Effect of Metal Specific Amino Acid Complexes and Inorganic Trace Minerals on Vitamin Stability in Premixes[1,2]

Gerald C. Shurson[3], Troy M. Salzer, Dean D. Koehler, and Mark H. Whitney

Department of Animal Science, University of Minnesota, St. Paul 55108

ABSTRACT: Stability of vitamin activity in a swine premix containing metal specific amino acid complexes, inorganic trace minerals, or no trace minerals was evaluated over a 120 d

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[3]To whom correspondence should be addressed: 385 AnSci/VetMed Bldg., 1988 Fitch Avenue, Univ. of Minnesota, St. Paul, MN 55108, phone (612) 625-2764, fax (612) 625-1210.

period. Two vitamin-trace mineral premixes containing either metal specific amino acid complexes or inorganic trace mineral sources were formulated to contain 200% of NRC (1988) sow requirements for iodine, copper, zinc, manganese, and iron. A separate vitamin premix containing no trace minerals served as the control. Premixes were stored in an environmentally-controlled feed storage room and samples were collected every month to determine vitamin activity. Minimal monthly vitamin stock losses (0-1%) were observed for all vitamins except B_{12} and choline chloride. Calcium pantothenate, vitamin E, riboflavin, biotin and niacin were most resistant to destruction, while vitamin K, vitamin A, pyridoxine, and thiamine mononitrate were subject to the greatest loss of activity during the 120 day storage period. Overall, vitamin stability in the inorganic trace mineral premix was lower than previously reported. Use of metal specific amino acid complexes in vitamin-trace mineral premixes significantly reduced the loss of vitamin A, vitamin K, vitamin B_{12}, thiamine mononitrate, folic acid, pyridoxine, and choline chloride ($P < .05$) compared to losses of vitamin activity in premixes containing inorganic trace minerals. Losses in vitamin A, B_{12}, thiamin, and choline were similar between the vitamin premix and the vitamin-complex trace mineral premix. Biotin activity was undetectable in the vitamin-complex trace mineral premix due to unexplained analytical interference. Each vitamin was ranked according to relative vitamin assay cost, loss in vitamin activity per month, and susceptibility to multiple stress factors. This ranking was used to identify vitamins that could represent overall vitamin activity in a premix and could be assayed at a reasonable cost for a feed manufacturing quality control program. Vitamin A was identified as the best indicator vitamin, followed by thiamine, vitamin K, and vitamin B_{12}. These results suggest that vitamin stability in swine vitamin-trace mineral premixes is improved when using metal specific amino acid

complexes compared to inorganic trace mineral sources. More liberal safety margins for vitamins may be needed when formulating vitamin-trace mineral premixes using inorganic sources of trace minerals.

Key Words: Pigs, Vitamin Stability, Complexes, Trace Minerals, Premixes

Introduction

Vitamins and trace minerals are required as co-factors in many metabolic processes necessary for efficient utilization of nutrients. Loss of vitamin activity in premixes and complete feeds during storage may account for hidden depressions in growth, feed efficiency, and disease resistance due to subclinical vitamin deficiencies. Unfortunately, many vitamins are relatively unstable compounds which undergo significant deterioration under normal storage conditions (Coehlo, 1991).

Individual vitamins have varying degrees of sensitivity to environmental degradation factors. Humidity (moisture), light, heat, pH, pelleting, extruding, and storage time (Scott, 1972; Bauernfeind, 1977; Gadient, 1986; Killeit, 1988; Coehlo, 1991) are important factors that affect vitamin stability in premixes. When vitamins are exposed to oxidizing agents such as mineral salts, the ionic charges hasten the rate of vitamin destruction. Adams (1972) found that a multivitamin premix containing inorganic trace minerals, when stored at 98°F for three months, lost 55% of its pyridoxine activity, compared to a 24% loss by a similar premix containing no trace minerals or choline chloride.

. Trace minerals are commonly supplied in swine premixes as highly reactive, inorganic mineral salts. However, several sources of more expensive chelated, amino acid complexed, and encapsulated trace mineral sources are commercially available. These organic trace mineral forms may have the ability to protect vitamins from destructive ionic charges which are associated with inorganic trace minerals.

If organic trace minerals reduce the rate of vitamin destruction, their use may allow for extended "safe" storage periods for premixes, lower safety margins for vitamins due to increased vitamin stability, and/or less potential for reductions in pig performance due to subclinical vitamin deficiencies. The objectives of this study were to determine the rate of vitamin losses in vitamin stock, vitamin premix, vitamin-inorganic trace mineral premix, and vitamin-complex trace mineral premix; to compare the effect of metal specific amino acid complexes and inorganic trace minerals on vitamin stability during a 120 day storage period; and to determine key vitamins to assay in feed manufacturing quality control programs.

Materials and Methods

Premix Formulation and Treatments

Two vitamin-trace mineral premixes were formulated to contain 200% of NRC (1988) requirements for iodine, copper, zinc, manganese, and iron for sows. Premixes were designed to be added at a rate of 0.50% of the diet (Table 1). These levels were chosen because sow premixes have the highest concentrations of vitamins and trace minerals compared to premixes for growing pigs, and "typical" commercial premixes are often formulated to provide dietary trace mineral levels near 200% of NRC (1988). Iodine was supplied by EDD organic iodine. Sodium selenite was also added to provide 1500 mg selenium per kg of premix. This supplied an amount of selenium equivalent to 0.3 ppm of the final diet. Both vitamin-trace mineral premixes were formulated to contain identical levels of trace minerals either from inorganic trace mineral sulfates or oxides, or from metal specific amino acid complexes (ZINPRO Corp, Eden Prairie, MN). Composition of premixes is shown in Table 1.

A vitamin premix containing no trace minerals was also formulated and served as a control. The vitamin premix and the two vitamin-trace mineral premixes were formulated to contain the same level of vitamins. Vitamin levels exceeded NRC (1988) and were chosen to represent "typical" industry levels based on an informal survey of vitamin levels in commercial premixes. Sources and amounts of each vitamin used in each premix are shown in Table 1. Choline chloride was added to all premixes. Ethoxyquin was added to each premix as an antioxidant. Rice by-product and calcite grits were added as carriers to each premix in different amounts to provide equal final premix batch size.

Premix Preparation and Storage

Premixes were manufactured at a commercial vitamin premix plant. Each of the three premixes was prepared in three separate 23 kg batches. Each batch represented one replicate and was divided into five-4.6 kg, plastic-lined paper bags. Each 4.6 kg bag was labeled to identify the vitamin premix, vitamin-inorganic trace mineral premix, and the vitamin-complex metal specific amino acid premix. Each bag was also labeled 1, 2, or 3 corresponding to the batch (replicate) that it represented. Samples (approximately 300 g) of each individual vitamin stock used to manufacture the premixes were also placed in sealed containers to allow monitoring of potency loss during the experimental period.

Premixes and vitamin stock samples were stored in an environmentally controlled feed storage room. Room temperature was maintained at 30.8°C throughout the experiment and daily high/ low temperatures were recorded over a four month storage period to verify constant temperature. The storage room remained dark during the storage period except for brief periods of sampling at each monthly interval. Humidity levels were measured but not controlled during the experimental period. Average and range of relative humidity during each month are given in Table 2.

Premix Sampling and Nutrient Assays

Upon arrival at the storage facility, pH of each premix in a 10% aqueous solution of distilled, deionized water, was measured. A 350 g subsample of premix was obtained from each of three replicate, sealed premix bags at d 0, 30, 60, 90, and 120 of the experiment, and 200g subsamples of the concentrated vitamin stock were collected on d 0, 60, and 120 and delivered via overnight mail to a commercial analytical lab. Samples were frozen upon receipt by the laboratory until assays could be performed. Each premix was assayed for vitamin activity and trace mineral content. Vitamin assays included: vitamin A, vitamin D, vitamin E, vitamin K, thiamine, riboflavin, pyridoxine, cyanocobalamin (B_{12}), niacin, folic acid, pantothenic acid, biotin, and choline. Trace mineral assays included: zinc, copper, iron, and manganese (except for the vitamin stock and control vitamin premix). Treatment and storage time differences were statistically analyzed using the GLM procedure of SAS with repeated measures design (SAS, 1985).

Results and Discussion

Loss of Vitamin Activity

Average monthly loss of activity for all vitamins during the 120 d storage period averaged about 5% per month. Individual values ranged from 0% (calcium pantothenate) to 10% (vitamin K) in the vitamin-inorganic trace mineral premix (Table 3). This is considerably higher than the 0.55% average vitamin loss that occurred in vitamin stock, the 3.1% average vitamin activity loss in the vitamin premix, and the 2.5% average loss of vitamin activity in the vitamin-complex metal specific amino acid premix. These results suggest that loss of vitamin activity can be reduced by approximately 40 to 50% by either mixing separate vitamin and inorganic trace mineral premixes or by using metal specific amino acid complexed trace minerals in a vitamin-trace mineral premix.

Some vitamins were more resistant to destruction than others. Vitamins that were most resistant to destruction included calcium pantothenate, vitamin E, riboflavin, biotin and niacin. Vitamins that were moderately resistant to oxidation included vitamin D_3, choline chloride, folic acid, and vitamin B_{12}. Vitamins that were subject to the greatest loss of activity were vitamin K, vitamin A, pyridoxine, and thiamine mononitrate. These results are somewhat different than relative stability and expected average losses per month described by BASF (Coehlo, 1991).

Our results showed that average choline chloride and vitamin B_{12} activity loss per month is more than four-fold greater than that reported by Coehlo (1991), in vitamin premixes containing choline chloride (Table 5). In fact, choline chloride and vitamin B_{12} appeared to be slightly more sensitive to oxidation than pantothenic acid and vitamin E, which are listed as being "high" stability vitamins according to Coehlo (1991) (Table 4). Furthermore, average vitamin loss per month was 6 to 7 times greater for riboflavin and niacin, and 8 to 9 times greater for biotin in the vitamin premix containing choline chloride compared to expected monthly losses reported by Coehlo (1991). Pyridoxine was also much less stable in the vitamin premix with choline chloride compared to the classification reported by Coehlo (1991), and in fact, was comparable to menadione in stability with approximately 6% activity loss each month.

In the vitamin-inorganic trace mineral premix, choline and vitamin B_{12} losses were 10 to 11 times greater per month than those reported by Coehlo (1991), whereas riboflavin, niacin, and biotin losses were only 2 to 3 times higher (Table 4). Pantothenic acid, vitamin E, thiamine mononitrate, pyridoxine, and vitamin A were the only vitamins in our study in which losses in vitamin activity in a premix containing trace minerals and choline were comparable to those reported by Coehlo (1991). Although the stability of vitamin sources used has a significant effect on stability in vitamin-trace mineral premixes, it should be noted that our study showed greater losses for several vitamins than those reported by Coehlo (1991). This may lead to suboptimal vitamin nutrition in field situations when prolonged premix storage occurs and minimal safety margins are used in formulating premixes.

pH of Premixes

Average pH values were 5.06, 4.17, and 2.22 for the vitamin premix (VP), vitamin-inorganic trace mineral premix (VITM), and vitamin-complexed trace mineral premix (VCTM), respectively. Since vitamin A and D_3 are considered to be sensitive to acid pH, and vitamin E, vitamin K, thiamine mononitrate, riboflavin, and pyridoxine are considered mildly sensitive to acid pH (Coehlo, 1991), it is surprising that average monthly losses of these vitamins in the VCTM were lower than vitamin losses observed in the VITM which had a much higher pH (Table 3). Based on these results, it appears that the lower average monthly vitamin losses in the VCTM were influenced to a greater extent by the effects of complexing on reducing oxidizing potential of the trace minerals than by the low pH of the premix.

Safety Margins

Safety margins for vitamin formulation should be based upon vitamin cost, presence/absence of trace minerals and choline chloride, anticipated storage time, feed processing environmental conditions, and anticipated rates of vitamin potency losses. Results from this study suggest that more liberal safety margins should be used when formulating VP or VITM, compared to safety margins based on estimated losses suggested by Coehlo (1991). This is necessary to ensure that vitamin nutrition does not limit pig health and performance. Vitamin losses would be expected to be even greater if pelleting, extruding, high humidity, or constant exposure to light were used in our study. Based on our results, recommended overages of vitamins in non-pelleted or non-extruded feed products should be at least equal to the average monthly losses for each of the three types of premixes evaluated in this study (Table 3). More realistically, safety margins should be two times greater than the average losses for each vitamin/month to account for humidity and other environmental factors associated with on-farm or feed mill storage conditions. If anticipated premix storage is more than one month, safety margins should be increased proportionately based on the anticipated number of months of premix storage.

Vitamin Assay Index

Vitamin assay costs are generally expensive and range from $15 (β-carotene) to $250 (vitamin D_3 by HPLC) per sample depending on the specific vitamin being assayed, its chemical form, the sensitivity of the assay, and the analytical laboratory chosen. The average assay cost per sample for most vitamins is $45 to $55 per sample. Since one of the objectives of this study was to determine which vitamin(s) would be indicative of relative nutritional value of a vitamin-trace mineral premix, we developed an evaluation system to identify these vitamins.

The primary factors that were used in selecting these indicator vitamins are assay cost, rate of vitamin activity loss, and sensitivity to multiple environmental factors. Table 5 shows comparisons and rankings of individual vitamin assay costs, average losses of vitamin activity per month, composite multiple stress factor sensitivities of each vitamin, and overall rankings for each vitamin representing equal contributions from each criteria. The relative cost ranking was based on an average assay cost for each vitamin from three widely recognized and reputable commercial laboratories that routinely conduct vitamin assays. The lowest cost vitamin assay was given a ranking of 1, and the highest vitamin assay cost was ranked 13. Average loss of vitamin activity/month rankings are based on the results from this study (Table 3) for VITM. Vitamin K exhibited the greatest loss per month and was ranked 1, whereas pantothenic acid had the least activity loss per month and was ranked 13. The composite multiple stress factor ranking is based on sensitivity to moisture, oxidation, reduction, heat, light, pH, and trace minerals as reported by Coehlo (1991), with each stress factor for a given vitamin being scored as follows: very sensitive = 1, sensitive = 2, mildly sensitive = 3, and resistant = 4. Scores for each stress factor were added and composite scores were ranked from lowest to highest.

Based on this evaluation system, vitamin A, thiamine, vitamin K, and vitamin B_{12}, are the top four choices, respectively, for monitoring vitamin losses/activity in premixes (Table 5). Of these four vitamins, vitamin A appears to be the best indicator vitamin because of its low assay cost, relatively high sensitivity to multiple stress factors and high expected activity losses per month of storage.

Metal Specific Amino Acid Complexes vs. Inorganic Trace Minerals on Vitamin Stability

Use of metal specific amino acid complexes in vitamin-trace mineral premixes resulted in reduced losses of vitamin A, vitamin K, vitamin B_{12}, thiamine mononitrate, folic acid, pyridoxine, and choline chloride ($P<.05$) compared to vitamin activity losses VITM (Table 6). Metal specific amino acid complexes also tended to reduce the loss of vitamin D and niacin compared to inorganic trace minerals ($P<.10$). It was somewhat surprising that choline chloride activity was preserved better in the VCTM because choline is considered to be resistant to trace mineral oxidation (Coehlo, 1991). In addition, vitamin K activity was better preserved in the VCTM than in the VP. As noted in Table 6, a substantial and increasing interference was observed for biotin assays in the VCTM premixes. The cause for this analytical problem is not known. However, this finding has implications when attempting to assay biotin in feed mixtures containing metal specific amino acid complexes. Results from this study clearly show that the use of metal specific amino acid complexes in vitamin-trace mineral premixes preserves vitamin activity compared to vitamin losses that occur when using traditional inorganic trace mineral sources in premixes.

Implications

The results of this study indicate that use of metal specific amino acid complexes significantly minimizes vitamin destruction in vitamin-trace mineral premixes comparable to vitamin activity losses in a vitamin premix containing no trace minerals. It appears that vitamin activity losses can be reduced by approximately 40 to 50% by either mixing and storing separate vitamin and inorganic trace mineral premixes before manufacturing complete feed, or by using metal specific amino acid complex trace minerals in a vitamin-trace mineral premix. Vitamin A, thiamine, vitamin K, and vitamin B_{12} appear to be the best "indicator vitamins" to use when monitoring vitamin activity losses in premixes. Observed losses in vitamin activity suggest that previously reported (Coehlo, 1991) safety margins for premixes may be low. Safety margins should probably be two times greater than the average losses for each vitamin per month to account for humidity and other environmental and processing factors associated with vitamin potency losses on-farm or during feed mill storage. If anticipated premix storage is more than one month, safety margins should be increased proportionately based upon anticipated number of months of premix storage.

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Table 1. Nutrient levels and sources of experimental premixes

Vitamin/ Mineral	Source	Source Concentration	Vitamin Premix (g)	Vitamin-Inorganic TM Premix (g)	Vitamin-Complex TM Premix (g)
Vitamin A	Rovimix A-650	650,000 IU/g	1.37	1.37	1.37
Vitamin D_3	Rovimix AD_3	A 650,000 IU/g D 325,000 IU/g	1.37	1.37	1.37
Vitamin E	Rovimix E	227,000 IU/g	13.12	13.12	13.12
Vitamin K	Hetrazeen MPB	45.5%	2.94	2.94	2.94
Vitamin B_{12}	Vitamin B_{12} 1%	10 mg/kg	0.78	0.78	0.78
Niacin	Niacin, 99%	99%	7.83	7.83	7.83
Riboflavin	Ribo 80% SD	80%	2.15	2.15	2.15
Thiamine	Rovimix B_1	91.9%	0.39	0.39	0.39
Folic Acid	Folic acid, 80%	80%	0.98	0.98	0.98
Pantothenic acid	Ca pantothenate	97.5%	5.09	5.09	5.09
Pyridoxine	B_6 HCl FG USP	82.3%	0.78	0.78	0.78
Choline	Choline chloride	60%	191.94	191.94	191.94
Biotin	Rovimix H 2%	2%	2.94	2.94	2.94
Copper	Copper sulfate	25.2%	-	8.03	-
Iron	Iron sulfate, 31%	30%	-	107.72	-
Zinc	Zinc oxide	72%	-	27.81	-
Manganese	Mn sulfate	27%	-	14.89	-
Copper	Cu Plex® 100	10%	-	-	19.98
Iron	METH-IRON®	15%	-	-	220.93
Zinc	ZINPRO® 180	18%	-	-	111.25
Manganese	MANPRO® 160	16%	-	-	25.07
Iodine	EDD organic I	79.5%	-	0.07	0.07
Selenium	Selenium 20-X		-	15.08	15.08
Carrier	Calcite grits		195.86	23.50	0.20
Carrier	Rice by-product		570.89	569.65	374.17
Antioxidant	Santoquin	66.67%	1.57	1.57	1.57
Total			1000.00	1000.00	1000.00

Table 2. Relative humidity levels during premix storage

Month	Dates	Avg. Relative Humidity	Range in Relative Humidity
1	9/8/95 to 10/9/95	38 %	25 - 58 %
2	10/10/95 to 11/9/95	34 %	23 - 52 %
3	11/10/95 to 12/9/95	24 %	15 - 41 %
4	12/10/95 to 1/9/96	22 %	12 - 40 %

Table 3. Average monthly loss (%) in vitamin activity during a 120-day storage period for vitamin and vitamin-trace mineral premixes

Vitamin	Vitamin Stock	Vitamin Premix	Vitamin-Inorganic Trace Min Premix	Vitamin-Complex Trace Min Premix	SE
Vitamin A	0.74	3.50[a]	8.97[b]	3.07[a]	1.06
Vitamin D_3	0.00	3.02	4.48	2.68	1.06
Vitamin E	0.88	1.61	1.12	1.39	0.63
Vitamin K	0.00	6.01[a]	10.16[b]	2.23[c]	0.53
Vitamin B_{12}	2.82	2.05[d]	5.43[e]	2.32[d]	0.83
Niacin	0.83	3.49	3.24	1.08	1.12
Riboflavin	0.00	3.33	2.74	2.69	0.66
Thiamine	0.00	2.64[a]	7.90[b]	4.08[c]	0.36
Folic Acid	0.35	2.19[a]	5.56[b]	4.34[b]	0.48
Ca pantothenate	0.00	0.00	0.00	0.06	0.64
Pyridoxine	0.32	5.87[a]	8.64[b]	4.94[a]	0.55
Choline chloride	1.25	2.14[d]	4.88[e]	3.13	0.62
Biotin	0.00	4.35	2.92	--	1.65

[a,b,c] Within a row, means lacking a common superscript letter differ ($P < .01$).

[d,e] Within a row, means lacking a common superscript letter differ ($P < .05$).

Table 4. Stability of vitamins in premixes and during pelleting and extrusion[a]

	Very High	**High**	**Moderate**	**Low**	**Very low**
Vitamin	Choline chloride	Riboflavin	Thiamine mono	Thiamine HCl	Menadione
	B_{12}	Niacin	Folic acid		
		Pant. Acid	Pyridoxine		
		E	D_3		
		Biotin	A		
			losses/month		
Premixes w/o choline and trace minerals	0	<0.5 %	0.5 %	1 %	2 %
Premixes w/ choline	<0.5 %	0.5 %	2 %	4 %	6 %
Premixes w/ choline and trace minerals	<0.5 %	1 %	8 %	15 %	30 %
Pelleting	1 %	2 %	6 %	10 %	26 %
Extrusion	1 %	5 %	11%	17%	50%

[a] Adapted from Coehlo, 1991.

Table 5. Comparison and ranking of vitamin assay costs, activity loss/month and sensitivity to multiple stress factors

Vitamin	Relative Cost Ranking	Loss in Vitamin Activity/Month Rank	Sensitivity to Multiple Stress Factors Rank	Overall Rank
Vitamin A	3	2	2	1
Vitamin D_3	13	8	2	9
Vitamin E	10	12	10	13
Vitamin K	12	1	1	3
Vitamin B_{12}	11	5	2	4
Niacin	4	9	13	12
Riboflavin	1	11	9	7
Thiamine	2	4	6	2
Folic acid	9	6	5	6
Pantothenic acid	7	13	6	11
Pyridoxine	7	3	8	5
Choline chloride	6	7	10	8
Biotin	5	10	10	10

Table 6. Effect of inorganic trace mineral and metal specific amino acid complexes in vitamin-trace mineral premixes on losses in vitamin activity during a 120 d storage period

Vitamin	Vitamin Premix	Vitamin-Inorganic TM Premix	Vitamin-Complex TM Premix	SE
Vitamin A, IU/kg	361,992[a]	938,293[b]	290,701[a]	121,805
Vitamin D, IU/kg	79,009	101,279	58,766	26,569
Vitamin E, IU/kg	636	451	546	251
Vitamin K, mg/kg	264[a]	326[a]	99[b]	35
Vitamin B_{12}, µg/kg	1.47[a]	9.09[b]	3.21[a]	1.23
Niacin, mg/kg	1555	1467	528	464
Riboflavin, mg/kg	310	242	264	59
Thiamine, mg/kg	165[a]	359[b]	238[a]	42
Folic acid, mg/kg	117[a]	279[b]	213[c]	24
Ca pantothenate, mg/kg	-260	-33	15	161
Pyridoxine, mg/kg	440	491[a]	356[b]	44
Choline chloride, g/kg	8.7[a]	23.3[b]	12.8[a]	3.1
Biotin, mg/kg	14.0	8.0	*	5.1

[a,b,c] Within a row, means lacking a common superscript letter differ ($P < .05$).

* Unexplained analytical interference prevented detection of biotin activity in complexed trace mineral premixes.