                  |
| Pooled SEM                                | 1.6                    |

**Source of variation**

|                      | Probability, *P*< |
|----------------------|-------------------|
| Premix Type          | 0.001             |
| Oil type             | 0.018             |
| Premix Type × Oil type| 0.330             |
| Storage condition    | 0.0001            |
| Premix Type × Storage condition | 0.0001          |
| Oil type × Storage condition | 0.310          |
| Premix Type × Oil type × Storage condition | 0.201          |
| Time                 | 0.001             |
| Premix Type × Time   | 0.075             |
| Oil type × Time      | 0.976             |
| Premix Type × Oil type × Time | 0.773          |
| Storage condition × Time | 0.0001          |
| Premix Type × Storage condition × Time | 0.573          |
| Oil type × Storage condition × Time | 0.825          |
| Premix Type × Oil type × Storage condition × Time | 0.488          |

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1Room temperature, the average temperature and relative humidity were 22.1 °C and 28.4%, respectively.
2High heat and high humidity, the average temperature and relative humidity were 39.5 °C and 78.8%, respectively.
3Included at 1% of the premixes.
4Mineral oil comprised of saturated aliphatic and alicyclic nonpolar hydrocarbons sourced as a byproduct of petroleum refining.
5Medium chain fatty acid, MCFA, comprised of a 1:1:1 blend of medium chain fatty acids (C6:0, C8:0, and C10:0).
6Percent vitamin stability was calculated by dividing the vitamin activity at day 30, day 60, or day 90 by the analyzed initial vitamin activity and then multiplying by 100.

Means within premix type × storage condition interaction followed by a different letter are significantly different (*P* ≤ 0.05).

Means within storage condition × storage time interaction followed by a different letter are significantly different (*P* ≤ 0.05).

Means within a main effect of oil type followed by a different letter are significantly different (*P* ≤ 0.05).

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Impact of storage conditions and premix type on water-soluble vitamin stability

oxidents. Based on the results of the current study, the order of environmental sensitivity under HTHH for water-soluble vitamins was cobalamin, riboflavin, pantothenic acid, and niacin from high to low. Whereas, the order of metal ion sensitivity for water-soluble vitamins was riboflavin, pantothenic acid, cobalamin, niacin from high to low. The MCFA did not affect the stability of water-soluble vitamins except cobalamin.

Experiment 2: Heat Pulse Treatment Experiment

Gadient (1986) reported that the riboflavin and niacin were slightly sensitive to heat while the pantothenic acid and cobalamin were moderately sensitive to heat during pelleting. The current study indicated that when premixes with either MO or MCFA were heated at 60 °C for 9 h and 27 min, the water-vitamin stability was more than 88% except riboflavin in VTM with MO and cobalamin in VP with MCFA. The result of current study was in agreement with Gadient’s study for riboflavin and niacin but disagreed for pantothenic acid and cobalamin. The result of the current study demonstrated that the percent riboflavin stability in VTM with MO was low (84.4%), which may be due to the impact of sulfate salt in VTM. Additionally, the stability of cobalamin was 81.3% in VP with MCFA, which may be caused by the impact of MCFA. Moreover, the pantothenic acid stability was 87.3% in VP when stored for 60 d and 86.7% in the VTM when stored for 30 d under high temperature and high humidity regardless of oil type. Riboflavin was stable in only vitamin premix with either MO or medium chain fatty acid (MCFA) up to 60 d regardless of storage condition. In addition, medium chain fatty acid did not influence water-soluble vitamin degradation during storage up to 90 d and in the heat pulse process except cobalamin. The vitamin stability was decreased by 5% to 12% after the vitamin premixes with 1% MO add was heated at 60 °C for approximately nine and a half hours.

CONCLUSION

The water-soluble vitamins were stable in both vitamin and vitamin trace mineral premix when stored at 22 °C with 28.4%RH up to 90 d for niacin and cobalamin or up to 60 d for pantothenic acid (84.8% stability) regardless of oil type. When premixes were stored at 39.5 °C with 78.8%RH, the niacin were stable up to 90 d while the cobalamin was dramatically decreased regardless oil type. Pantothenic acid had a stability of 87.3% in VP when stored for 60 d and 86.7% in the VTM when stored for 30 d under high temperature and high humidity regardless of oil type. Riboflavin was stable in only vitamin premix with either MO or medium chain fatty acid (MCFA) up to 60 d regardless of storage condition. In addition, medium chain fatty acid did not influence water-soluble vitamin degradation during storage up to 90 d and in the heat pulse process except cobalamin. The vitamin stability was decreased by 5% to 12% after the vitamin premixes with 1% MO add was heated at 60 °C for approximately nine and a half hours.

ACKNOWLEDGMENT

This is a contribution no. 20-256-J from the Kansas Agric. Exp. Sta., Manhattan 66506.
Conflict of interest statement
There are no conflicts of interest to disclose.

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