

Article

Broiler Breeders Fed Diets Supplemented with Conventional or Lipid Matrix Microencapsulated Trace Minerals at Standard or High Levels: Part I. Influence on Production, Skeletal Integrity, and Intestinal Histomorphology of Broiler Breeders [†]

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[†] This manuscript is part of a Ph.D. thesis by the first author, available online at

<https://repository.lib.ncsu.edu/items/c0b8e542-a3f9-4fec-bade-e87dbb5b22> (accessed on 24 March 2025).

Abstract: Lipid matrix microencapsulation is hypothesized to improve bioavailability for broiler breeders supplemented with normal and protect against excessive levels of inorganic trace minerals. At 27 weeks (wk), nine females and two males were assigned to each of the 12 floor pens. Each pen was randomly assigned to dietary treatments (trt) in a factorial arrangement of two mineral premix forms, free (FRTM) and microencapsulated (MITM), and two mineral premix supplement dosages (100% and 300% of Aviagen recommendations). At 55 wk termination, 15 hens/trt were randomly selected to assess tibia and jejunum morphology. Breeders receiving MITM improved hen day % (HD %), hen housed (HH%), and FCR but produced the lightest chicks at hatch. The TM form had no significant effect on histology, but TM 100% had greater villus height (VH), crypt depth (CD), and villi area than TM 300% ($p < 0.5$). No significant treatment effects on bone weight, length, and bone mass density were observed. However, MITM treatments increased tibia thickness, and FRTM supplementation increased ash% and Cu content. Hens fed 300% TM had higher bone Mn content than 100% TM, whereas hens fed MI100 had the highest Fe content. Feeding lipid matrix microencapsulated trace minerals to broiler breeders does have positive impacts on the production of eggs, but no effects were observed in the histomorphology of the jejunum or tibia bone parameters.



Academic Editor: Ilias Giannenas

Received: 2 December 2024

Revised: 4 March 2025

Accepted: 17 March 2025

Published: 6 April 2025

Citation: Malheiros, D.M.; Malheiros, R.D.; Anderson, K.E.; Ferket, P.R. Broiler Breeders Fed Diets Supplemented with Conventional or Lipid Matrix Microencapsulated Trace Minerals at Standard or High Levels: Part I. Influence on Production, Skeletal Integrity, and Intestinal Histomorphology of Broiler Breeders. *Poultry* **2025**, *4*, 17. <https://doi.org/10.3390/poultry4020017>

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Keywords: broiler breeder; nutrition; histomorphology; microencapsulated premix; trace minerals; bone quality

1. Introduction

Supplemental trace minerals, although constituting a small portion of feed, are paramount for normal metabolism, health, and productive performance in animals. Trace mineral supplementation is particularly important for broiler breeders because they are managed over an extended period as compared to their offspring for meat production, which necessitates robust biological fitness particularly during peak egg production. Zinc, copper, manganese, and selenium are critical in various biological functions. These trace minerals are key cofactors for the antioxidant enzymes that control metabolic oxidative stress [1], among many other metabolic enzymes. Zinc, for instance, activates hundreds of enzymes and influences reproductive performance in animals. It significantly impacts growth, tissue development, repair, skeletal development, immune competence, and gene

expression. However, excessive calcium and phosphorus in the diet can antagonize zinc, potentially leading to deficiencies if not balanced properly [2–6]. Alongside zinc, manganese and copper play crucial roles in broiler breeder performance and embryo development. Deficiencies in these minerals result in lower egg production rates, poor hatchability, and compromised eggshell and bone strength [7–11]. Copper, for instance, is vital in luteinizing hormone (LH) synthesis by prostaglandin E2 (PGE2), and it helps maintain LH and follicle-stimulating hormone (FSH) concentrations in serum, which are pivotal for ovarian development, ovulatory synchrony, and fertility [12,13]. These trace minerals (TMs) also play a very important role in the fertility of broiler breeders as they are involved in the regulation of many hormone excretions, immune defense systems, and intermediary metabolism [14,15].

Trace minerals are typically supplemented to feed in small quantities, so it is very important that these minerals are supplemented in the correct quantities and properly mixed throughout the feed as premixes. Conventional trace mineral premixes are typically blends of inorganic trace mineral salts with limestone and other non-nutritive carriers. Because inorganic trace minerals are pro-oxidant catalysts, they are often not included in premixes with vitamins and other nutrients that are susceptible to oxidative degradation unless they include antioxidants [16] used within a few weeks of premix and feed manufacture and consumption by the animal [17]. In contrast, the microencapsulation of vitamin and mineral premixes in a lipid matrix has been demonstrated to have superior handling and storage stability characteristics without the need for non-nutritive carriers [18]. Dietary supplementation of microencapsulation of trace mineral premixes could be particularly beneficial to support the reproductive performance of broiler breeder hens.

Because of controlled feeding to manage body weight and associated reproductive performance, genetic companies and commercial poultry nutritionists recommend that broiler breeders be fed higher dietary supplementation levels of trace minerals than recommended by NRC (1994) to avoid marginal deficiencies [7]. But excessive trace mineral supplementation of highly soluble inorganic trace minerals leads to lower mineral bioavailability and potentially a decrease in animal performance because of marginal toxicity or nutrient antagonisms [18] that lead to excessive environmental emission [8]. However, dietary supplementation of inorganic trace minerals microencapsulated in a slow-release lipid matrix [19] may improve bioavailability without overwhelming saturable mineral transporters or reduce excessive trace mineral–nutrient antagonisms, thus improving the egg quality, hatchability, and progeny quality of broiler breeders.

The aim of this experiment was to assess the effects of high and normal dietary inclusion levels of inorganic trace minerals supplied as protected microencapsulated (MITM) or free premix forms (FRTM) on broiler breeder production parameters, gut, and skeletal parameters. We hypothesized that MITM at normal or higher dietary inclusion levels would enhance breeder performance and mitigate adverse effects of excessive TM supplementation as compared to FRTM.

2. Materials and Methods

2.1. Housing and Management

All experimental procedures on live animals used in this experiment were approved by the North Carolina State University Animal Care and Use Committee (IACUC Protocol # 15-061A). All the animals were received as a donation from a genetic company that commercializes broiler breeders in the USA, AVIAGEN INC. (Huntsville, AL, USA). The birds used in this experiment were 27 weeks of age at the start of the trial, after being raised by standard husbandry practices for broiler breeder pullets and cockerels. For the duration of the experiment, the hens were raised at the NC State Prestage Department

of Poultry Science live animal care facility. A total of 108 Ross 708 females, and 24 Ross HY males were randomly distributed among 12 pens (1.8 m by 1 m; about 0.163 m² per bird), with 9 females and 2 males per pen, so there were 3 replicate pens per treatment. Pens were also randomized in the room to ensure an even distribution of floor pens. Hens were housed in an environmentally controlled room maintained at approximately 25 °C and 77% relative humidity. Each pen had a nest box with 6 nest spaces, and water was available ad libitum through a Plasson® bell drinker. Each pen was supplied with a female feeder that had a screen to exclude males from consuming the female feed, and a male feeder raised to a level out of reach for the females to consume the male feed was included. The amount of feed was issued daily to each feeder in accordance with the breeder recommendations. The floor pen litter was composed of soft pine shavings that were renewed when necessary and supplemented with fresh shavings twice per week or as needed to maintain litter quality and maintain environmental ammonia emission below 10–15 ppm. The broiler breeders received 16 h of fluorescent light (30–60 lux) and 8 h of dark. Eggs were collected manually twice daily, and egg production was recorded using a pen. This experiment was designed to evaluate 4 dietary treatments consisting of a factorial arrangement of 2 mineral premix forms (free {FRTM} and microencapsulated {MITM}) and 2 mineral premix supplement dosages (100% and 300% of Aviagen recommendations): (1) free 100% (FR100), (2) microencapsulated 100% (MI100), (3) free 300% (FR300), and (4) microencapsulated 300% (MI300). The free premix forms were prepared as usually by blending free active ingredients with a non-nutritive ground rice hull carrier and limestone. The lipid-matrix-protected premix form was prepared by microencapsulating active TM ingredients in a proprietary hydrogenated vegetable oil matrix (Jefo Nutrition, Inc., Saint Hyacinthe, QC, Canada).

Tables A1–A3 illustrate the three feed phases for each treatment group, which were formulated to meet or exceed the breeder nutrient recommendation for the respective sexes according to the Ross Parent stock Handbook (2018) and supplied to the birds in mash form. The dietary treatment feeds were manufactured from a common basal feed that did not include the vitamin and mineral premixes, palmitic acid, and a non-nutritive filler (vermiculite), to which the respective vitamin premixes and filler were added. Palmitic acid was added to FR300 and FR100 at an inclusion rate to standardize all diets to contain the same dietary level of hydrogenated vegetable oil as provided by the MI300 diet. The formulation of each dietary treatment for the hen feed was balanced to have identical nutrient composition except for the trace mineral dose level. All roosters received the same balanced diet formulated to meet or exceed the recommendations from the AVIAGEN guide of ROSS Broiler Breeders 708 (2700 kcal metabolizable energy/kg, 16.2% crude protein, 0.58% Ca, and 0.76% P). In this experiment, the vitamins in the MITM treatments were also supplemented in the same microencapsulation technology to mitigate any potentially negative mineral–vitamin interactions. All samples were collected in the same order to reduce confounding effects.

2.2. Productive Performance

Broiler breeder performance was evaluated using key performance indicators like hen-day egg production (HD%), hen-housed egg production (HH%), the feed conversion ratio (FCR), eggs per hen per week, and chick weight at hatch. Hen-day egg production was calculated as the total number of eggs produced per pen, per day divided by the number of live hens in that pen and then multiplied by 100 in order to account for daily egg production while adjusting for mortality. Hen-housed egg production was determined as the total number of eggs produced per pen divided by the number of hens housed at the start of the trial and then multiplied by 100; it provides a measure of cumulative production

regardless of mortality. The feed conversion ratio was expressed as the total feed intake in kilograms divided by the total dozen eggs produced, serving as an indicator of feed efficiency. The number of eggs per hen per week was determined by dividing the total number of eggs produced per pen by the number of weeks in lay.

2.3. Jejunum Mucosal Histomorphology

At termination, when the hens were 55 weeks of age, 5 productive hens were randomly selected from each pen (15 hens per treatment) and euthanized by cervical dislocation. Within 5 min of confirmed death of the sampled bird, approximately a 5 cm section from the middle of the jejunum (identified as the section between the end of the duodenal loop and the Meckel's diverticulum) was removed, opened longitudinally, and fixed immediately in a buffered 10% formalin solution for 48 h. Methodologies were performed in accordance with previous works [20]. Samples were then washed in 70% ethanol to remove the fixing solution, dehydrated in increasing alcohol concentrations, clarified in xylol, and embedded in paraffin. Semiseriated 5-µm transverse thick histological sections were stained with hematoxylin–eosin, and microscope slides were assembled with Canada balsam. A light microscope (LEICA-DMR; Leica Camera AG, Solms, Germany) was used to visualize the stained sections on slides at 4X magnification, and images were captured through Image Tools to measure the villus height (VH), upper villi width, bottom villi width, crypt depth (CD), and muscularis mucosae thickness using AmScope™ ×86 software version 3.7.3036 (AMSCOPE, Orange County, CA, USA). The villi surface was calculated using 10 readings per replicate per variable according to the following formulas: VH/CD and

$$\text{Villi surface} = \left(\frac{\text{upper villi width} + \text{Bottom Villi width}}{2} \right) \text{Villus Height}$$

2.4. Bone Mineral Composition and Characteristics

Methodologies were adapted from previous works [21]. After the jejunum sample was collected within the critical 5 min period after confirmed death for each of the 15 hens per treatment as described above, the left tibia was dissected and stored frozen at −20 °C until subsequent analysis. Upon refrigerated thawing, the flesh was removed from the bones, weighed, and measured for bone length and diaphysis diameter using digital calipers with a scale sensitivity of 0.01 mm. Bone strength and bending moment were then determined using the TA.HDPlus texture analyzer machine (Stable Micro Systems, Hamilton, MA, USA) with a 250 kg load cell. The bone mass density was assessed using dual-energy X-ray absorptiometry (DEXA, SCHICK, accuDEXA BMD Assessment System, Long Island City, NY, USA). The mineral composition of these bones was then determined through chemical digestion, and trace mineral composition was determined through inductively coupled plasma mass spectrometry (ICP-MS, 5800 ICP-OES, Agilent, Santa Clara, CA, USA).

2.5. Statistical Analysis

All data were statistically analyzed as a 2 × 2 factorial randomized design with 3 replicate pens as the experimental unit per treatment group. Data were analyzed using one-way ANOVA (JMP 15 software; SAS Inst. Inc.; Cary, NC, USA), and the means were then statistically distinguished using Tukey's multiple range test. The main and interaction factor effects and treatment group effects were considered significant at $p < 0.05$. Treatments were unblinded to the researchers for the duration of the experiment.

3. Results

Broiler breeder performance was assessed using percent hen-day egg production (HD%), percent hen-housed egg production (HH%), kg feed/dozen eggs, and eggs per

hen per week (Table 1). There were no significant premix form X trace mineral dose interaction effects nor main effects of trace mineral dose observed on any of the performance measurements. The microencapsulated form of the supplemented trace minerals resulted in significantly ($p < 0.05$) better key performance indicators for HD%, HH%, FCR (kg feed/dozen eggs), and eggs per hen regardless of the dose. Hen-day (HD) and hen-house (HH) egg production rates were higher for the microencapsulated form of the trace minerals than the free form (71.7% vs. 66.2% and 71.7 vs. 66.0, respectively, $p < 0.005$), but no significant differences were observed between the doses or treatment combinations. Microencapsulation significantly improved feed conversion (kg feed/dozen eggs) by approximately 9%, but increased supplementation level did not improve it. However, the weights of chicks from hens subjected to the MITM treatments were significantly less than hens subjected to the FRTM treatments (41.9 g vs. 43.2 g, $p < 0.05$).

Table 1. Effect of dietary supplementation of trace minerals in two forms (microencapsulated (MI) and free (FR) trace minerals) and two doses (100% and 300%) on broiler breeder performance from 26 to 55 weeks of age ¹.

		Egg Production %		Feed Conversion		
		HD ²	HH ³	kg Feed/Dozen of Eggs	Eggs/hen (per week)	Chick Weight (g)
TM Form						
	MI	71.71 ^a	71.66 ^a	2.63 ^b	5.02 ^a	41.94 ^b
	FR	66.21 ^b	66.01 ^b	2.85 ^a	4.63 ^b	43.17 ^a
TM Dose						
	100%	69.95	69.78	2.70	4.89	42.39
	300%	67.98	67.89	2.78	4.76	42.70
Treatment	Dose					
MI	300%	70.93	70.93	2.69	4.96	45.86
FR	300%	66.60	64.86	2.87	4.66	47.35
MI	100%	72.87	72.39	2.57	5.10	44.64
FR	100%	67.69	67.16	2.82	4.74	45.34
		<i>p</i> -value				
TM Form		0.0019	0.0014	0.0140	0.0019	0.0422
TM Dose		0.2618	0.2843	0.3386	0.2618	0.5824
TM Form * TM Dose		0.8154	0.8099	0.6115	0.8169	0.1442
SEM ⁴		1.24	1.24	0.062	0.087	0.43

^{a,b} Means within a column with a different superscript differ significantly ($p < 0.05$). ¹ Means are an average of 3 pens per treatment, with each pen containing 9 hens and 2 roosters. ² Hen-day egg production = 100((eggs produced)/(# hens)). ³ Hen-house egg production = 100((eggs produced)/(# hens housed)). ⁴ Standard error of the mean with 8 degrees of freedom.

The effect of the premix form and the TM dose on the jejunum mucosa histomorphology is presented in Table 2. There were no significant form X dose interaction effects nor premix form effects observed. However, highly significant ($p < 0.005$) TM dose effects were observed on the villus height, crypt depth, and surface area. Hens fed the high dose of TM (300% of industry recommendations) had a 25% lower villus height, a 23% lower crypt depth, and a 31% lower villus surface area than hens fed the standard dose of TM (100% of industry recommendations). There were no significant treatment effects observed on the villus bottom and top width, villus height/crypt depth ratio, and muscularis thickness.

Table 2. Effect of dietary supplementation of trace minerals in two forms (microencapsulated (MI) and free (FR) trace minerals) and two doses (100% and 300%) on the jejunum mucosa histomorphology of broiler breeders ¹.

		Villus Height (VH, μm)	Villus Tip Width (μm)	Villus Bottom Width (μm)	Crypt Depth (CD, μm)	Muscularis (μm)	Area (μm^2)	Ratio (VH/CD)
TM Form								
	MI	857.04	159.48	186.90	128.38	203.89	154,637	6.91
	FR	834.17	156.97	170.95	132.75	210.22	141,458	6.43
TM Dose								
	100%	968.46 ^a	166.74	186.91	147.73 ^a	212.54	175,103 ^a	6.76
	300%	722.76 ^b	149.72	170.94	113.40 ^b	201.57	120,992 ^b	6.58
Treatment	Dose							
	MI 300%	733.35	152.76	183.65	112.52	187.09	129,606	6.78
	FR 300%	712.16	146.67	158.22	114.28	216.06	112,377	6.38
	MI 100%	980.74	166.20	190.15	144.24	220.69	179,668	7.04
	FR 100%	956.18	167.27	183.68	151.21	204.39	170,539	6.49
					<i>p</i> -value			
TM Form		0.6641	0.8255	0.3037	0.6262	0.7376	0.4742	0.2997
TM Dose		<0.0001	0.1391	0.3029	0.0003	0.5624	0.0045	0.6801
TM Form * TM Dose		0.9744	0.7534	0.5397	0.7713	0.2340	0.8256	0.8729
SEM		37.05	8.02	10.86	6.31	13.30	12,934	0.32

^{a,b} Means within a column with a different superscript differ significantly ($p < 0.05$). ¹ Means are an average of 15 birds per treatment with 10 villi measurements per hen.

The effect of the premix form and the TM dose on the physical characteristics of the tibia bone is presented in Table 3. In general, there were no significant form X TM dose effects or TM main effects on tibia bone characteristics observed in sampled hens at the termination of the study period. The main effect of the TM form on tibia thickness was significant, favoring the MITM treatments over the FRTM treatments (7.82 mm v. 7.62 mm, $p < 0.05$). There were no treatment effects observed on the fresh tibia length, weight, bone thickness, bending moment, or bone mass density determined by DEXA.

The effect of the premix form and the TM dose on tibia bone ash and select mineral content is presented in Table 4. The concentrations (mg/g) of P, Ca, and Zn in the tibia bones of the broiler breeders were not significantly ($p > 0.05$) affected by the dietary treatment. Although there were no significant treatment interaction effects nor main effects of the TM dose on bone ash percentage, there was a significant main effect of the premix form, favoring the free form over the microencapsulated form (35.8% vs. 34.0%, $p < 0.05$). Likewise, birds fed diets with the free premix form had bones with significantly higher Cu content than those fed the microencapsulated form of the premix (0.000407 mg Cu/g bone vs. 0.000237 mg Cu/g bone, $p < 0.05$). Only a significant form X dose interaction effect was observed on the Fe content in the broiler breeder bones, whereas this interaction effect approached significance for the Mn content. At normal TM dose levels, the Fe and Mn content of the bone increased due to microencapsulation but decreased at a high dietary TM dose. Interestingly, only the Mn content in the bones of broiler breeders was significantly increased as the TM dose increased.

Table 3. Effect of dietary supplementation of trace minerals in two forms (microencapsulated (MI) and free (FR) trace minerals) and two doses (100%, 300%) on the physical characteristics of the tibia bone of broiler breeders at 55 weeks of age ¹.

		Length (mm)	Diaphysis Diameter (mm)	Fresh Weight (g)	Bending Moment (mm)	Bone Strength (N)	BMD ²
TM Form							
MI		116.95	7.82 ^a	18.04	0.1276	350.39	0.6907
FR		117.98	7.62 ^b	18.05	0.1299	351.17	0.6871
TM Dose							
100%		118.19	7.76	17.91	0.1280	347.84	0.6847
300%		116.69	7.69	18.18	0.1296	353.92	0.6933
Treatment	Dose						
MI	300%	116.24	7.81	18.16	0.1248	342.35	0.6975
FR	300%	117.18	7.57	18.2	0.1347	366.28	0.6888
MI	100%	117.66	7.84	17.91	0.1304	358.43	0.6839
FR	100%	118.71	7.68	17.90	0.1255	337.15	0.6856
		<i>p</i> -value					
TM Form		0.3088	0.0354	0.9757	0.6671	0.9280	0.8731
TM Dose		0.1340	0.4829	0.5120	0.7614	0.6616	0.7050
TM Form * TM Level		0.9571	0.6635	0.9529	0.2027	0.1316	0.8142
SEM		0.68	0.067	0.29	0.0040	1.1	0.015

^{a,b} Means within a column with a different superscript differ significantly ($p < 0.05$). ¹ Means are an average of 15 bones per treatment. ² Bone mass density.

Table 4. Effect of dietary supplementation of trace minerals in two forms (microencapsulated (MI) and free (FR) trace minerals) and two doses (100%, 300%) on the tibia bone ash content and mineral composition of broiler breeders at 55 weeks of age ².

Treatment	Ash%	P	Ca	Cu	Fe	Mn	Zn
mg/g ³							
TM Form							
MI							
FR							
TM Dose							
100%							
300%							
Treatment	Dose						
MI	300%	34.50	19.06	42.13	0.00017	0.01427 ^{ab}	0.00191
FR	300%	35.74	17.93	39.62	0.00029	0.01614 ^{ab}	0.00220
MI	100%	33.53	18.87	41.94	0.00021	0.01640 ^a	0.00188
FR	100%	35.85	18.60	42.41	0.00032	0.01400 ^b	0.00176
<i>p</i> -value							
TM Form		0.0330	0.1298	0.2880	0.0143	0.0787	0.4123
TM Dose		0.5990	0.6036	0.1781	0.3077	0.9991	0.0216 ¹
TM Form * TM Dose		0.5075	0.3510	0.1211	0.3336	0.0113 ¹	0.0595
SEM		0.5703	0.3208	0.6657	0.0000478	0.0005625	0.000072

^{a,b} Means within a column with a different superscript differ significantly ($p < 0.05$). ¹ Means are an average of 15 bones per treatment. ² Means are an average of 3 pens per treatment, with each pen containing 9 hens and 2 roosters. ³ mg of mineral per g of ash.

4. Discussion

This study demonstrated that the broiler breeders fed diets supplemented with MITM had the greatest effect on performance parameters, independent of the dietary trace mineral dose. Egg production, the feed conversion ratio, and the hatchability rate were significantly improved by the premix microencapsulation treatments. The benefit of protecting reactive inorganic trace minerals in a lipid matrix demonstrates a promising technology for broiler breeders. Indeed, the economic return from the increased production efficiency and chick yield likely surpasses the added cost of microencapsulating the premix, not including the savings that may occur because of improved nutrient stability and handling characteristics. This is especially important as these are broiler breeders who produce broiler chicks as part of the integrated poultry meat industry. With a relatively small investment in feed manufacturing, hens fed the microencapsulated mineral premix had greater egg production, which in turn would result in more broilers for the meat industry. In comparison to the other treatments that had a higher level of minerals or are free mineral premix forms, the superior breeder hen reproductive performance of the MITM-fed hens may have been due to less antagonistic effects of inorganic trace minerals on vitamins and other critical nutrients. In this trial, broiler breeder hens fed MITM produced an average of 11 more eggs per hen during the 26 to 55 week period of lay than hens fed FRTM; this is a substantial difference observed where each hen has an increased potential for progeny production. Additionally, Richards et al. [5] reported that feeding higher levels of trace minerals could potentially decrease animal performance. However, the results reported herein does not fully agree with the findings by Richards et al. [5], as the performance parameters observed were not affected significantly by the dose of TM supplemented. Although the progeny from hens fed MITM weighed a mean of 1.2 g less than the progeny from FRTM-fed broiler breeders ($p < 0.05$), this difference may not be realized by market age.

The effects of MITM on broiler breeder performance are in accordance with previous research [7], where amino acid–mineral complexes were used as a means of protection for the minerals and showed improvements in broiler breeder performance. As MITM is a novel form of protection, few references are available for comparison, and other forms of protection are used as a basis for comparison.

Enterocytes are highly prolific and have a high turnover rate relative to other cells in the body. They serve a critical barrier function and are essential for digestion and nutrient assimilation, so histomorphological evaluation of the enteric villi is a means to assess mucosal distress. Previous studies [22,23] have attributed higher VH/CD ratios to mucosal distress, which is indicative of a lower rate of villus tip sloughing of enterocytes relative to their generation in the crypts. Similarly, Ma et al. [24] observed that birds fed organic TMs had lower crypt depths and higher VH/CD ratios in the ileum as compared to inorganic (free) trace minerals. In contrast, we did not observe significant differences among TM form treatments for jejunal VH, CD, or the VH/CD ratio, which may be a different response than observed by other researchers who only evaluated the ileum [24]. However, dietary TM dose did affect mucosal histomorphology, regardless of the premix form. The excessive dietary trace mineral dose (300%) significantly reduced the villus height, crypt depth, and villi surface area, without an effect on the villus height/crypt depth ratio or muscularis thickness that would otherwise suggest possible enteric distress. Although one would conclude that a 44% decrease in villi area could compromise the capacity to absorb nutrients, this was apparently not observed as there was no TM dose effect on feed conversion nor egg production rate. Instead, the high inorganic TM dose may have suppressed enteric microflora competition for nutrients, so a compensatory response to greater villi surface area was not needed [19]. Although MITMs are inorganic, they have an organic coating

matrix that protects the trace minerals from reactions in the gut while still retaining their inorganic characteristics on the enteric microbiome.

Bone is a primary body reserve for minerals, particularly in egg-laying breeders that require diurnal mobilization of minerals for proper eggshell formation. The physical characteristics of tibia bone mineralization is a typical assessment measured to assess the bioavailability of dietary minerals. No significant treatment effects were observed on the physical characteristics of the tibia bone, including length, weight, bending moment, bone breaking strength, and BMD (Table 3). It is noteworthy that tibia bone thickness was significantly greater among hens fed the microencapsulated form than those fed the free form of trace minerals. Greater bone thickness may provide greater capacity for medullary bone function for mineral mobilization and blood cell formation. The bone ash and mineral concentrations of the bone (Table 4) are standard indicators of dietary mineral bioavailability. The microencapsulation of trace minerals was observed to significantly reduce percent ash and mg copper/g ash content in bone as compared to the free form of trace minerals, but this response may be due to more mineral mobilization to satisfy the eggshell formation demand due to greater egg production rates observed without affecting the bone integrity. Only the Mn content of tibia was increased as the trace mineral dose increased. However, a significant mineral form X dose interaction was observed only on the iron content of the bone. Bone iron content was significantly increased due to the microencapsulation of trace minerals at the normal dose of trace minerals but not at the high dose level. This observation may be related to increased bone marrow observed among the MI treatments, as it correlates with increased tibia bone width.

5. Conclusions

In conclusion, feeding MITM to broiler breeders has great potential to improve broiler breeder egg production and efficiency. Although the microencapsulation of trace mineral premixes may modify bone mineralization, it may remediate potential antagonistic mineral–nutrient interactions and thus yield significant benefits in the reproductive performance of broiler breeders. Lipid matrix microencapsulation of premixes is demonstrated to be a novel micro-ingredient supplementation technology that favors stability through storage and feed manufacturing as well as poultry production efficiency and sustainability.

Author Contributions: Conceptualization, P.R.F. and R.D.M.; methodology, D.M.M.; formal analysis, D.M.M.; investigation, D.M.M. and R.D.M.; resources, D.M.M., R.D.M. and P.R.F.; data curation, D.M.M.; writing—original draft preparation, D.M.M.; writing—review and editing, D.M.M., P.R.F. and K.E.A.; supervision, P.R.F.; project administration, R.D.M.; funding acquisition, P.R.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Jefe Nutrition Inc., and The APC was funded by Peter Ferket.

Institutional Review Board Statement: All experimental procedures on live animals used in this experiment were approved by the North Carolina State University Animal Care and Use Committee (IACUC Protocol # 15-061A, approval date 06/16/2017).

Informed Consent Statement: Not Applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgments: I would like to acknowledge Nc State University for their continued support in conducting research and allowing students to participate in hands-on animal work.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Appendix A

Table A1. Experimental diets fed to broiler breeders from 27 to 34 weeks of age.

Ingredient	Experimental Treatments			
	MI300	FR300	MI100	FR100
	----- % of Diet -----			
Corn	65.18	65.18	65.18	65.18
Soybean Meal (47.5% CP)	19.12	19.12	19.12	19.12
Limestone fine	7.06	7.06	7.06	7.06
Wheat Middlings	6.55	6.55	6.55	6.55
Poultry Fat	0.50	0.50	0.50	0.50
Mono-Dicalcium Phosphate	0.48	0.48	0.48	0.48
Salt	0.31	0.31	0.31	0.31
DL-Methionine	0.17	0.17	0.17	0.17
Sodium Bicarbonate	0.14	0.14	0.14	0.14
Choline Chloride (60%)	0.06	0.06	0.06	0.06
L-Threonine	0.03	0.03	0.03	0.03
Quantum Blue Phytase 750 FTU	0.02	0.02	0.02	0.02
	0	0	0	0
Protected Vitamin (PV)	0.078 ¹	0	0.078 ¹	0
Protected Mineral (PM)	0.306 ³	0	0.092 ²	0
Free Vitamin (FV)	0	0.05 ¹	0	0.05 ¹
Free Mineral (FM)	0	0.20 ³	0	0.06 ²
Palmitic Acid	0	0.134	0.117	0.134
Vermiculite	0	0.0	0.097	0.140
Total Ingredients	100.0	100.0	100.0	100.0
Calculated values:				
Metabolizable Energy, kcal/kg	2800	2800	2800	2800
Crude Protein, %	15.0	15.0	15.0	15.0
Dig Lysine, %	0.65	0.65	0.65	0.65
Dig Methionine + Cyst(e)ine, %				
Dig Threonine, %				
Crude Fat (ether extract), %	3.28	3.28	3.28	3.28
Ca, %	2.85	2.85	2.85	2.85
Non-phytate P, %	0.58	0.58	0.58	0.58

¹ Each kilogram of PV at a dietary inclusion of 0.078% or FV at a dietary inclusion of 0.05% supplied the following per kg of complete feed: vitamin A, 13,000 IU; cholecalciferol, 5850 IU; alpha-tocopherol, 84.5 IU; niacin, 78 mg; pantothenic acid, 23 mg; riboflavin, 8.5 mg; pyridoxine, 4.2 mg; menadione, 4 mg; folic acid, 2.5 mg; thiamin, 3.25 mg; biotin, 0.234 mg; vitamin B₁₂, 0.02 mg; and ethoxyquin. ² Each kilogram of PM at a dietary inclusion of 0.092% or FM at a dietary inclusion of 0.06% supplied the following per kg of complete feed: 76 mg Zn as ZnO, 76 mg Mn as MnO, 44 mg Fe as FeSO₄·H₂O, 8.5 mg Cu as CuSO₄, 0.85 mg I as Ca(IO₃)₂, and 0.27 mg Se as Na₂SeO₃. ³ Each kilogram of PM at a dietary inclusion of 0.306% or FM at a dietary inclusion of 0.20% supplied the following per kg of complete feed: 251 mg Zn as ZnO, 252 mg Mn as MnO, 148 mg Fe as FeSO₄·H₂O, 30 mg Cu as CuSO₄, 2.85 mg I as Ca(IO₃)₂, and 0.88 mg Se as Na₂SeO₃.

Appendix B

Table A2. Experimental diets fed to broiler breeders from 35 to 49 weeks of age.

Ingredient	Experimental Treatments			
	MI300	FR300	MI100	FR100
	----- % of Diet -----			
Corn	66.25	66.35	66.35	66.35
Soybean Meal (47.5% CP)	17.8	17.8	17.8	17.8
Limestone fine	7.65	7.65	7.65	7.65
Wheat Middlings	6.24	6.24	6.24	6.24

Table A2. Cont.

Ingredient	MI300	Experimental Treatments		
		FR300	MI100	FR100
		----- % of Diet -----		
Poultry Fat	0.5	0.5	0.5	0.5
Mono-Dicalcium Phosphate	0.39	0.39	0.39	0.39
Salt	0.28	0.28	0.28	0.28
Sodium Bicarbonate	0.19	0.19	0.19	0.19
DL-Methionine	0.15	0.15	0.15	0.15
Choline Chloride (60%)	0.06	0.06	0.06	0.06
Selenium Premix NCSU	0.05	0.05	0.05	0.05
Quantum Blue Phytase 750 FTU	0.02	0.02	0.02	0.02
L-Threonine	0.02	0.02	0.02	0.02
	0	0	0	0
Protected Vitamin (PV)	0.078 ¹	0	0.078 ¹	0
Protected Mineral (PM)	0.306 ³	0	0.092 ²	0
Free Vitamin (FV)	0	0.05 ¹	0	0.05 ¹
Free Mineral (FM)	0	0.20 ³	0	0.06 ²
Palmitic Acid	0	0.134	0.117	0.134
Vermiculite	0	0	0.097	0.14
Total Ingredients	100	100	100	100
Calculated values:				
Metabolizable Energy, kcal/kg	2800	2800	2800	2800
Crude Protein, %	14.4	14.4	14.4	14.4
Dig Lysine, %	0.62	0.62	0.62	0.62
Dig Methionine + Cyst(e)ine, %				
Dig Threonine, %				
Crude Fat (ether extract), %	3.28	3.28	3.28	3.28
Ca, %	3.05	3.05	3.05	3.05
Non-phytate P, %	0.58	0.58	0.58	0.58

¹ Each kilogram of PV at a dietary inclusion of 0.078% or FV at a dietary inclusion of 0.05% supplied the following per kg of complete feed: vitamin A, 13,000 IU; cholecalciferol, 5850 IU; alpha-tocopherol, 84.5 IU; niacin, 78 mg; pantothenic acid, 23 mg; riboflavin, 8.5 mg; pyridoxine, 4.2 mg; menadione, 4 mg; folic acid, 2.5 mg; thiamin, 3.25 mg; biotin, 0.234 mg; vitamin B₁₂, 0.02 mg; and ethoxyquin. ² Each kilogram of PM at a dietary inclusion of 0.092% or FM at a dietary inclusion of 0.06% supplied the following per kg of complete feed: 76 mg Zn as ZnO, 76 mg Mn as MnO, 44 mg Fe as FeSO₄·H₂O, 8.5 mg Cu as CuSO₄, 0.85 mg I as Ca(IO₃)₂, and 0.27 mg Se as Na₂SeO₃. ³ Each kilogram of PM at a dietary inclusion of 0.306% or FM at a dietary inclusion of 0.20% supplied the following per kg of complete feed: 251 mg Zn as ZnO, 252 mg Mn as MnO, 148 mg Fe as FeSO₄·H₂O, 30 mg Cu as CuSO₄, 2.85 mg I as Ca(IO₃)₂, and 0.88 mg Se as Na₂SeO₃.

Appendix C

Table A3. Experimental diets fed to broiler breeders from 50 to 55 weeks of age.

Ingredient	MI300	Experimental Treatments		
		FR300	MI100	FR100
		----- % of Diet -----		
Corn	67.17	67.17	67.17	67.17
Soybean Meal (47.5% CP)	17.97	17.97	17.97	17.97
Limestone fine	8.20	8.20	8.20	8.20
Wheat Middlings	4.73	4.73	4.73	4.73
Poultry Fat	0.50	0.50	0.50	0.50
Mono-Dicalcium Phosphate	0.37	0.37	0.37	0.37
Salt	0.28	0.28	0.28	0.28
Sodium Bicarbonate	0.19	0.19	0.19	0.19

Table A3. Cont.

Ingredient	Experimental Treatments			
	MI300	FR300	MI100	FR100
	----- % of Diet -----			
DL-Methionine	0.15	0.15	0.15	0.15
Choline Chloride (60%)	0.06	0.06	0.06	0.06
Selenium Premix NCSU	0.05	0.05	0.05	0.05
Quantum Blue Phytase 750 FTU	0.02	0.02	0.02	0.02
L-Threonine	0.02	0.02	0.02	0.02
Protected Vitamin (PV)	0.078 ¹	0	0.078 ¹	0
Protected Mineral (PM)	0.306 ³	0	0.092 ²	0
Free Vitamin (FV)	0	0.05 ¹	0	0.05 ¹
Free Mineral (FM)	0	0.20 ³	0	0.06 ²
Palmitic Acid	0	0.134	0.117	0.134
Vermiculite	0	0.0	0.097	0.140
Total Ingredients	100.0	100.0	100.0	100.0
Calculated values:				
Metabolizable Energy, kcal/kg	2800	2800	2800	2800
Crude Protein, %	15.0	15.0	15.0	15.0
Dig Lysine, %	0.65	0.65	0.65	0.65
Dig Methionine + Cyst(e)ine, %				
Dig Threonine, %				
Crude Fat (ether extract), %	3.28	3.28	3.28	3.28
Ca, %	2.85	2.85	2.85	2.85
Non-phytate P, %	0.58	0.58	0.58	0.58

¹ Each kilogram of PV at a dietary inclusion of 0.078% or FV at a dietary inclusion of 0.05% supplied the following per kg of complete feed: vitamin A, 13,000 IU; cholecalciferol, 5850 IU; alpha-tocopherol, 84.5 IU; niacin, 78 mg; pantothenic acid, 23 mg; riboflavin, 8.5 mg; pyridoxine, 4.2 mg; menadione, 4 mg; folic acid, 2.5 mg; thiamin, 3.25 mg; biotin, 0.234 mg; vitamin B₁₂, 0.02 mg; and ethoxyquin. ² Each kilogram of PM at a dietary inclusion of 0.092% or FM at a dietary inclusion of 0.06% supplied the following per kg of complete feed: 76 mg Zn as ZnO, 76 mg Mn as MnO, 44 mg Fe as FeSO₄·H₂O, 8.5 mg Cu as CuSO₄, 0.85 mg I as Ca(IO₃)₂, and 0.27 mg Se as Na₂SeO₃. ³ Each kilogram of PM at a dietary inclusion of 0.306% or FM at a dietary inclusion of 0.20% supplied the following per kg of complete feed: 251 mg Zn as ZnO, 252 mg Mn as MnO, 148 mg Fe as FeSO₄·H₂O, 30 mg Cu as CuSO₄, 2.85 mg I as Ca(IO₃)₂, and 0.88 mg Se as Na₂SeO₃.

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