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Handling Characteristic Evaluation of Free, Standard and Lipid Microencapsulated Vitamin and Mineral Premixes

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Abstract: Feed ingredients can change physically or chemically when exposed to moisture, heat or pressure. These changes impact how well they flow through a bin and disperse throughout the feed. Microencapsulation is a relatively new technique used to reduce reactivity and improve storage and handling characteristics (HC) of nutrients. The authors hypothesize that lipid matrix microencapsulation of free vitamin and mineral (VM) premixes significantly improve their HC making them more desirable products for feed mills. Triplicate samples of free, standard and lipid microencapsulated VM premixes were evaluated for the following HC: particle size (d_{gw}), particle size variability (S_{gw}), flowability (measured by angle of repose (AOR) and minimum orifice diameter (MOD)), lumping, compressibility, bulk and tapped density, solubility and hygroscopic percent change in weight (day 0-1, 0-3, 0-5, 5-9, 0-9). Results were analyzed in a 3 × 2 factorial (JMP Pro 14) of form (free, standard, microencapsulated) by type (vitamin, mineral). Microencapsulated VM had the highest d_{gw} (611.0 μm and 722.7 μm, respectively, $p = 0.002$); free and microencapsulated vitamins had the smallest S_{gw} (1.67 and 1.49, respectively, $p < 0.001$). Microencapsulated premixes had significantly lower AOR ($p < 0.001$) and MOD values ($p < 0.001$) than other premixes, indicating improved flowability. From days 0-3 and 0-5, microencapsulated premixes absorbed approximately half as much moisture (1.62% and 2.24%, respectively) than the free (3.48% and 5.04%, respectively) or standard (3.74% and 5.26%, respectively) premixes ($p = 0.001$). The benefits gained from lipid matrix microencapsulation technology not only improve the HC of animal feed additives, but also improve the stability of VM premixes.

Key words: Lipid microencapsulation, vitamin, mineral, poultry, swine, flowability.

1. Introduction

The handling characteristics (HC) of feed premixes and additives are essential to the efficiency of animal feed manufacturing. Feed additive companies strive for good bioavailability and palatability, and HC are not usually their only concern. This can result in vitamin and mineral (VM) premixes, enzyme additives, probiotics, prebiotics and other essential feed additives of low dietary inclusion levels bridging in bins and causing issues at the feed mill that result in poor dispersibility in the feed [1]. There are measurable properties of feed ingredients that can be used to estimate relative HC at a feed mill. These properties are broken up into three categories: the physical properties of feed ingredients, the physical effects of storage on feed ingredients, and the hydration stability of feed ingredients. There are adequate data evaluating the effects and importance of feed ingredient physical properties [2-4], but little research has been done to evaluate the effects and importance of storage or hydration stability [5]. The physical properties of feed additives include particle size distribution and flowability. Determining the mean diameter of the particles in microns (d_{gw}) as well as how variable those particles are in size (S_{gw}) is an important way to evaluate the particle size distribution of a feed ingredient [6]. Flowability is an estimation of how well a feed ingredient will flow through the

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equipment at a feed mill during animal feed manufacturing. Measuring the angle of repose (AOR) and the minimum or critical orifice diameter are two of the simplest ways to determine the flowability of a feed ingredient [7, 8]. Both of these physical property measurements help estimate relative feed mill performance and flow characteristics of various feed ingredients.

Storage and transport can have degrading effects on feed additives, premixes and other ingredients. A lumping test evaluates the effect of high heat in a low moisture environment, such as being stored in bags during hot weather, and rates the effects on a subjective scale. A compression test evaluates the effects of pressure on the ingredient, such as being stacked in bags on a pallet, and also rates the effects on a subjective scale [9]. Percent compressibility of an ingredient can be determined by measuring the initial (bulk) and final tapped densities and can also be used as an estimation of flowability. The Carr's Compressibility Index and Hausner Ratio are commonly used to express the percent compressibility and flow of powders and feed additives [10, 11]. The stability of a feed ingredient when exposed to water or moisture can also be used to evaluate relative stability among feed ingredients. The percent insoluble fraction of ingredients can be used to quickly compare the relative hydration stability of various feed additives and premixes. Hygroscopicity is defined as the ability of a product or ingredient to react with moisture in the air by absorbing or releasing water vapor. Knowing the hygroscopic properties of feed additives is important because the way various ingredients react with moisture has a strong impact on their flowability, dispersibility in the feed, lumping and compressibility [11].

No product form can assure complete nutrient stability, but the advanced product forms now available to commercial feed and premix manufacturers provide superior stability. Free VM additives are commonly mixed with a carrier to form commercial "standard" VM premixes. Free and standard premixes are susceptible to nutrient degradation due to a lack of protection from the environment. Microencapsulation can be used to retain bioactivity in a product during storage, protect the nutrients from chemical interactions, increase shelf-life, guard against light-induced reactions and/or oxidation or allow for the controlled release of nutrients [12].

It was hypothesized that lipid matrix microencapsulation of VM premixes will significantly improve their HC compared to free and standard VM premixes, making them more desirable products for animal feed manufacturers. The objective of this experiment was to quantify the physical characteristics, physical effects of storage and hydration stability of free, standard and lipid matrix microencapsulated poultry and swine VM premixes. To the authors' knowledge, there is no published literature that evaluates different animal VM premix forms for their relative HC. The materials and methods described in this manuscript can be used as a procedure for measuring and comparing the relative handling properties of different feed ingredients.

2. Materials and Methods

Nine VM premixes (Table 1) were evaluated for their HC and data were analyzed comparing the three premix forms (free, standard and microencapsulated). The following HC were evaluated in triplicate for each premix: particle size (d_{gw}, μm), particle size variability (S_{gw}), AOR (°), minimum orifice diameter (MOD, mm), lumping score (1-6), compression score (1-6), bulk density (kg/m^3), tapped density (kg/m^3), Carr's Compressibility Index (%), Hauser's ratio, percent insoluble fraction (%), and hygroscopic percent change in weight (day 0-1, 0-3, 0-5, 5-9, 0-9). The carrier for all standard premixes was rice hulls and each of the three samples for each standard premix was from different manufacturing lots. Free and microencapsulated premixes were only manufactured

in one lot, specifically for experimental purposes. To make the microencapsulated premixes, the free VM premixes were encapsulated in a hydrogenated vegetable oil based on the technique detailed in U.S. Patent WO2018089516 [13]. All 27 premix samples were divided using a riffle divider to approximately 500 g for each analysis. The 500 g sample was then divided using a riffle divider to reach the appropriate sample size requested for each analysis.

2.1 Physical Properties

Particle size was determined according to the procedures in ASAE S319.2 and S319.4 [14]. To evaluate the particle size distribution of each sample, 100 g was sieved with a brass sieve stack (14-sieves) containing sieve agitators with bristle sieve cleaners and rubber balls measuring 16 mm in diameter (Table 2). A dispersing agent (Model SSA-58, Gilson Company, Inc., Lewis Center, OH, USA; 0.5 g) was mixed with the sample and the sample was placed on the top sieve. The sieve stack was placed in a Ro-Tap machine (Model RX-29, W. S. Tyler Industrial Group, Mentor, OH, USA) and shaken for 10 min. Subsequently, each sieve was individually weighed without the sieve agitator(s) to obtain the weight of the sieve. The sieves were then cleaned and weighed again to determine the weight of the sample from each sieve. The weight of the dispersing agent was not subtracted from the weight of the pan as specified in ASAE S319.2. Sieves were cleaned after each analysis

Table 1 Nine premixes evaluated for their physical properties, effects of storage, hydration stability and handling characteristics (HC).

Form	Type	Premix name
Free	Vitamin	Free Poultry Vitamin Premix
	Mineral	Free Poultry Trace Mineral Premix
Standard	Vitamin	NCSU Poultry Vitamin Premix
	Vitamin	NCSU Swine Sow/Pig Vitamin Premix
	Vitamin	NCSU Market Swine Vitamin Premix
	Mineral	NCSU Poultry Trace Mineral Premix
	Mineral	NCSU Swine Trace Mineral Premix
Microencapsulated	Vitamin	Lipid Matrix Microencapsulated Vitamin Premix
	Mineral	Lipid Matrix Microencapsulated Trace Mineral Premix

Table 2 Sieve and sieve agitator arrangement for particle size distribution analysis.

US sieve number	Sieve opening (μm)	Sieve agitator(s)
4	4,760	None
6	3,360	None
8	2,380	None
12	1,680	Three rubber balls
16	1,190	Three rubber balls
20	840	Three rubber balls
30	590	One rubber ball, one bristle sieve cleaner
40	420	One rubber ball, one bristle sieve cleaner
50	297	One rubber ball, one bristle sieve cleaner
70	210	One rubber ball, one bristle sieve cleaner
100	149	One bristle sieve cleaner
140	105	One bristle sieve cleaner
200	74	One bristle sieve cleaner
270	53	One bristle sieve cleaner
Pan	37	None

with a vacuum and a stiff bristle sieve cleaning brush. Calculations for the d_{gw} were performed according to the equations listed and described in ASAE standard S319.4 (which are identical to S319.2) and the S_{gw} according to ASAE S319.2. Two different ASAE versions were used because ASAE S319.4 allows for variations in sieving time but ASAE S319.2 uses the traditional S_{gw} calculations, which does not include units for S_{gw} [14].

Flowability was measured in two ways, by the AOR and MOD. The AOR was measured according to a modified version of the procedure used by Syamsu *et al*. [15]. To measure the AOR, 200 g of each sample was allowed to flow from a funnel 12 cm above a free-standing platform, 5 cm in diameter. The angle between the free-standing platform of the sample pile and the height of the pile (AOR) was calculated by taking the inverse tangent of the height of the pile divided by the platform radius. The MOD was determined using a powder flowability test instrument (Flodex Model WG-0110, Paul N. Gardner Company, Inc., Pompano Beach, FL, USA). To measure the MOD, 50 g of each sample was allowed to flow through a stainless-steel funnel with a 0.6 cm diameter into a cylinder. The samples rested for 30 s in the cylinder and were then evaluated based on their ability to flow through an opening in a horizontal disc at the bottom of the cylinder. Each disc was 6 cm in diameter and the interior hole (orifice) diameter ranged from 4 mm to 34 mm. A negative result was recorded when the sample did not flow through the opening in the disc or formed a cylindrical hole (Fig. 1). The disc was then replaced by one with a larger hole size diameter until a positive result was observed. A positive result was recorded when the material flowed through the disc opening forming an inverted cone shape (Fig. 2). If a positive result was observed, the disc was replaced by one with a smaller hole size diameter until a negative result was observed. Three positive results consecutively on the same disc size were used to determine the MOD (mm) of each sample.

Fig. 1 Depiction of negative result for minimum orifice diameter (MOD).

Fig. 2 Depiction of positive result for MOD.

2.2 Physical Impacts of Storage

The lumping score and compression score were measured according to a modified version of the procedure listed in the *BASF Vitamin Quality School Handbook*, 1994 [16]. To evaluate the lumping tendency of each sample, 10 g was measured into a 50 mL centrifuge tube. Tubes were tightly closed and stored in a heating oven at 122 °F (50 °C) for 24 h. Tubes were then removed from the oven and allowed to cool. The samples were poured out of the tubes and scored on a subjective scale from one to six (Table 3).

To evaluate the compression capacity of each sample, 15 g was poured into a hollow cylinder standing on a plane surface. The surface of each sample was smoothed and a solid cylinder weighing 1,250 g was placed on top. After 24 h at ambient temperature, the solid cylinder was carefully removed, and the hollow cylinder was slowly lifted up. The results were rated on a subjective scale from one to six (Table 4).

Percent compressibility was determined by measuring the bulk and tapped densities (kg/m^3) of each premix [15]. About 50 g of each sample was poured into a 150 mL graduated cylinder and the exact weight (g) and volume (mL) were recorded. These were used to calculate the premixes' bulk density. The cylinder was then tapped until no further change in the volume was observed. The weight (g) and volume (mL) were again recorded. This represented the premixes' tapped density. The Carr's Compressibility Index ($C = 100 \times (1 - \text{bulk/tapped})$) and Hausner Ratio ($H$ =tapped/bulk) were calculated for each sample.

2.3 Hydration Stability

To determine the percent insoluble fraction of each premix, 10 g was mixed in a beaker with 15 mL distilled water for 1 min. Samples were then poured through a filter paper to drain off the water. The filter paper was left in ambient conditions for 3 d to dry and the residue on the paper was weighed. The weight of the premix remaining on the filter paper was divided by the starting weight to determine the percent insoluble fraction of each premix.

Hygroscopicity was measured according to a modified version of the procedure used by Hemmingsen *et al*, 2008 [5]. For each sample, 10 g was measured into a petri dish and stored in a CO_2 incubator (Model 5300, Precision Scientific, Chicago, IL, USA) at 95 °F (35 °C) and 99% relative humidity (RH) for 5 d. The weight of each sample was recorded on days 0, 1, 3 and 5. The percent increase in weight (%) of each sample was calculated after each day. Samples were then taken out of the high temperature, high humidity environment and allowed to be exposed to ambient conditions (70 °F (21 °C), 50% RH) for 4 d. The weight of each sample was again recorded on days 6 and 9 and used to calculate the percent decrease in weight (%) of each sample. Percent change in weight for each time period was calculated by taking the difference of the two weights, dividing by the original weight and multiplying by 100.

Table 3 Scoring description chart for animal feed ingredient, additive and premix lumping test evaluation.

Score	Description
1	Product flows as freely as starting material
2	Product flows freely when tapped
3	Product flows freely as tapped; a few small lumps are observed
4	Product barely flows; when tapped lumps are observed
5	Product does not flow, when tapped and removed from container the sample falls apart in soft lumps
6	Product does not flow at all; sample has formed one solid lump

Table 4 Scoring description chart for animal feed ingredient, additive and premix compression test evaluation.

Score	Description
1	Sample collapses completely upon removing cylinder
2	Tablet only collapses when touched
3	Tablet collapses in lumps when touched
4	Tablet is formed and needs gentle force to break
5	Tablet is formed and needs force to break
6	Formed tablet only broke by strong force

2.4 Statistical Analysis

Results were analyzed in JMP Pro 14 (SAS Institute, Inc., Cary, NC, USA) in a 3 × 2 factorial analysis of variance (ANOVA) of form (free, standard or microencapsulated) by type (vitamin or mineral) with three replicates per premix. Interactions were removed from the model if $p > 0.05$. The Tukey's honest significant difference (HSD) test was used to determine differences in means. MOD, lumping score and compression score results are integer count data and were therefore analyzed using the Generalized Linear Regression personality in JMP Pro 14 with a Poisson link function.

3. Results and Discussion

3.1 Physical Properties

Premix mixability in mash feed is improved when premixes have a d_{gw} similar to the d_{gw} of the complete mash feed and less variation in particle size, S_{gw}, but it is difficult to recommend one correct method for measuring particle size and distribution due to the potential alterations in the procedure [17]. Since all premixes in this study were analyzed using the same procedure, results can be compared relatively. There was a significant form × type interaction for the d_{gw} with the microencapsulated VM premixes having a significantly larger d_{gw} than the standard VM premixes and free vitamin premix, which were significantly larger than the free mineral premix (p = 0.002) (Table 5). There were significant form effect and type effect in the S_{gw} of the premixes, but no significant interaction effect was observed (Table 5). Standard premixes, regardless of type, had a significantly larger S_{gw} (2.50) than free and microencapsulated premixes (1.73 and 1.61, respectively) ($p < 0.001$), with the mineral premixes having more variability in particle size than the vitamin premixes ($p < 0.001$) (Table 5). Standard premixes typically have more variability in particle size due to the addition of a carrier, and minerals typically have more variability than vitamins due to the different versus minerals [9]. Mash poultry and swine feed often have a d_{gw} between 600 μm and 900 μm [18], indicating that the lipid microencapsulated VM premixes are likely to have the best mixability due to the fact that they have a d_{gw} that fits within that micron range and the particles in the microencapsulated premixes are all very similar in size.

Flowability is difficult to estimate exactly because ingredient flow behavior is solely a function of the manufacturing equipment used and the physical properties of the feedstuffs [19]. Because of this, it is more useful to estimate the relative flowability among

Table 5 Premix physical property results[+].

Form	Type	d_{gw} (μm)	S_{gw}	Angle of repose (AOR, °)	Minimum orifice diameter (MOD, mm)
Free	Vitamin	$200.33^{b} \pm 29.07$	$1.67^{B} \pm 0.07$	$29.23^{b} \pm 1.09$	$12^{A} \pm 0.31$
	Mineral	$78.67^{c} \pm 29.07$	$1.78^{B} \pm 0.07$	$38.59^{a} \pm 1.09$	$18^{A} \pm 0.31$
Standard	Vitamin	$246.11^{b} \pm 16.78$	$2.33^{A} \pm 0.04$	$32.22^{b} \pm 0.63$	$14^{A} \pm 0.18$
	Mineral	$256.50^{b} \pm 20.56$	$2.69^{A} \pm 0.05$	$35.99^{a} \pm 0.77$	$19^{A} \pm 0.22$
Microencapsulated	Vitamin	$611.00^{a} \pm 29.07$	$1.49^{B} \pm 0.07$	$21.78^{c} \pm 1.09$	$5^{B} \pm 0.31$
	Mineral	$722.67^{a} \pm 29.07$	$1.74^{B} \pm 0.07$	$19.78^{c} \pm 1.09$	$5^{B} \pm 0.31$
p-value (form)		< 0.001	< 0.001*	< 0.001	< 0.001*
p-value (type)		0.995	< 0.001	< 0.001	0.004
p-value (form × type)		0.002*	-	< 0.001*	-

[+] Standard deviations (mean ± standard deviation) for $n = 3$ samples.

[a, b, c, d] Means with no common superscripts are significantly different ($p < 0.05$).

[A, B] Form means (vitamin and mineral (VM) averaged together) with no common superscripts are significantly different ($p < 0.05$).

* p-value corresponding to the superscripts in that column.

feed ingredients using analyses such as AOR or MOD. A smaller AOR and smaller MOD both correspond to improved bin flowability. Regardless of form, mineral premixes had a significantly larger AOR ($p < 0.001$) and MOD ($p = 0.004$) than the vitamin premixes (Table 5). The microencapsulated VM premixes had a significantly smaller AOR than the free vitamin and standard vitamin premixes, which were significantly smaller than the free mineral and standard mineral premixes ($p < 0.001$) (Table 5). Regardless of type, microencapsulated premixes had a significantly lower MOD (5 mm) than premixes of the free (15 mm) or standard (16.5 mm) forms ($p < 0.001$) (Table 5). The significant reduction in AOR and MOD values observed with the microencapsulated VM premixes corresponds to a significant improvement in flowability in the feed mill. These results demonstrate that poor flowing free premixes and additives could be microencapsulated in a hydrogenated lipid matrix to improve HC and make products that are more compatible with feed mills.

3.2 Physical Impacts of Storage

The lumping test estimates a premix's tendency to lump and clump when exposed to heat during storage. No significant differences in lumping scores were observed between premixes (Table 6). The compression test determines a premix's tendency to form a solid cake when exposed to pressure, like being stacked in bags on a pallet. There was a significant form effect on the compression score with the microencapsulated premixes having a significantly higher compression score than the free and standard premixes ($p = 0.001$) (Table 6). A higher compression score for the microencapsulated premixes is expected because of the nature of the hydrogenated lipid matrix.

The Carr's Compressibility Index or percent compressibility ($C = 100 \times (1 - \text{bulk/tapped})$ or $C = 100 \times ((\text{tapped} - \text{bulk})/\text{tapped})$) and Hausner Ratio ($H = \text{tapped/bulk}$) are frequently used as indicators of the flowability of a powder or feed additive [10, 11]. For a feed additive with a good flowability, the bulk density and tapped density would be close in value, therefore the Carr's index would be small and the Hausner Ratio would be close to a value of 1.0 [9]. There was a significant form × type interaction for the premix bulk density, tapped density, Carr's Compressibility Index and Hausner Ratio (Table 6). The free mineral premix had the largest bulk density followed by the standard mineral, the microencapsulated mineral, the microencapsulated vitamin, the standard vitamin, and the free vitamin premix, all being significantly

Table 6 Premix physical impacts of storage results[+].

Form	Type	Lumping score (1-6)	Compression score (1-6)	Bulk density (kg/m^3)	Tapped density (kg/m^3)	Carr's Compressibility Index (%)	Hausner Ratio
Free	Vitamin	3.16 ± 0.30	2.33^{B} ± 0.24	517.25^{f} ± 10.81	559.72^{d} ± 14.19	7.58^{c} ± 0.95	1.08^{c} ±0.01
	Mineral			1,282.61^{a} ± 10.81	1,579.30^{a} ± 14.19	18.75^{a} ± 0.95	1.23^{a} ± 0.01
Standard	Vitamin	2.22 ± 0.20	1.67^{B} ± 0.16	566.71^{e} ± 6.24	655.56^{c} ± 8.19	13.56^{b} ± 0.55	1.16^{b} ± 0.01
	Mineral			1,234.76^{b} ± 7.65	1,531.40^{a} ± 10.04	19.34^{a} ± 0.67	1.24^{a} ± 0.01
Microencapsulated	Vitamin	3.67 ± 0.30	4.67^{A} ± 0.24	646.58^{d} ± 10.81	663.90^{c} ± 14.19	2.59^{d} ± 0.95	1.03^{cd} ± 0.01
	Mineral			974.12^{c} ± 10.81	986.93^{b} ± 14.19	1.29^{d} ± 0.95	1.01^{d} ± 0.01
p-value (form)		0.166	0.001*	< 0.001	< 0.001	< 0.001	< 0.001
p-value (type)		0.469	0.622	< 0.001	< 0.001	< 0.001	< 0.001
p-value (form × type)		-	-	< 0.001*	< 0.001*	< 0.001*	< 0.001*

[+] Standard deviations (mean ± standard deviation) for $n = 3$ samples.

[a, b, c, d, e, f] Means with no common superscripts are significantly different ($p < 0.05$).

[A, B] Form means (VM averaged together) with no common superscripts are significantly different ($p < 0.05$).

* *p*-value corresponding to the superscripts in that column.

different ($p < 0.001$). The free mineral and standard mineral premixes had significantly greater tapped densities than the microencapsulated mineral, and all these forms of mineral supplements were significantly greater than the microencapsulated vitamin and standard vitamin, which were significantly greater than the free vitamin premix ($p < 0.001$). The microencapsulated VM premixes had a significantly lower percent compressibility than the free vitamin premix, which was significantly lower than the standard vitamin, which was significantly lower than the free mineral and standard mineral premixes ($p < 0.001$). The microencapsulated mineral premix had a significantly lower Hausner Ratio than the free vitamin (both being similar to the microencapsulated vitamin), which was lower than the standard vitamin, and was lower than the free mineral and standard mineral premixes ($p < 0.001$). Interestingly, the microencapsulated VM premixes had the highest compression score but the lowest percent compressibility, likely because the compression test involved a weighted object compacting the ingredient for a longer period of time whereas the Carr's index utilized gravity and tapping force to compact the ingredient. The microencapsulated VM premixes had significantly lower Carr's index and Hausner Ratio values than all other premixes, suggesting a better flowability than premixes of the free or standard forms, which was also demonstrated by the AOR and MOD results. Lipid microencapsulation equilibrates the bulk densities of the vitamins and minerals, making them physically more similar, and improves the handling properties and flowability.

3.3 Hydration Stability

Measuring the solubility of a premix or feed additive is an easy way to estimate an ingredient's chemical reactivity when exposed to water or moisture [20]. Solubility results are presented as the percent insoluble fraction of each premix (Table 7). Microencapsulated VM premixes were essentially insoluble and actually increased in weight, indicating some water absorption, but no change in physical or textural appearance was observed after the premixes had been dried on the filter paper ($p = 0.003$). This increase in weight could be due to the premixes not having enough time to dry, although they all dried for the same amount of time and appeared and felt like no moisture was remaining when they were weighed. The insoluble fraction of the standard vitamin and free mineral premixes were similar to the microencapsulated VM premixes as well as the free vitamin premix. The

Table 7 Premix hydration stability results[+].

Form	Type	Insoluble fraction (%)	Hygroscopic percent change in weight (%)				
			D0-1	D0-3	D0-5	D5-9[1]	D0-9
Free	Vitamin	$78.89^{bc} \pm 4.82$	1.52 ± 0.35	$3.48^{A} \pm 0.42$	$5.04^{A} \pm 0.46$	$-4.48^{d} \pm 0.18$	$1.03^{b} \pm 0.52$
	Mineral	$88.81^{abc} \pm 4.82$				$-3.08^{c} \pm 0.18$	$1.09^{b} \pm 0.51$
Standard	Vitamin	$90.29^{ab} \pm 2.78$	1.82 ± 0.23	$3.74^{A} \pm 0.27$	$5.26^{A} \pm 0.29$	$-3.74^{c} \pm 0.10$	$0.91^{b} \pm 0.30$
	Mineral	$75.77^{c} \pm 3.41$				$-1.78^{b} \pm 0.13$	$3.81^{a} \pm 0.37$
Microencapsulated	Vitamin	$100.86^{a} \pm 4.82$	0.75 ± 0.35	$1.62^{B} \pm 0.42$	$2.24^{B} \pm 0.46$	$-1.56^{ab} \pm 0.18$	$0.38^{b} \pm 0.51$
	Mineral	$105.78^{a} \pm 4.82$				$-1.06^{a} \pm 0.18$	$1.43^{b} \pm 0.51$
p-value (form)		< 0.001	0.057	0.001*	< 0.001*	< 0.001	0.003
p-value (type)		0.621	0.113	0.521	0.579	< 0.001	0.002
p-value (form × type)		0.003*	-	-	-	< 0.001*	0.008*

[+] Standard deviations (mean ± standard deviation) for $n = 3$ samples.

[1] During D5-9 samples were kept at ambient temperature with no added humidity.

[a, b, c, d] Means with no common superscripts are significantly different ($p < 0.05$).

[A, B] Form means (VM averaged together) with no common superscripts are significantly different ($p < 0.05$).

* *p*-value corresponding to the superscripts in that column.

standard mineral had the smallest insoluble fraction, at 75.77%, and was statistically similar to the free VM. This means that almost 25% of the standard trace mineral premixes, commonly included in poultry and swine diets, are soluble and therefore reactive when exposed to moisture. This increase in reactivity results in a lower bioavailability of nutrients, which often causes swine and poultry nutritionists to compensate for the loss by increasing inclusion levels of these premixes in animal diets [21]. Higher inclusion levels of trace mineral premixes can have negative impacts on palatability, gut health and the environment [22]. Lipid microencapsulation protects premix nutrients from chemical reactions involving water, suggesting an increase in the bioavailability of nutrients.

The hygroscopic nature of premixes and feed additives is very important to feed manufactures because it influences flowability and the general handling ability of ingredients at a feed mill [5, 16]. Ingredients like salt, choline chloride and dried distiller's grains are notorious for being very hygroscopic, often bridge in bins, slow the production rate, and reduce the efficiency of feed manufacturing. Premix hygroscopicity results are shown in Table 7. A significant form effect was observed on percent change in weight for days 0-3 ($p = 0.001$) and days 0-5 ($p < 0.001$), with the microencapsulated premixes increasing significantly less in weight than the standard and free premixes, regardless of type. Even though the numerical change in weight is minimal, it is important to note that the free and standard premixes absorbed over twice the amount of moisture than the microencapsulated premixes for days 0-1, 0-3 and 0-5. There was a significant form × type interaction for the premix percent change in weight for days 5-9 with the microencapsulated mineral premix decreasing significantly less in weight than the free mineral and standard vitamin premixes, which decreased less in weight than the standard mineral and microencapsulated vitamin premixes. The microencapsulated mineral premix decreased the least in weight between day 5 and day 9 and was statistically similar to the microencapsulated vitamin ($p < 0.001$). From days 0-9 all premixes absorbed more moisture than they released, and there was a significant form × type interaction effect. The standard mineral retained the most in moisture weight and increased in weight significantly more than all other premixes. The free and standard premixes increased the most in weight from days 0-5 and decreased the most in weight from days 5-9. The larger decrease in weight of the free and standard vitamin premixes from days 5-9 could be from moisture loss as well as other volatile losses from chemical reactions resulting in 0-9 day results similar to the encapsulated premixes. These data show that lipid microencapsulated VM premixes absorb less moisture from their surroundings than standard or free premixes suggesting that microencapsulated premixes may be more protected from the environment and potential chemical interactions.

4. Conclusions

The HC of feed ingredients, and especially feed additives and premixes, are essential to the efficiency of animal feed manufacturing. Lipid matrix microencapsulation by means of U.S. Patent WO2018089516 improves HC of VM premixes by significantly increasing the average particle size (d_{gw}), by reducing the AOR and MOD measurements resulting in improved flowability, by reducing the percent compressibility (calculated with the bulk and tapped densities) and by reducing the amount of moisture uptake and loss resulting in improved hygroscopic characteristics. The various handling characteristic materials and methods in this manuscript can be used as a procedure for measuring the relative handling properties of different feed ingredients and additives.

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