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Impact of Storage Conditions and Premix Type on Fat-Soluble Vitamin Stability

Abstract

The objective of Exp. 1 was to determine the impact of 0, 30, 60, or 90 d storage time on fat-soluble vitamin stability when vitamin premix (VP) and vitamin trace mineral premix (VTM) are blended with 1% inclusion of medium chain fatty acids (MCFA; 1:1:1 blend of C6:C8:C10) or mineral oil (MO) with different environmental conditions. Treatments were arranged as a 2 × 2 × 2 × 4 factorial, with 2 premix type (VP or VTM), 2 oil type (MO or MCFA), 2 storage conditions [room temperature (RT) or high-heat, high-humidity (HTHH)] and 3 storage times (30, 60, or 90 d). Samples were stored at room temperature in a temperature-controlled laboratory (approximately 72°F) for RT or in an environmentally-controlled chamber set at 104°F and 75% humidity for HTHH. For Exp. 1, there was a premix type × oil type × storage time interaction of vitamin A (P = 0.002). Vitamin A was stable in VP mixed with MCFA and VTM mixed with MO when stored from 0 to 90 d. Increasing the storage time continued to degrade vitamin A in VP mixed with MO and VTM mixed with MCFA. There was a premix type × storage condition interaction (P < 0.01). When premixes were stored under HTHH, the VTM had greater vitamin A stability as compared to VP. However, there was no difference for vitamin A stability between VP and VTM when stored under RT. There was an oil type × storage condition interaction (P < 0.01). The premixes with MO had a higher vitamin A stability compared to the premixes with MCFA when stored at RT. However, there was no difference for vitamin A stability between premix with MO and MCFA when stored at HTHH. There was a storage condition × time interaction (P < 0.01). When premixes were stored at HTHH, the vitamin A stability decreased as storage time increased to d 90. However, there was no difference in vitamin A stability as storage time increased to d 90 when stored at RT. Vitamin D3 stability was increased (P < 0.002) when stored at RT compared to premixes stored at HTHH. There was a decrease in vitamin D3 stability as storage time increased (P = 0.002) from d 30 to 60; however, there was no further decrease from d 60 to 90. There was a storage condition × time interaction (P < 0.001) for vitamin E stability. Vitamin E was stable at both RT and HTHH up to 30 d. However, the degradation rate of vitamin E was faster when premixes were stored under HTHH versus RT after 30 d of storage. The objective of Exp. 2 was to determine the effect of d of MCFA addition and premix type on fat-soluble vitamin stability after exposure to a heat pulse process. Treatments consisted of a 2 × 2 factorial, with 2 premix types (VP or VTM) and 2 oil types (MO or MCFA). All treatments were heated in an environmentally-controlled chamber at 140°F and 20% humidity. Vitamin A stability was reduced (P = 0.030) in premixes containing MCFA after premixes were heated at 140°F. The premix type did not affect the stability of vitamins A and D3. However, after the heat pulse treatment, vitamin E stability was reduced (P = 0.030) in VP compared to VTM.

Keywords

vitamin stability, vitamin storage, premix stability

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Impact of Storage Conditions and Premix Type on Fat-Soluble Vitamin Stability

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Summary

The objective of Exp. 1 was to determine the impact of 0, 30, 60, or 90 d storage time on fat-soluble vitamin stability when vitamin premix (VP) and vitamin trace mineral premix (VTM) are blended with 1% inclusion of medium chain fatty acids (MCFA; 1:1:1 blend of C6:C8:C10) or mineral oil (MO) with different environmental conditions. Treatments were arranged as a 2 × 2 × 2 × 4 factorial, with 2 premix type (VP or VTM), 2 oil type (MO or MCFA), 2 storage conditions [room temperature (RT) or high-heat, high-humidity (HTHH)] and 3 storage times (30, 60, or 90 d). Samples were stored at room temperature in a temperature-controlled laboratory (approximately 72°F) for RT or in an environmentally-controlled chamber set at 104°F and 75% humidity for HTHH. For Exp. 1, there was a premix type × oil type × storage time interaction of vitamin A ($P = 0.002$). Vitamin A was stable in VP mixed with MCFA and VTM mixed with MO when stored from 0 to 90 d. Increasing the storage time continued to degrade vitamin A in VP mixed with MO and VTM mixed with MCFA. There was a premix type × storage condition interaction ($P < 0.01$). When premixes were stored under HTHH, the VTM had greater vitamin A stability as compared to VP. However, there was no difference for vitamin A stability between VP and VTM when stored under RT. There was an oil type × storage condition interaction ($P < 0.01$). The premixes with MO had a higher vitamin A stability compared to the premixes with MCFA when stored at RT. However, there was no difference for vitamin A stability between premix with MO and MCFA when stored at HTHH. There was a storage condition × time interaction ($P < 0.01$). When premixes were stored at HTHH, the vitamin A stability decreased as storage time increased to d 90. However, there was no difference in vitamin A stability as storage time increased to d 90 when stored at RT. Vitamin D3 stability was increased ($P < 0.002$) when stored at RT compared to premixes stored at HTHH. There was a decrease in vitamin D3 stability as storage time increased ($P = 0.002$) from d 30 to 60; however, there was no further decrease from d 60 to 90. There was a storage condition × time interaction ($P < 0.001$) for vitamin E stability. Vitamin E was stable at both RT and HTHH up

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to 30 d. However, the degradation rate of vitamin E was faster when premixes were stored under HTHH versus RT after 30 d of storage. The objective of Exp. 2 was to determine the effect of d of MCFA addition and premix type on fat-soluble vitamin stability after exposure to a heat pulse process. Treatments consisted of a 2 × 2 factorial, with 2 premix types (VP or VTM) and 2 oil types (MO or MCFA). All treatments were heated in an environmentally-controlled chamber at 140°F and 20% humidity. Vitamin A stability was reduced ($P = 0.030$) in premixes containing MCFA after premixes were heated at 140°F. The premix type did not affect the stability of vitamins A and D3. However, after the heat pulse treatment, vitamin E stability was reduced ($P = 0.030$) in VP compared to VTM.

Introduction

Vitamins are essential components for metabolism of protein, carbohydrates, and fat. Vitamin deficiencies could affect animal performance by decreasing growth rate or increasing the incidence of reproductive failures and osteoporosis. There are many factors that can influence the stability of vitamins in premixes such as vitamin source, temperature, water content, pH, time, presence of choline, oxygen, light, and catalytic minerals.[4] Typically, vitamin concentrations in complete feed are dependent on vitamins provided by the premix. These concentrations can be affected by storage conditions, storage time, and feed manufacturing process.

Pure vitamin production is limited to certain countries; therefore, they must be imported by a majority of countries, including the United States. Previous research has demonstrated that pathogenic viruses such as porcine epidemic diarrhea virus (PEDV) and African swine fever virus (ASFV) can survive in certain feed ingredients and feed additives under simulated transport conditions.[5] Therefore, precautionary steps need to be considered in order to reduce the risk of disease transmission through feed. Feed additives, temperature, and exposure time are options to consider. For instance, previous research has demonstrated that 1% of a medium chain fatty acid blend (MCFA) effectively mitigated PEDV in feed ingredients.[6] However, the negative effects the pathogen reducing procedures have on vitamin stability need to be determined. Therefore, the first objective of this experiment was to determine the impact of storage time on fat-soluble vitamin stability when a vitamin premix (VP) and vitamin trace mineral premix (VTM) are blended with 1% inclusion of MCFA (1:1:1 blend of C6:C8:C10) or mineral oil (MO) with different environmental conditions. In addition to feed additives, pathogens could be eliminated by a combination of temperature and exposure time. For instance, ASFV can be inactivated at 140°F for 20 min, while PEDV activity can be reduced about 5.5 $\log_{10}$ when heated at 140°F for 30 min.[7] Thus,

[4] DSM Vitamin Nutrition Compendium. Vitamin stability. Available at: https://www.dsm.com/markets/anh/en_US/Compendium.html.

[5] Dee., S., F. Bauermann, M. Niederwerder, A. Singrey, T. Clement, M. DeLima, C. Long, G. Patterson, M. Shehan, A. Stoian, V. Petrovan, C.K. Jones, J. De Jong, J. Ji., G Spronk, J. Hennings, J. Zimmerman, B. Rowland, E. Nelson, P. Sundberg, D. Diel, and L. Minion. 2018. Survival of viral pathogens in animal feed ingredients under transboundary shipping models. PLoS ONE. doi: 10.1371/journal.pone.0194509.

[6] Cochrane, R.A. 2018. Interventional strategies to reduce biological hazards in animal feeds. PhD dissertation. Available at: https://krex.k-state.edu/dspace/handle/2097/39014.

[7] Hofmann, M., and Wyler, R. 1989. Quantitation, biological and physicochemical properties of cell culture-adapted porcine epidemic diarrhea coronavirus (PEDV). Vet. Microbiol. 20, 131–142. doi: 10.1016/0378-1135(89)90036-9.

exposing ingredients to increased temperature for a short period of time (a heat pulse process) could be an opportunity to prevent pathogen movement from a high-risk area to a clean area. However, one concern is that a heat pulse treatment could denature fat-soluble vitamins in the premix. Therefore, the second objective of this experiment was to determine the effect of heat pulse treatment and MCFA addition on fat-soluble vitamin stability with two premix types.

Procedures

A VP and VTM were manufactured for use in both experiments (Table 1). Both premixes contained phytase, and phytase stability results are presented in Saensukjaroenphon et al.[8] Briefly, ingredients were mixed for 5 min in 105 lb batches using a 3 ft^3 paddle mixer (Figure 1; Davis model 2014197-SS-S1, Bonner Springs, KS). Then, each premix was equally discharged into 3 separate 35 lb aliquots. A 5.5 lb subsample of each aliquot was taken to create a 16.5 lb experimental premix treatment. The 16.5 lb premixes were mixed for 10 s using a mixer (Hobart model HL-200, Troy, OH) 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 0.16 lb of a 1:1:1 commercial blend of C6:0, C8:0, and C10:0 MCFA (PMI Nutritional Additives, Arden Hills, MN) or 0.16 lb of MO were added using a pressurized hand-held sprayer with a fine hollow cone spray nozzle (UNIJET model TN-SS-2, Wheaton, IL). The premixes were mixed for an additional 90 s post oil application. The mixed samples were divided to obtain 8 individual 2 lb samples, which were placed in single-lined paper bags. This process was repeated to yield three replicates per treatment.

Experiment 1

Treatments were arranged as a 2 × 2 × 2 × 4 factorial, with 2 premix types (VP or VTM), 2 oil types (MO or MCFA), 2 storage conditions [room temperature (RT) or high-heat, high-humidity (HTHH)] and 3 storage times (30, 60, or 90 d). Samples were stored at room temperature in a temperature-controlled laboratory (approximately 72°F) for RT or in an environmentally-controlled chamber (Caron model 6030, Marietta, OH) set at 104°F and 75% humidity for HTHH. The sample bags were pulled out at day 0, 30, 60 and 90 for room temperature (RT) condition and at day 30, 60, and 90 for high temperature and high humidity (HTHH) condition. The actual storage temperature and humidity for both conditions were collected using a data logger (HOBO model Onset U12-012, Bourne, MA). For the room temperature condition, the average temperature was 71.6, 71.8, and 71.8°F; and the average relative humidity was 28.4, 23.0, and 33.7% for d 0 to 30, 31 to 60, and 61 to 90, respectively. For the HTHH condition, the average temperature was 103.1, 103.1, and 103.1°F; and the average relative humidity was 78.3, 79.0, and 79.1% for d 0 to 30, 31 to 60, and 61 to 90, respectively. The individual premix samples were riffle-divided twice to yield two 0.5 lb sub-samples that were sent to the DSM Nutritional Products for laboratory analysis of vitamin A (AOAC 974.29.45.1.02), D3 (AOAC 2011.12), and E (AOAC 971.30). Previous research determined that the lower assay tolerance of vitamin E is

[8] Saensukjaroenphon, M.; Evans, C. E.; Stark, C. R.; and Paulk, C. B. (2020) "Effect of Pellet Cooling Method, Sample Preparation, Storage Condition, and Storage Time on Phytase Activity of a Swine Diet," Kansas Agricultural Experiment Station Research Reports: Vol. 6: Iss. 10. https://doi.org/10.4148/2378-5977.8007.

82%.[9] Therefore, values greater than or equal 82% are not considered reportable in this experiment. The vitamin concentration at d 0, which was the initial concentration, was reported in international unit (IU) per lb. The results of vitamin concentration at d 30, d 60, and d 90 were reported in percent stability, which was calculated by dividing the vitamin concentration by the initial vitamin concentration and then multiplying by 100.

Experiment 2

Treatments consisted of a 2 × 2 factorial, with 2 premix types (VP or VTM) and 2 oil types (MO or MCFA). All treatments were heated in an environmentally-controlled chamber (Caron model 6030, Marietta, OH) at 140°F and 20% humidity. The sample bags were pulled out after they were stored for 11 h and 48 min. The data logger (HOBO model Onset U12-012, Bourne, MA) was placed within the sample bag at approximately midlevel, and the remaining sample was placed on top to ensure the data logger reflected true sample temperature. The premix temperature reached 140°F after 2 h and 21 min in the chamber. The samples were held at 140°F for 9 h and 27 min. The individual premix samples were riffle-divided twice to yield two 0.5 lb sub-samples that were sent to commercial laboratories for analysis similar to Exp. 1.

Statistical Analysis

The initial vitamin concentration was analyzed using the GLIMMIX procedure of SAS with mixing batch serving as the experimental unit. Treatments were analyzed as a 2 × 2 factorial, with main effect of premix type (VP or VTM) and oil type (MO or MCFA). Treatment differences were considered significant at $P < 0.05$.

For Experiment 1, data were analyzed using the GLIMMIX procedure of SAS with sample storage bag serving as the experimental unit. Treatments were analyzed as a 2 × 2 × 2 × 4 factorial, with main effects of premix type (VP or VTM), oil type (MO or MCFA), storage conditions (RT or HTHH), and storage time (30, 60, or 90 d). Treatment differences were considered significant at $P < 0.05$.

For Experiment 2, data were analyzed using the GLIMMIX procedure of SAS with mixing batch serving as the experimental unit. Treatments were analyzed as a 2 × 2 factorial, with main effects of premix type (VP or VTM) and oil type (MO or MCFA). Treatment differences were considered significant at $P < 0.05$.

Results and Discussion

The initial concentration of vitamins A, D3, and E was reported in Table 2 for VP with MO, VP with MCFA, VTM with MO, and VTM with MCFA. The formulated vitamin concentration was 407,514, 163,005, and 4,347 IU per lb for vitamin A, D3 and E, respectively. Therefore, the initial concentration of three fat-soluble vitamins was more than 91% of formulated concentration for all four premixes.

[9] Frye, T.M. 1994. The performance of vitamins in multicomponent premixes. Proc. Roche Technical Symposium, Jefferson, Georgia.

Experiment 1

There were no four-way interactions among combinations of oil type, premix type, storage condition and storage time ($P > 0.200$) for vitamin A. There was no evidence of an oil type × premix type × storage condition, oil type × storage condition × time, or premix type × storage condition × time interaction ($P > 0.332$) for stability of vitamin A. There was a premix type × oil type × storage time interaction of vitamin A ($P = 0.002$; Table 3). Vitamin A was stable in VP mixed with MCFA and VTM mixed with MO when stored from 0 to 90 d, while increasing the storage time continued to degrade vitamin A in VP mixed with MO and VTM mixed with MCFA. There was no evidence of an oil type × premix type, oil type × time, premix type × time interaction for stability of vitamin A ($P > 0.051$). There was a premix type × storage condition interaction ($P < 0.01$). When premixes were stored HTHH, the VTM had greater vitamin A stability as compared to VP. However, there was no difference for vitamin A stability between VP and VTM when stored at RT. There was an oil type × storage condition interaction ($P = 0.009$). The premixes with MO had a higher vitamin A stability compared to the premixes with MCFA when stored under RT. However, there was no difference for vitamin A stability between premix with MO and MCFA when stored at HTHH. There was a storage condition × time interaction ($P < 0.01$) for vitamin A. When premixes were stored HTHH, the vitamin A stability decreased as storage time increased to d 90. However, there was no difference in vitamin A stability as storage time increased to d 90 when stored at RT.

There were no four-way, three-way, or two-way interactions among combinations of oil type, premix type, storage condition, and storage time ($P > 0.073$) for vitamin D3. There was no evidence of main effects ($P > 0.424$) of oil type or premix type on vitamin D stability. However, vitamin D3 stability was affected ($P < 0.002$) by the storage condition and time (Table 4). The premixes stored under RT had a higher vitamin D3 stability compared to the premixes stored under HTHH. There was a decrease in vitamin D3 stability as storage time increased ($P = 0.002$) from d 30 to 60; however, there was no further decrease from d 60 to 90.

There were no four-way or three-way interactions among combinations of oil type, premix type, storage condition and storage time ($P > 0.073$) (Table 5) for vitamin E. There was no evidence of an oil type × storage condition, or oil type × time interaction ($P > 0.244$) for stability of vitamin E. There were interactions ($P < 0.016$) for premix type × oil type, premix type × storage condition, and premix type × storage time for vitamin E stability. However, these interactions were not considered reportable because the percent stability of all treatments was 82% and above which was above the lower assay tolerance of vitamin E (82%) previously reported.[10] In addition, there was a storage condition × time interaction ($P < 0.001$) for vitamin E stability. Vitamin E was stable under both RT and HTHH up to 30 d. However, the degradation rate of vitamin E was faster when premixes were stored under HTHH versus RT after 30 days of storage.

Experiment 2

There was no evidence of interaction between oil type and premix type ($P > 0.287$) for the stability of fat-soluble vitamins (Table 6). The oil type did not affect ($P > 0.732$) the stability of vitamins D3 and E. However, vitamin A stability was reduced ($P = 0.030$)

in premixes containing MCFA after premixes were heated at 140°F for 9 h and 27 min. The premix type did not affect ($P > 0.074$) the stability of vitamins A and D3. However, after the heat pulse treatment, vitamin E stability was reduced ($P = 0.030$) in VP compared to VTM.

In conclusion, the fat-soluble vitamins were stable when mixed with both vitamin and vitamin trace mineral premix and stored at 71.6°F with 28.4% RH. When premixes were stored at 103°F with 78.8% RH, vitamins A and D3 were stable up to 30 d while vitamin E was stable up to 60 d. In addition, MCFA did not negatively affect fat-soluble vitamin degradation during storage up to 90 d and in the heat pulse process. The vitamin stability was greater than 90% after the premixes were heated at 140°F for approximately 9.5 h. If both chemical treatment (MCFA) and heat pulse treatment have similar efficiency at neutralizing or reducing the target pathogen, the process of chemical treatment could become a more practical practice.

Brand names appearing in this publication are for product identification purposes only. No endorsement is intended, nor is criticism implied of similar products not mentioned. Persons using such products assume responsibility for their use in accordance with current label directions of the manufacturer.

Table 1. Composition of vitamin premix and vitamin trace mineral premix

	Vitamin trace mineral premix		Vitamin premix	
Ingredients	Inclusion, %	Batch, lb	Inclusion, %	Batch, lb
KSU swine vitamin[1]	54.35	57.08	54.35	57.08
KSU trace mineral[2]	32.60	34.24	---	---
Masonry sand	---	---	32.60	34.24
HiPhos GT5000[3]	8.70	9.13	8.70	9.13
Belfeed B 1100 MP[4]	4.35	4.56	4.35	4.56
Total	100.00	105.01	100.00	105.01

[1]Composition per lb: 749,796 IU vitamin A, 299,998 IU vitamin D3, 8,000 IU vitamin E, 6.03 mg vitamin B12, 600 mg menadione, 1,500 mg riboflavin, 5,000 mg d-pantothenic acid, and 9,000 mg niacin. Rice hulls and calcium carbonate are carriers in the premix.
[2]Composition per lb: 33 g iron, 33 g zinc, 10 g manganese, 5 g copper, 90 g iodine, and 90 g selenium. Calcium carbonate is a carrier in the premix.
[3]Composition per lb: 2,267,985 FYT phytase (*Aspergillus oryzae*).
[4]Composition per lb: 44,452 U xylanase (*Bacillus subtilis*).

Table 2. The analyzed fat-soluble vitamin concentrations of initial samples (sampled immediately after mixing, d 0)

	Vitamin premix		Vitamin trace mineral premix	
Item	Mineral oil[1]	MCFA[2]	Mineral oil	MCFA
Vit. A, IU/lb	405,557	395,790	406,464	410,193
Vit. D3, IU/lb	154,065	158,679	154,995	148,863
Vit. E, IU/lb	4,097	4,196	4,364	4,347

[1]Included at 1% of the premixes; comprised of saturated aliphatic and alicyclic nonpolar hydrocarbons sourced as a byproduct of petroleum refining.
[2]Included at 1% of the premixes; comprised of a 1:1:1 blend of medium chain fatty acids (C6:0, C8:0, and C10:0; PMI Nutritional Additives, Arden Hills, MN).

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

Item				
Premix type	Oil type[1]	Storage time, days	Storage condition	Vitamin A stability,[6] %
Interaction				
Vitamin premix	Mineral oil[2]	30		91.8^{bc}
Vitamin premix	Mineral oil	60		88.5^{c}
Vitamin premix	Mineral oil	90		77.6^{d}
Vitamin premix	MCFA[3]	30		84.0^{cd}
Vitamin premix	MCFA	60		91.1^{bc}
Vitamin premix	MCFA	90		90.2^{bc}
Vitamin trace mineral premix	Mineral oil	30		98.9^{ab}
Vitamin trace mineral premix	Mineral oil	60		98.9^{ab}
Vitamin trace mineral premix	Mineral oil	90		92.8^{bc}
Vitamin trace mineral premix	MCFA	30		104.3^{a}
Vitamin trace mineral premix	MCFA	60		86.4^{cd}
Vitamin trace mineral premix	MCFA	90		83.8^{cd}
Pooled SEM				3.4
Vitamin premix			RT[4]	102.9^{k}
Vitamin premix			HTHH[5]	71.5^{m}
Vitamin trace mineral premix			RT	105.6^{k}
Vitamin trace mineral premix			HTHH	82.8^{l}
Pooled SEM				2.0
	Mineral oil		RT	107.6^{p}
	MCFA		RT	100.9^{q}
	Mineral oil		HTHH	75.2^{r}
	MCFA		HTHH	79.0^{r}
	Pooled SEM			2.0
		30	RT	104.6^{x}
		60	RT	104.3^{x}
		90	RT	103.8^{x}
		30	HTHH	84.9^{y}
		60	HTHH	78.1^{y}
		90	HTHH	68.4^{z}
		Pooled SEM		2.4

continued

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

Item				
Premix type	Oil type[1]	Storage time, days	Storage condition	Vitamin A stability,[6] %
Source of variation				
Oil type				0.453
Premix type				0.001
Oil type × premix type				0.051
Storage condition				0.001
Oil type × storage condition				0.009
Premix type × storage condition				0.031
Oil type × premix type × storage condition				0.679
Time				0.003
Oil type × time				0.382
Premix type × time				0.059
Oil type × premix type × time				0.002
Storage condition × Time				0.008
Oil type × storage condition × time				0.332
Premix type × storage condition × time				0.349
Oil type × premix type × storage condition × time				0.121

[1]Included at 1% of the premixes.
[2]Mineral oil comprised of saturated aliphatic and alicyclic nonpolar hydrocarbons sourced as a byproduct of petroleum refining.
[3]Medium chain fatty acid, MCFA, comprised of a 1:1:1 blend of medium chain fatty acids (C6:0, C8:0, and C10:0; PMI Nutritional Additives, Arden Hills, MN).
[4]Room temperature, the average temperature and relative humidity were 71.8°F and 28.4%, respectively.
[5]High heat and high humidity (HTHH), the average temperature and relative humidity were 103.1°F and 78.8%, respectively.
[6]Percent vitamin stability was calculated by dividing the vitamin activity at d 30, 60, or 90 by the analyzed initial vitamin activity and then multiplying by 100.
[a-d]Means within premix type × oil type × storage time interaction followed by a different letter are significantly different ($P \leq 0.05$).
[k-m]Means within premix type × storage condition interaction followed by a different letter are significantly different ($P \leq 0.05$).
[p-r]Means within oil type × storage condition interaction followed by a different letter are significantly different ($P \leq 0.05$).
[x-z]Means within storage condition × storage time interaction followed by a different letter are significantly different ($P \leq 0.05$).

Table 4. Effect of the premix type, oil type, storage temperature, and storage time on vitamin D3 stability for storage condition samples

Item				
Storage condition	Storage time, days	Premix type	Oil type[3]	Vitamin D3 stability,[6] %
Interaction				
RT[1]	30			92.3
RT	60			86.8
RT	90			89.4
HTHH[2]	30			87.8
HTHH	60			80.2
HTHH	90			77.0
Pooled SEM				2.0
Main effect				
RT				89.5[a]
HTHH				81.7[b]
Pooled SEM				1.2
	30			90.1[a]
	60			83.5[b]
	90			83.2[b]
	Pooled SEM			1.4
		Vitamin premix		86.2
		Vitamin trace mineral premix		84.9
		Pooled SEM		1.2
			Mineral oil[4]	85.8
			MCFA[5]	85.3
			Pooled SEM	1.2

continued

Table 4. Effect of the premix type, oil type, storage temperature, and storage time on vitamin D3 stability for storage condition samples

Item				
Storage condition	Storage time, days	Premix type	Oil type[3]	Vitamin D3 stability,[6] %
Source of variation				
Oil type				0.752
Premix type				0.424
Oil type × premix type				0.781
Storage condition				<0.0001
Oil type × storage condition				0.339
Premix type × storage condition				0.721
Oil type × premix type × storage condition				0.793
Time				0.002
Oil type × time				0.465
Premix type × time				0.959
Oil type × premix type × time				0.676
Storage condition × time				0.141
Oil type × storage condition × time				0.421
Premix type × storage condition × time				0.282
Oil type × premix type × storage condition × time				0.073

[1]Room temperature, the average temperature and relative humidity were 71.8°F and 28.4%, respectively.
[2]High heat and high humidity, the average temperature and relative humidity were 103.1°F and 78.8%, respectively.
[3]Included at 1% of the premixes.
[4]Mineral oil comprised of saturated aliphatic and alicyclic nonpolar hydrocarbons sourced as a byproduct of petroleum refining.
[5]Medium chain fatty acid, MCFA, comprised of a 1:1:1 blend of medium chain fatty acids (C6:0, C8:0, and C10:0; PMI Nutritional Additives, Arden Hills, MN).
[6]Percent vitamin stability was calculated by dividing the vitamin activity at d 30, 60, or 90 by the analyzed initial vitamin activity and then multiplying by 100.
[a-b]Means within a main effect of storage condition followed by a different letter are significantly different ($P \leq 0.05$).

Table 5. Effect of the premix type, oil type, storage temperature, and storage time on vitamin E stability for storage condition samples

Item				
Storage condition	Storage time, days	Premix type	Oil type[3]	Vitamin E stability,[6] %
Interaction				
RT[1]	30			96.9[a]
RT	60			91.0[b]
RT	90			87.9[c]
HTHH[2]	30			96.0[a]
HTHH	60			83.9[d]
HTHH	90			79.6[e]
Pooled SEM				2.0
Main effect				
		Vitamin premix		87.1[l]
		Vitamin trace mineral premix		91.3[k]
		Pooled SEM		0.5
			Mineral oil[4]	88.1[y]
			MCFA[5]	90.4[x]
			Pooled SEM	0.5

continued

Table 5. Effect of the premix type, oil type, storage temperature, and storage time on vitamin E stability for storage condition samples

Item				
Storage condition	Storage time, days	Premix type	Oil type[3]	Vitamin E stability,[6] %
Source of variation				
Oil type				0.002
Premix type				0.001
Oil type × premix type				0.016
Storage condition				0.001
Oil type × storage condition				0.542
Premix type × storage condition				0.001
Oil type × premix type × storage condition				0.200
Time				0.001
Oil type × time				0.244
Premix type × time				0.008
Oil type × premix type × time				0.609
Storage condition × time				0.001
Oil type × storage condition × time				0.776
Premix type × storage condition × time				0.310
Oil type × premix type × storage condition × time				0.628

[1]Room temperature, the average temperature, and relative humidity were 71.8°C and 28.4%, respectively.
[2]High heat and high humidity, HTHH the average temperature and relative humidity were 103.1°F and 78.8%, respectively.
[3]Included at 1% of the premixes.
[4]Mineral oil comprised of saturated aliphatic and alicyclic nonpolar hydrocarbons sourced as a byproduct of petroleum refining.
[5]Medium chain fatty acid, MCFA, comprised of a 1:1:1 blend of medium chain fatty acids (C6:0, C8:0, and C10:0; PMI Nutritional Additives, Arden Hills, MN).
[6]Percent vitamin stability was calculated by dividing the vitamin activity at d 30, 60, or 90 by the analyzed initial vitamin activity and then multiplying by 100.
[a-d]Means within storage condition × storage time interaction followed by a different letter are significantly different ($P \leq 0.05$).
[k-l]Means within a main effect of premix type followed by a different letter are significantly different ($P \leq 0.05$).
[x-y]Means within a main effect of oil type followed by a different letter are significantly different ($P \leq 0.05$).

Table 6. Effect of the premix type and oil type on vitamin stability of premix subjected to a pulse of high temperature (140°F)

Item		Percent stability[4]		
Premix type	Oil type[1]	Vitamin A	Vitamin D3	Vitamin E
Interaction				
Vitamin premix	Mineral oil[2]	106.5	109.3	95.8
Vitamin premix	MCFA[3]	90.4	105.7	94.6
Vitamin trace mineral premix	Mineral oil	103.3	94.7	100.0
Vitamin trace mineral premix	MCFA	97.0	99.4	100.0
	Pooled SEM	4.3	5.1	1.6
Main effect				
Vitamin premix		98.4	107.5	95.2[y]
Vitamin trace mineral premix		100.1	97.1	100.0[x]
Pooled SEM		3.0	3.6	1.1
	Mineral oil	104.9[a]	102.0	97.9
	MCFA	93.7[b]	102.6	97.3
	Pooled SEM	3.0	3.6	1.1
Source of variation				
Oil type × premix type		0.287	0.435	0.712
Oil type		0.030	0.911	0.732
Premix type		0.700	0.074	0.016

[1]Included at 1% of the premixes.
[2]Mineral oil comprised of saturated aliphatic and alicyclic nonpolar hydrocarbons sourced as a byproduct of petroleum refining.
[3]Medium chain fatty acid, MCFA, comprised of a 1:1:1 blend of medium chain fatty acids (C6:0, C8:0, and C10:0; PMI Nutritional Additives, Arden Hills, MN).
[4]Percent vitamin stability was calculated by dividing the vitamin activity at d 30, 60, or 90 by the analyzed initial vitamin activity and then multiplying by 100.
[a-b]Means within a main effect of oil type followed by a different letter are significantly different ($P \leq 0.05$).
[x-y]Means within a main effect of premix type followed by a different letter are significantly different ($P \leq 0.05$).

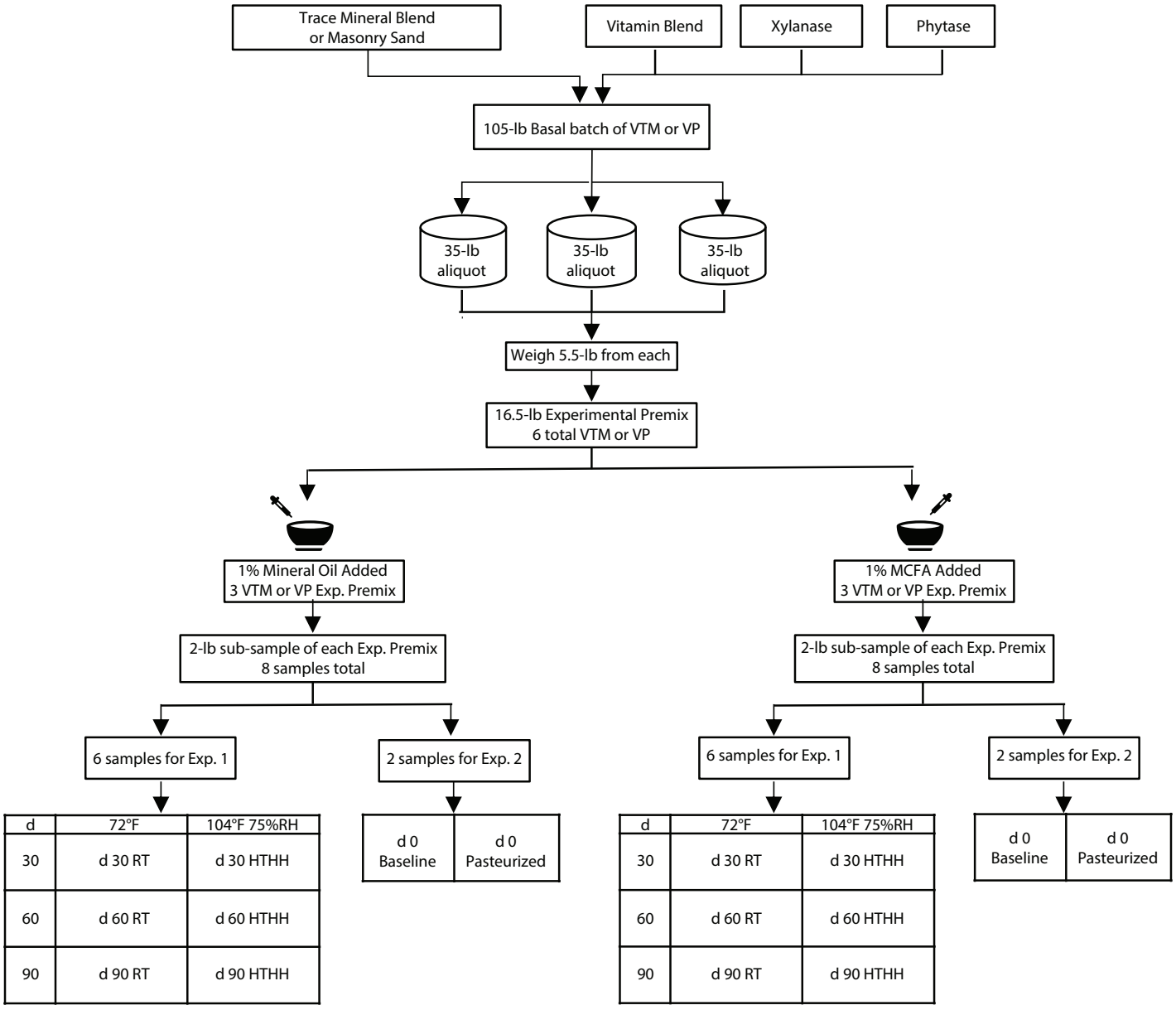

Figure 1. Flow chart of mixing steps used to create experimental treatments. Ingredients were mixed for 5 min in 105 lb batches using a 3 ft^3 paddle mixer (Davis model 2014197-SS-S1, Bonner Springs, KS). Then, each premix was equally discharged into 3 separate 35 lb aliquots. A 5.5 lb subsample of each aliquot was taken to create a 16.5 lb experimental premix treatment. The 16.5 lb premixes were mixed for 10 s using a mixer (Hobart model HL-200, Troy, OH). 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 medium chain fatty acids (MCFA; PMI Nutritional Additives, Arden Hills, MN) or 0.16 lb of mineral oil (MO) were added using a pressurized hand-held sprayer with a fine hollow cone spray nozzle (UNIJET model TN-SS-2, Wheaton, IL). The premixes were mixed for an additional 90 s post oil application. The mixed samples were divided to obtain 8 individual 900 g samples, which were placed in single-lined paper bags. Samples were then stored at room temperature in a temperature-controlled laboratory (approximately 72°F) or in an environmentally-controlled chamber (Caron model 6030, Marietta, OH) set at 104°F and 75% relative humidity (RH). In addition, separate samples were heated in an environmentally-controlled chamber (Caron model 6030, Marietta, OH) at 140°F and 20% RH.