PROTECTED VITAMIN AND MINERAL PREMIXES MAINTAIN PERFORMANCE OF COMMERCIAL PIGS AT REDUCED INCLUSION RATES

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Executive Summary

Pigs have a dietary requirement for certain inorganic elements and vitamins. A wide range of minerals are required by the pig and the functions of these inorganic elements are extremely diverse, ranging from structural functions in tissues to a wide variety of regulatory functions. Meeting the mineral requirements of the pig will be influenced by the bioavailabilities of minerals in feed ingredients, with supplementation required if dietary levels are deemed too low or bioavailability is low.

In practice, the deficiencies of vitamins and minerals within the diet are overcome using specifically formulated vitamin and mineral premixes, utilising high bioavailable vitamins and minerals to reduce the associated cost of digestion. These premixes are generally mixed within a carrier such as Prebase™, millrun or limestone to allow for an inclusion of a standard mass within the larger diet volume. An alternative approach is the encapsulation of important ingredients to avoid degradation prior to their arrival at the site of absorption.

This research project involved two experiments, one in weaner pigs and one in finisher pigs, with identical experimental designs. The experiments were a 2 x 2 factorial design with the first factor being the level of standard commercial loose-carrier premix, 100% (2.0 kg/t) or 70% (1.4 kg/t) of normal commercial inclusion rates, and the second factor being the inclusion of an encapsulated vitamin and mineral supplement at 0 or 0.6 kg/t.

The project sought to conserve the use of vitamins and minerals through their encapsulation in a protection matrix, such that they will avoid degradation prior to reaching sites of absorption within the small intestine, with the aim to reduce the inclusion rate of vitamins and minerals in the diet. Findings from this study showed no significant differences in performance in both weaners and finishers when the standard vitamin and mineral premix was reduced to 70% of normal inclusion rates, although there was a tendency for poorer production during some periods measured. Including the encapsulated premix maintained, but did not enhance, finisher pig performance when fed at 70% of the normal inclusion rate. However, when an encapsulated vitamin and mineral supplement was offered to weaners on top of the standard commercial rate of a loose-carrier vitamin and mineral premix, there was a 4 to 5% (0.6-0.8 kg) improvement in bodyweight at 28 days after weaning.

Evidence from this study suggests that this improvement may be achieved through an anti-inflammatory response within the gastrointestinal tract, which is supported by work in broilers. A significant reduction in the level of calprotectin was seen in weaners receiving the encapsulated premix on top of the standard level of a loose-carrier vitamin and mineral premix. In finishers, feeding 100% of the commercial level of vitamins and minerals tended to reduce the calprotectin concentration, whilst the inclusion of the encapsulated premix resulted in a statistically significant reduction in calprotectin. Reducing the inclusion rate of the standard premix tended to increase the level of inflammation in both studies, as indicated by calprotectin concentrations. The lack of any performance gains in finishers, despite a reduction of inflammation using the encapsulated vitamin and mineral supplement, suggests a greater degree of robustness and ability to cope with some level of inflammation.

This study suggests that the use of a supplementary level of an encapsulated vitamin and mineral premix to protect degradation and chelation of “free” vitamins and minerals appears warranted, as a result of reduced inflammation in the weaner pig and improved post-weaning performance.

Table of Contents

1. Introduction

Pigs have a dietary requirement for certain inorganic elements and vitamins. A wide range of minerals are required by the pig from relatively large quantities of calcium and phosphorus through to trace elements such as molybdenum and vanadium (NRC, 1998). The functions of these inorganic elements are extremely diverse, ranging from structural functions in tissues to a wide variety of regulatory functions. Meeting the mineral requirements of the pig will be influenced by the bioavailabilities of minerals in feed ingredients, with supplementation required if dietary levels are deemed too low or bioavailability is low.

Vitamins are organic compounds distinct from amino acids, carbohydrates, and lipids that are required in minute amounts for normal growth and reproduction (NRC, 1998). Some vitamins are not required in the diet because they can be synthesized readily from other feed or metabolic constituents, or by microorganisms in the intestinal tract, and they play an important role in nutrient metabolism. In feed ingredients, vitamins exist primarily as precursor compounds or coenzymes that may be bound or complexed in some manner, thus converting these to a usable and absorbable form requires some level of digestion.

In practice, the deficiencies of vitamins and minerals within the diet are overcome using specifically formulated vitamin and mineral premixes, using highly bioavailable vitamins and minerals to reduce the associated cost of digestion. These premixes are generally mixed within a carrier such as Prebase™, millrun or limestone to allow for an inclusion of a standard mass within the larger diet volume. These vitamins and minerals are then subject to the normal conditions of milling, which may include heating and/or pressure within the pelleting process (Gadient, 1986; Reddy and Love, 1999), storage in sometimes less than ideal conditions (Shurson *et al.*, 1996), and digestion within the gastrointestinal tract.

Encapsulation of important ingredients to avoid degradation prior to their arrival at the site of absorption is widely practiced within the livestock industries (Jyothi Sri *et al.*, 2012), and can consist of a product within a gel, oil, or fat, i.e., an outer protective layer that avoids degradation or digestion. A particular form of encapsulation of vitamins and minerals was used in this project, which embeds the product of interest within a matrix of triglycerides, shielding ingredients from degradation and allowing for progressive release within the gastrointestinal tract. This process should result in more of the active ingredients being available at the site(s) of digestion allowing for the inclusion of lower levels of the ingredients in the original formulations.

This project aimed to investigate the ability to utilise such an encapsulation technology to reduce the levels of included vitamins and minerals, on the presumption there would be reduced degradation prior to reaching absorption sites within the small intestine, thereby improving the cost effectiveness of production. A secondary aim was to evaluate the ability of an encapsulated vitamin and mineral premix to alter indices of inflammation, given the strong roles vitamins and minerals can play in the immune response of pigs.

2. Methodology

Animal use within this project was approved by the CHM Alliance Pty Ltd Animal Ethics Committee (CHM PP 133/20).

This research project involved two experiments, one in weaner pigs and one in finisher pigs, with identical experimental designs. The experiments were a 2 x 2 factorial design with the first factor being the level of standard commercial loose-carrier premix, 100% or 70% of normal commercial inclusion rates, and the second factor being the inclusion of an encapsulated vitamin and mineral supplement at 0 or 0.6 kg/t (the minimum inclusion rate suggested by the supplier).

Weaner experiment
Five-hundred and sixty male and female weaner pigs (~20 days of age, 6.42 ± 0.05 kg, 50% male, 50% female) entered the experiment over a four-week period. The pigs were sorted by sex, visually sorted by size and assigned to pens (n=14) within a commercial research facility. Pigs within each pen were weighed and allocated to one of four treatments using a randomised block design, resulting in 10 replicates per treatment, with the pen as the replicate.

A common first stage weaner diet (14.85 MJ digestible energy (DE) per kg, 0.89 g standard ileal digestible Lysine per MJ DE, Appendix 1) was fed to all treatments, with diets only differing in the volume of standard commercial premix and the addition of an encapsulated vitamin and mineral supplement (PorciPro (PP), Jefo Nutrition Inc., Saint-Hyacinthe, Québec, Canada, Appendix 3).

The four treatments were:

- A positive control treatment (100%) containing 2.0 kg/t of a standard commercial loose-carrier vitamin and mineral premix.
- A treatment based on the positive control (100% + PP), which included 2.0 kg/t of the standard commercial loose-carrier vitamin and mineral premix and 0.6 kg/t of the encapsulated vitamin and mineral supplement.
- A negative control treatment (70%) which included 1.4 kg/t of the standard commercial loose-carrier vitamin and mineral premix.
- A treatment based on the negative control (70% + PP), which included 1.4 kg/t of the standard commercial loose-carrier vitamin and mineral premix and 0.6 kg/t of the encapsulated vitamin and mineral supplement.

Feed was offered to the pigs via a multispace, round, transition pan feeder (TIGSA Transit plus, PGSaludables, Barcelona, Spain) on an *ad libitum* basis, with the mass of feed delivered recorded, as well as the feed refusal at the end of the week to correspond with weigh events. Water was also available *ad libitum* and delivered through the combination of one nipple and one bowl drinker per pen and was also monitored weekly.

On day 28 of the experiment, one animal per pen was identified and a blood sample was collected via jugular venepuncture into SST tubes, centrifuged, and serum was suctioned off into multiple aliquots and frozen. Blood samples were shipped to Murdoch University for analysis. Upon reviewing the growth performance results and comparing it with other studies

in this area, blood samples were analysed for markers of inflammation and antioxidant capacity with the circulating levels of Haptoglobin, Calprotectin, C-Reactive Protein and Total Antioxidant Capacity measured (Table 1).

Table 1. Methodology and kits for the measurement of markers of inflammation and antioxidant capacity.

	Description
Haptoglobin	AHL – AU480 (DPIRD – AHL) Haptoglobin: In-House Method NTM-62 based on Comparative Haematology International (1991) 9:117-124, An automated Biochemical Assay for Haptoglobin: Prevention of Interference from Albumin, P.D Eckersall et al. (and patent # W01999024833 A1).
Calprotectin	MBS033848; CP ELISA kit; Porcine Calprotectin ELISA Kit (Resolving Images).
C-Reactive Protein	Cat no. RDSDY2648, R&D Systems Porcine C-Reactive Protein/CRP DuoSet ELISA, 15 Plate, Per Set (Invitro Technologies). Cat. No. RDSDY999, R&D Systems Substrate Reagent Pack (8 vials Color A, 8 vials Color B), Per Kit (Invitro Technologies).
Total Antioxidant Capacity	STA-360 OxiSelect™ Total Antioxidant Capacity (TAC) Assay Kit (Cell Biolabs).

Statistical analysis of growth performance and sample analysis was performed utilizing a general analysis of variance in GenStat 21st edition (VSN International Ltd, Hemel Hempstead, HP2 4TP, UK), with an unbalanced treatment structure utilized for sample analysis where needed. Differences in removals were determined by Chi-square analysis, also in GenStat 21st edition. Statistically significant differences were determined at $P < 0.05$.

Finisher experiment

Two-hundred and sixty-four male and female pigs (~15 weeks of age, 55.6 ± 0.35 kg, 50% male, 50% female) entered the experiment over a two-week period. The pigs were sorted by sex and assigned to pens (n=11) within a commercial research facility. Pigs within each pen were weighed and allocated to one of four treatments using a randomised block design, resulting in 10 replicates per treatment, with the pen as the replicate.

A common finisher diet (13.50 MJ digestible energy (DE) per kg, 0.64 g standard ileal digestible Lysine per MJ DE, Appendix 2) was fed to all treatments, with diets only differing in the volume of standard commercial premix and the addition of an encapsulated vitamin and mineral supplement (PorciPro (PP), Jefo Nutrition Inc., Saint-Hyacinthe, Québec, Canada, Appendix 3).

The four treatments were:

- A positive control treatment (100%) containing 2.0 kg/t of a standard commercial loose-carrier vitamin and mineral premix.
- A treatment based on the positive control (100% + PP), which included 2.0 kg/t of the standard commercial loose-carrier vitamin and mineral premix and 0.6 kg/t of the encapsulated vitamin and mineral supplement.
- A negative control treatment (70%) which included 1.4 kg/t of the standard commercial loose-carrier vitamin and mineral premix.
- A treatment based on the negative control (70% + PP), which included 1.4 kg/t of the standard commercial loose-carrier vitamin and mineral premix and 0.6 kg/t of the encapsulated vitamin and mineral supplement.

Feed was offered to the pigs via a standard “penguin” type feeder on an *ad libitum* basis, with the mass of feed delivered recorded via a FeedLogic smart feeding system, with feed

remaining in the feeder at day 21 and day 42 estimated to account for feed refusal. Water was also available *ad libitum* and delivered through three nipple drinkers per pen.

Growth performance was measured over the 42-day period, with the first cut of pigs from each pen going to slaughter at this time (~20 weeks of age). Pigs were marketed on a set weight, rather than a set time basis, and they remained on their respective treatment diets until they reached market weight. Carcase weight (hot-standard carcase weight, HSCW) and backfat depth at the P2 site were recorded for pigs at slaughter, however an error in recording resulting in full data only being available for the third (and final) cut.

On day 41 of the experiment, one animal per pen was identified and a blood sample was collected via jugular venepuncture into SST tubes, centrifuged and serum was suctioned off into multiple aliquots and frozen. Blood samples were shipped to Murdoch University for analysis. As for the weaner study, upon reviewing the growth performance results and comparing it with other studies in this area, blood samples were analysed for markers of inflammation and antioxidant capacity with the circulating levels of Haptoglobin, Calprotectin, C-Reactive Protein and Total Antioxidant Capacity measured (Table 1).

Statistical analysis of growth performance, carcase characteristics and sample analysis were performed utilizing a general analysis of variance in GenStat 21st edition (VSN International Ltd, Hemel Hempstead, HP2 4TP, UK), with an unbalanced treatment structure utilized for sample analysis where needed. Data were analysed to determine any interaction between the treatments and sex, with no significant interactions being observed. Differences in removals were determined by Chi-square analysis, also in GenStat 21st edition. Statistically significant differences were determined at $P < 0.05$.

3. Outcomes

Weaner experiment

The 30% reduction in standard vitamin and mineral premix had no significant effect on the performance of weaner pigs at any stage during the experimental period (Table 2), although there was a trend ($P = 0.072$) for slower growth in week 3 in pigs receiving diets with the lower premix amount, accompanied by a corresponding trend ($P = 0.090$) for deteriorated feed conversion. Calprotectin levels tended to be higher ($P = 0.061$) in pigs receiving the reduced level of standard vitamin and mineral premix, suggesting greater inflammation.

The inclusion of an encapsulated premix into weaner diets also had limited effect, although the number of removals in pigs receiving the encapsulated premix was lower ($P = 0.10$). Pigs receiving the encapsulated premix tended to have a higher weight at the end of week 3 ($P = 0.092$) and the end of the experiment ($P = 0.099$), associated with a significant improvement in feed intake and growth rate in week 3 of the experiment.

When looking at the interaction between treatments, those pigs that received the encapsulated premix in addition to 100% of the standard premix had a significantly increased growth rate across the whole experiment period compared with all other treatments, resulting in this treatment having a 0.6 to 0.8 kg heavier exit weight ($P = 0.044$). These results reflect an increased growth rate during week four of the experiment ($P = 0.019$).

This improvement in growth is supported by a significant reduction in the level of calprotectin measured in pigs receiving the encapsulated premix on top of the standard level of the loose-carrier vitamin and mineral premix (Table 2).

Finisher experiment
The 30% reduction in standard loose-carrier vitamin and mineral premix had no effect on the performance of finisher pigs at any stage during the experimental period (Table 3). Pigs receiving 100% of the standard premix tended ($P = 0.093$) to have a higher feed intake in the first stage with a concurrent increase in growth rate. The 30% reduction in the standard vitamin and mineral premix, as in the weaner experiment, tended to result in an increased level of calprotectin ($P = 0.095$).

The encapsulated premix also had no significant main effects on growth performance. There was an increase ($P = 0.011$) in the P2 fat depth of slaughter pigs in the third cut of pigs that received the encapsulated premix, but when looking at all the slaughter pigs, this effect was no longer observed ($P > 0.05$).

Feeding 100% of the commercial level of vitamins and minerals showed a trend ($P = 0.095$) for a reduced calprotectin concentration, whilst the inclusion of the encapsulated premix resulted in a significant reduction in calprotectin. There were no interactions between the levels of standard premix and the inclusion of the encapsulated premix. There was a tendency ($P = 0.063$) for all treatments to have a lower concentration of circulating C-reactive protein compared to the “control” (100% commercial level of vitamins and minerals) treatment (Table 3).

Table 2. The growth performance and blood analysis of weaner pigs fed diets containing 100% or 70% of a standard premix, with (+PP) or without (-) the addition of 0.6 kg/t of an encapsulated premix.

	Standard premix				Encapsulated premix				Interaction					
	100%	70%	SED	*P* value	-	+PP	SED	*P* value	100%	100% +PP	70%	70% +PP	SED	*P* value
Weight, kg														
d 0	6.4	6.4	0.04	0.34	6.4	6.4	0.04	0.77	6.4	6.4	6.4	6.4	0.06	0.84
d 7	7.0	7.0	0.05	0.97	7.0	7.0	0.05	0.47	6.9	7.1	7.0	7.0	0.07	0.17
d 14	9.1	9.1	0.09	0.93	9.1	9.2	0.09	0.36	9.0	9.2	9.1	9.1	0.13	0.23
d 21	12.0	11.9	0.15	0.31	11.8	12.1	0.15	0.092	11.8	12.3	11.9	11.9	0.21	0.16
d 28	15.9	15.6	0.21	0.19	15.6	15.9	0.21	0.099	15.5[a]	16.3[b]	15.7[a]	15.6[a]	0.30	0.044
Average daily gain, kg/d														
d 0-7	0.085	0.079	0.006	0.35	0.080	0.084	0.006	0.55	0.079	0.091	0.082	0.076	0.009	0.15
d 8-14	0.305	0.304	0.009	0.92	0.302	0.308	0.009	0.47	0.299	0.312	0.304	0.305	0.013	0.55
d 15-21	0.413	0.392	0.011	0.072	0.391[a]	0.415[b]	0.011	0.033	0.393	0.432	0.388	0.397	0.015	0.19
d 22-28	0.555	0.535	0.014	0.16	0.537	0.553	0.014	0.26	0.530[a]	0.580[b]	0.544[ab]	0.526[a]	0.020	0.019
Average daily feed intake, kg/d														
d 0-7	0.14	0.14	0.005	0.92	0.14	0.14	0.005	0.61	0.13[ab]	0.14[ab]	0.14[b]	0.13[a]	0.008	0.040
d 8-14	0.35	0.34	0.009	0.50	0.34	0.35	0.009	0.24	0.33	0.36	0.34	0.34	0.012	0.095
d 15-21	0.52	0.51	0.012	0.37	0.50[a]	0.53[b]	0.012	0.047	0.50	0.53	0.50	0.52	0.018	0.72
d 22-28	0.73	0.72	0.014	0.46	0.71	0.73	0.014	0.11	0.70	0.75	0.72	0.71	0.20	0.059
Feed conversion ratio, kg/kg														
d 0-7	1.71	1.84	0.102	0.18	1.82	1.73	0.102	0.34	1.77	1.64	1.88	1.81	0.145	0.81
d 8-14	1.15	1.12	0.028	0.40	1.14	1.13	0.028	0.93	1.13	1.16	1.14	1.11	0.039	0.40
d 15-21	1.26	1.29	0.020	0.090	1.28	1.27	0.020	0.70	1.28	1.24	1.28	1.31	0.029	0.12
d 22-28	1.31	1.35	0.020	0.12	1.33	1.33	0.020	0.73	1.33	1.30	1.32	1.37	0.028	0.077
Total growth performance, d 0-28														
ADG	0.339	0.328	0.007	0.13	0.327	0.340	0.007	0.10	0.325[a]	0.354[b]	0.330[a]	0.326[a]	0.011	0.043
ADFI	0.43	0.42	0.009	0.45	0.42	0.43	0.009	0.10	0.42	0.45	0.42	0.42	0.013	0.10
FCR	1.27	1.30	0.013	0.057	1.29	1.28	0.013	0.77	1.28	1.26	1.29	1.30	0.018	0.23
Markers of inflammation and antioxidant capacity														
Haptoglobin, g/L	0.71	0.62	0.133	0.51	0.71	0.63	0.133	0.52	0.79	0.63	0.63	0.62	0.188	0.59
TAC	295.1	273.6	14.69	0.15	295.2	273.5	14.69	0.15	308.1	282.2	282.3	264.8	20.78	0.78
CRP, mg/L	10.4	12.1	3.11	0.60	10.6	11.9	3.11	0.70	8.2	12.6	13.1	11.1	4.39	0.32
Calprotectin, ng/mL	10.9	13.7	1.45	0.061	12.9	11.7	1.45	0.42	13.0[b]	8.8[a]	12.8[ab]	14.7[b]	2.05	0.041
Removals														
	3	3	$x^2(1)=0.00$, P=1.00		5	1	$x^2(1)=2.70$, P=0.10		3	0	2	1	$x^2(3)=3.37$, P=0.34	

[a,b]Means within a row, within effect, with different superscripts differ significantly (P < 0.05); SED, standard error difference of the means; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; TAC, total antioxidant capacity; CRP, C-reactive protein.

Table 3. The growth performance and blood analysis of finisher pigs fed diets containing 100% or 70% of a standard premix, with (+PP) or without (-) the addition of 0.6 kg/t of an encapsulated premix.

	Standard premix				Encapsulated premix				Interaction					
	100%	70%	SED	*P* value	-	+PP	SED	*P* value	100%	100% +PP	70%	70% +PP	SED	*P* value
Weight, kg														
d 0	55.6	55.5	0.67	0.95	55.6	55.6	0.67	0.96	55.7	55.5	55.5	55.6	0.94	0.82
d 21	75.7	74.8	0.74	0.23	75.1	75.4	0.74	0.74	75.9	75.5	74.3	75.2	1.05	0.42
d 42	100.7	100.3	1.33	0.76	100.9	100.2	1.33	0.61	101.3	100.2	100.4	100.2	1.87	0.75
Average daily gain, kg/d														
d 0-21	0.957	0.916	0.021	0.061	0.931	0.942	0.021	0.63	0.963	0.952	0.900	0.932	0.030	0.31
d 22-28	1.164	1.189	0.066	0.70	1.198	1.154	0.066	0.51	1.182	1.145	1.215	1.163	0.093	0.91
Average daily feed intake, kg/d														
d 0-21	2.33	2.24	0.052	0.093	2.30	2.27	0.052	0.53	2.32	2.33	2.27	2.20	0.074	0.44
d 22-28	2.89	3.07	0.137	0.20	3.00	2.96	0.137	0.76	2.96	2.82	3.05	3.10	0.194	0.52
Feed conversion ratio, kg/kg														
d 0-21	2.44	2.45	0.075	0.87	2.48	2.41	0.075	0.41	2.42	2.46	2.53	2.37	0.107	0.18
d 22-28	2.52	2.62	0.172	0.56	2.54	2.60	0.172	0.70	2.53	2.51	2.54	2.70	0.244	0.58
Total growth performance, d 0-28														
ADG	1.062	1.054	0.039	0.84	1.066	1.049	0.039	0.67	1.074	1.050	1.059	1.049	0.055	0.86
ADFI	2.61	2.66	0.067	0.49	2.66	2.62	0.067	0.58	2.64	2.58	2.67	2.65	0.095	0.71
FCR	2.48	2.54	0.106	0.57	2.50	2.51	0.106	0.94	2.48	2.48	2.53	2.55	0.149	0.91
All slaughter data														
N.					64	65								
HSCW, kg					82.9	82.8	0.94	0.87						
P2, mm					12.1	12.1	0.45	0.95						
Third-cut slaughter data														
N.	26	27			26	27			12	14	14	13		
HSCW, kg	85.4	84.4	1.18	0.43	85.0	84.8	1.18	0.92	85.5	85.3	84.5	84.4	1.67	0.98
P2 fat, mm	12.5	12.6	0.64	0.98	11.7[a]	13.4[b]	0.64	0.011	12.2	12.9	11.3	13.9	0.90	0.13
Markers of inflammation and antioxidant capacity														
Haptoglobin, g/L	0.59	0.62	0.081	0.71	0.57	0.64	0.081	0.41	0.56	0.62	0.59	0.65	0.115	0.99
TAC	306.5	322.5	31.38	0.61	304.9	324.1	31.38	0.54	312.6	300.3	297.1	347.9	44.38	0.32
CRP, mg/L	12.0	9.5	2.46	0.30	12.5	8.9	2.46	0.15	16.2	7.9	8.9	10.0	3.47	0.063
Calprotectin, ng/mL	9.1	11.2	1.25	0.095	11.4[a]	8.9[b]	1.25	0.049	9.9	8.3	13.0	9.5	1.77	0.44

[a,b]Means within a row, within effect, with different superscripts differ significantly ($P < 0.05$); SED, standard error difference of the means; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; HSCW, hot standard carcase weight; TAC, total antioxidant capacity; CRP, C-reactive protein.

4. Application of Research

The weaning process is one of the major stress events in a pig's life that can contribute to intestinal and immune system dysfunction (Campbell *et al.*, 2013), resulting in a poor transition to solid feed and a check in growth. The consequences associated with weaning also induce a deleterious effect on intestinal barrier function (Boudry *et al.*, 2004; Moeser *et al.,* 2007). This study showed that the use of an encapsulated vitamin and mineral supplement, in addition to a standard inclusion rate of a normal commercial vitamin/mineral premix, reduced the level of inflammation, as evidenced by a reduction in circulating calprotectin levels. In turn, this may have contributed to improved growth performance and the heavier exit weight.

Calprotectin is a biomarker that assesses inflammation in the gut, with elevated levels of calprotectin indicating the migration of neutrophils to the intestinal mucosa which occurs during intestinal inflammation (Costa *et al.*, 2003). The application of an encapsulated vitamin and mineral supplement to a standard inclusion rate of commercial vitamin/mineral premix resulted in reduced levels of calprotectin in weaner pigs, suggesting a protective effect from its use. In grower-finisher pigs, the main effect of the encapsulated vitamin/mineral premix, in the absence of any growth-promoting effects, indicates a similar immune modulating influence was likely in action. In both weaner and grower pigs a trend of reduced calprotectin levels was observed when the standard vitamin and mineral premix was reduced, suggesting broad reductions in vitamin and mineral premix levels leave animals potentially vulnerable to increased inflammation.

Data from the current study indicate that the observed production and immune effects support previous work in broiler chickens with this same encapsulated vitamin and mineral supplement. Bortoluzzi *et al.* (2021) reported a 4% increase in body weight across the first 35 days of life, with increased expression of immune-related genes and elevations in glutathione reductase activity, indicating this encapsulated vitamin and mineral supplement played a role in modulating the immune and antioxidant defense system of the birds.

5. Conclusion

This project sought to conserve the use of vitamins and minerals through their encapsulation in a protection matrix, such that they will avoid degradation prior to reaching sites of absorption within the small intestine, with the aim to reduce the inclusion rate of vitamins and minerals in the diet improving the cost effectiveness of pig production. The findings from this study showed that there was no significant difference in growth performance in both weaners and finishers when the standard vitamin and mineral premix was reduced to 70% of normal inclusion rates, although there was a tendency for slower growth during some periods measured. The inclusion of the encapsulated premix maintained, but did not enhance, the performance of pigs fed 70% of the normal inclusion rate. Reducing the inclusion rate of the standard premix tended to increase the level of inflammation in both studies, as indicated by calprotectin concentration, with the addition of the encapsulated premix appearing to have some positive influence in the finisher study.

However, when an encapsulated vitamin and mineral supplement was offered on top of the standard commercial rate of a loose-carrier vitamin and mineral premix, improved performance was observed, with pigs having a 4 to 5% improvement in weight at 28 days

after weaning. Evidence from this study suggests that this improvement may have been achieved through an anti-inflammatory response within the gastrointestinal tract, which is supported by work in young broilers. Whilst the reduction of inflammation using the encapsulated vitamin and mineral supplement was also observed in finisher pigs, the lack of any performance gain suggests a greater degree of robustness of the finisher pig and its ability to cope with some level of inflammation.

6. Limitations/Risks

The research was conducted within a commercial research facility with exceptional management, a strict adherence to space allowances and a generally high level of health. Whilst we are confident that the results seen here are true, results may differ with differing management, health status or nutritional approaches.

This study was conducted with commercial premixes that are custom-mixed for the SunPork Group, hence results may vary based on specific inclusions within premixes.

7. Recommendations

As a result of the outcomes in this study the following recommendations have been made:

- The use of a supplementary level of an encapsulated vitamin and mineral premix to protect degradation and chelation of "free" vitamins and minerals appears warranted, resulting in reduced inflammation in the weaner pig and improved post-weaning performance.
- The blanket reduction of the quantity of standard vitamin and mineral premix is not recommended. Whilst it did not result in any deleterious growth performance in either weaner or finisher pigs, there was a tendency for the inflammatory marker calprotectin to be increased in the reduced level of premix.
- If a reduction in vitamin and mineral premix is undertaken, extra care should be taken to ensure that those nutrients possessing anti-inflammatory and/or antioxidant characteristics are consumed at adequate levels.

8. References

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Appendices

Appendix 1: Weaner experiment diets

	Diet W1	Diet W2	Diet W3	Diet W4
Vitamin/mineral premix	100%	100%	70%	70%
PorciPro	-	30%	-	30%
Ingredients (%)				
Wheat	51.98	51.92	52.04	51.98
Biscuit meal	5.00	5.00	5.00	5.00
Soybean meal	7.75	7.75	7.75	7.75
Soy protein concentrate	3.35	3.35	3.35	3.35
Full-fat soybean	2.25	2.25	2.25	2.25
Blood meal	2.00	2.00	2.00	2.00
Meat meal	3.35	3.35	3.35	3.35
Fish meal	5.00	5.00	5.00	5.00
Whey powder	12.50	12.50	12.50	12.50
Hilyses	2.00	2.00	2.00	2.00
Canola oil	3.00	3.00	3.00	3.00
Salt	0.200	0.200	0.200	0.200
Zinc oxide	0.100	0.100	0.100	0.100
Betaine	0.100	0.100	0.100	0.100
DL-Methionine	0.155	0.155	0.155	0.155
Lysine HCl	0.400	0.400	0.400	0.400
L-Threonine	0.105	0.105	0.105	0.105
L-Tryptophan	0.035	0.035	0.035	0.035
L-Isoleucine	0.015	0.015	0.015	0.015
Xylanase	0.050	0.050	0.050	0.050
Phytase	0.0075	0.0075	0.0075	0.0075
Phytomolecule blend	0.020	0.020	0.020	0.020
Acidifier	0.400	0.400	0.400	0.400
Sweetener	0.030	0.030	0.030	0.030
Vitamin/mineral premix	0.200	0.200	0.140	0.140
PorciPro	-	0.060	-	0.060
Analysis				
Energy, MJ DE/kg	14.85	14.85	14.85	14.85
SID Lysine, g/MJ DE	0.89	0.89	0.89	0.89
Protein (%)	22.6	22.6	22.6	22.6

Appendix 2: Finisher experiment diets

	Diet F1	Diet F2	Diet F3	Diet F4
Vitamin/mineral premix	100%	100%	70%	70%
PorciPro	-	30%	-	30%
Ingredients (%)				
Barley	47.35	47.29	47.41	47.35
Wheat	38.75	38.75	38.75	38.75
Soybean meal	7.05	7.05	7.05	7.05
Blood meal	0.75	0.75	0.75	0.75
Meat meal	3.15	3.15	3.15	3.15
Vegetable oil	1.00	1.00	1.00	1.00
Limestone	0.700	0.700	0.700	0.700
Salt	0.250	0.250	0.250	0.250
DL-Methionine	0.090	0.090	0.090	0.090
Lysine HCl	0.420	0.420	0.420	0.420
L-Threonine	0.120	0.120	0.120	0.120
L-Tryptophan	0.010	0.010	0.010	0.010
Xylanase	0.050	0.050	0.050	0.050
Phytase	0.0075	0.0075	0.0075	0.0075
Deodorase	0.100	0.100	0.100	0.100
Vitamin/mineral premix	0.200	0.200	0.140	0.140
PorciPro	-	0.060	-	0.060
Analysis				
Energy, MJ DE/kg	13.50	13.50	13.50	13.50
SID Lysine, g/MJ DE	0.64	0.64	0.64	0.64
Protein (%)	15.9	15.9	15.9	15.9

Appendix 3: PorciPRO Product Technical Sheet

PorciPRO™

Vitamin and Mineral supplement for swine

Product description

PorciPRO™ is a Vitamin and Mineral supplement formulated and desingned exclusively for swine feeds.

Ingredients

Hydrogenated palm glycerides, Manganous oxide, Vitamin E supplement, Iron sulfate, Zinc oxide, Fumaric acid, Niacin supplement, Tryptophan, Cooper Sulfate, Sorbic acid, Malic acid, Citric acid, Dried *Bacillus subtilis* fermentation extract, Calcium Pantothenate, Riboflavin, Biotin, Vitamin A supplement, Menadione Nicotinamide Bisulfite, Vitamin D_3 supplement, Dried extracted *Streptomyces* fermentation solubles, Calcium Iodate, Pyridoxine Hydrochloride, Thyme extract, Thiamine Mononitrate, Folic acid, Vitamin B12 supplement, Vanillin, Sodium Selenite, Eugenol, Dried *Aspergillus niger* fermentation product, Dried *Aspergillus oryzae* fermentation product, Calcium Stearate, Silicon Dioxide, Calcium Carbonate.

Physical properties

Appearance: Granules
Color: Brown to gray

Guaranteed analysis

Vitamin E (min.)	40 000 UI / kg
Manganese (actual)	48 000 mg / kg
Zinc (actual)	44 000 mg / kg
Iron (actual)	22 000 mg / kg
Cooper (actual)	4 000 mg / kg
Iodine (actual)	600 mg / kg
Selenium (actual)	120 mg / kg

Directions for use

Swine feeds: 600 gr - 2500 gr / MT

Storage and shelf life

Store **PorciPRO™** in a cool and dry environment away from the sunlight and other sources of heat.
Shelf life: 36 months from manufacuring date if stored in its original packaging.

Packaging

Available in 25 kg multilayer paper bags with a sealed plastic film.

TECHNICAL SHEET
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VER. 20200921

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Appendix 4: APSA XVIII Extended Abstract

36. Supplementation of a protected complex of biofactors and antioxidants improves performance of weaner pigs

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Introduction: The weaning process is one of the major stress events in a pig's life that can contribute to intestinal and immune system dysfunction (Campbell et al., 2013), resulting in a poor transition to solid food and check in growth. Supplementation with specific vitamins, antioxidants and other compounds that regulate biological functions (biofactors) has been shown to improve performance in early life broilers (Bortoluzzi et al., 2021) through anti-inflammatory and improved immune response and appears to be one method by which we can ease this transition in the pig. We hypothesised that the supplementation of a protected complex of biofactors and antioxidants, P(BF + AOx), would improve the performance of weaner pigs, with a secondary hypothesis that the protection of this complex would allow the reduction in vitamin and mineral premix inclusion levels.

Material and methods: Five hundred and sixty male and female pigs (20 days of age, 6.42 ± 0.05 kg, 50:50 male:female) entered the experiment over 4 weeks, were sorted by sex and size and assigned to pens (n = 14). Pigs within each pen were weighed and allocated to one of four treatments using a randomised block design, resulting in 10 replicates per treatment, with pen as the replicate. A first stage weaner diet (14.9 MJ digestible energy (DE)/kg, 0.89 g standardised ileal digestible lysine/MJ DE) was fed to all treatments, with diets only differing in the volume of commercial vitamin and mineral premix and the addition of P(BF + AOx) (Jefo Nutrition Inc, Saint-Hyacinthe, Québec, Canada). The *Control* treatment contained a standard inclusion rate of vitamin and mineral premix (VMP, 2 kg/t) and the *Control + P(BF + AOx)* treatment had the addition of 0.6 kg/t of P(BF + AOx) to the control diet. The *RedVMP* treatment reduced the commercial VMP to 70% of normal levels (1.4 kg/t), with the *RedVMP + P(BF + AOx)* treatment having the reduced VMP inclusion rate (1.4 kg/t) plus 0.6 kg/t of P(BF + AOx). Performance data were analysed by ANOVA with treatment as a fixed factor, entry week as blocking factor and entry weight as a covariate (Genstat, 20th ed. VSN International, Hemel Hempstead, UK), with pairwise differences between treatments determined by LSD ($P < 0.05$).

Results: Pigs receiving the P(BF + AOx) treatment on top of the standard commercial premix (*Control + P(BF + AOx)* grew faster ($P < 0.05$) across the whole experimental period, resulting in a higher weight 28 days after weaning (0.9 kg, $P = 0.021$; Table 1). Reducing the VMP by 30% (*RedVMP*) did not impact growth performance when compared to the *Control*, with the P(BF + AOx) complex showing no effect at this reduced level. The improved growth rate of the *Control + P(BF + AOx)* treatment would appear to be a combination of both improved efficiency and improved intake with both parameters nearing significance at the $P < 0.10$ level.

Conclusion and implications: These results support our primary hypothesis with supplementation of P(BF + AOx) resulting in improved performance of weaner pigs; however, our secondary hypothesis was not proven, with no differences observed when VMP level was reduced. The use of such a complex would appear to support the transition of the weaned pig.

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Table 1
Growth performance (ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio) of weaner pigs fed diets differing in the inclusion rate of vitamin and mineral premix, *Control* 2 kg/t, *RedVMP* 1.4 kg/t, with or without the addition of a protected complex of biofactors and antioxidants, *+P(BF + AOx)*, 0.6 kg/t.

	Control	Control + P(BF + AOx)	RedVMP	RedVMP + P(BF + AOx)	SED	*P* value
Entry weight, d 0 (kg)	6.39	6.42	6.43	6.44	0.064	0.69
Exit weight, d 28 (kg)	15.5[a]	16.4[b]	15.6[a]	15.6[a]	0.28	0.021
ADG, d 0-28 (kg/d)	0.325[a]	0.355[b]	0.329[a]	0.327[a]	0.010	0.021
ADFI, d 0-28 (kg/d)	0.414	0.446	0.423	0.425	0.012	0.102
FCR, d 0-28 (kg/d)	1.28	1.26	1.29	1.30	0.016	0.094

[a,b]Means within a row with different superscripts differ significantly ($P < 0.05$).