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Effects of Oregano Essential Oil and Zinc Oxide on Nursery Pig Growth Performance, Apparent Crude Protein and Dry Matter Digestibility, and Fecal Dry Matter

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Summary

A total of 375 weanling pigs (DNA 600 × 241; initially 12.8 ± 0.03 lb) were used in a 42-d study to evaluate the effects of an oregano essential oil in water or feed on growth performance, digestibility of crude protein (CP) and dry matter (DM), and fecal dry matter in nursery pigs. Pens of pigs were randomly allotted to one of five dietary treatments in a generalized randomized block design with five pigs per pen and 15 pens per treatment (five replications per BW category). Diets were corn-soybean meal-based and arranged in a 2 × 2 + 1 factorial. Main effects were no added or added pharmacological levels of Zn (ZnO added to achieve Zn levels of 3,000 and 2,000 ppm in phases 1 and 2, respectively) and no added Orego-Stim Plus Liquid 2X (OSPL; Anpario, Worksop, United Kingdom) or added Orego-Stim Plus Liquid 2X provided through the drinking water system (delivered at a final dilution of approximately 1:8,000). The additional treatment included 0.03% of Orego-Stim Plus Powder 2X (OSPP; Anpario, Worksop, United Kingdom) in the feed without pharmacological Zn. Thus, the five treatments consisted of 1) positive control (PC) with pharmacological levels of Zn; 2) negative control (NC) without pharmacological levels of Zn; 3) PC + OSPL; 4) NC + OSPL; and 5) NC + OSPP. Treatment diets were fed in three phases from d 0 to 10 (phase 1), d 10 to 24 (phase 2), and d 24 to 42 (phase 3). On d 0 and 42, a serum sample was collected from the same gilt per pen for *Mycoplasma hyopneumoniae* S/P ratios. On d 10 and 21, fecal samples were collected from the same three randomly selected pigs in each pen to determine fecal DM. Additionally on d 21, fecal samples were used to determine the apparent total tract digestibility (ATTD) of DM and CP. During phase 1 (d 0 to 10), pigs fed diets containing pharmacological levels of Zn from ZnO had increased ADG ($P = 0.046$) and tended to have increased ADFI and d 10 BW ($P \leq 0.080$) compared with pigs fed diets without ZnO. During phase 2 (d 10 to 24), a similar response was observed where pigs fed diets containing pharmacological levels of Zn from ZnO had increased ADG,

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ADFI, and d 24 BW ($P \leq 0.001$) compared with pigs fed diets without ZnO. Additionally, pigs fed the PC diet had increased ADG, ADFI, and BW ($P < 0.05$) compared with pigs fed NC + OSPP. There were no differences in phases 1 or 2 when OSPL was added in the water. In phase 3 (d 24 to 42), pigs previously fed ZnO had reduced ADG and worsened F/G ($P \leq 0.041$) compared with pigs not previously fed pharmacological levels of Zn. Furthermore, pigs previously fed the PC tended to have decreased ($P < 0.10$) ADG compared with pigs fed the NC + OSPP. Overall (d 0 to 42), feeding pharmacological levels of Zn in phases 1 and 2 did not result in overall performance differences. Additionally, no significant differences were observed for OSPL. For fecal DM, a tendency for an NC + OSPL versus NC + OSPP × day interaction was observed ($P = 0.068$), where pigs fed NC + OSPP had greater fecal DM on d 10 ($P = 0.024$), with no difference observed on d 21. Zinc oxide increased ATTD of DM and CP ($P < 0.001$), whereas pigs receiving OSPL had lower CP digestibility ($P = 0.015$) and tended to have lower DM digestibility ($P = 0.090$) than those without OSPL. Additionally, pigs fed NC + OSPP had increased ($P < 0.001$) ATTD of DM and CP compared with pigs receiving the NC and NC + OSPL. No significant treatment differences were observed for *M. hyopneumoniae* S/P ratios. In conclusion, pharmacological ZnO improved early nursery growth performance and digestibility, but OSPL resulted in no benefits. Additionally, NC + OSPP resulted in greater fecal DM during the early nursery and improved digestibility compared with NC + OSPL. Further research is warranted under commercial settings.

Introduction

Weaning is a stressful event, often resulting in reduced voluntary feed intake, impaired gut health, and post-weaning diarrhea. To manage these challenges, pharmacological levels of zinc from zinc oxide (ZnO) have been included in nursery diets due to its ability to reduce diarrhea and improve performance. However, the inclusion of pharmacological ZnO has been restricted or banned in several countries, including the European Union, because of environmental concerns related to zinc accumulation and potential links to antimicrobial resistance. As a result, alternatives to ZnO are being researched, with phytogenic additives such as essential oils gaining interest. Essential oils are plant-derived compounds with reported antimicrobial, antioxidant, and immunomodulatory properties. By reducing pathogenic stress in the gastrointestinal tract, mitigating oxidative damage, and supporting immune function, they may offer benefits during the post-weaning period.

Orego-Stim is an oregano essential oil product that can be incorporated into feed or delivered through water lines. Studies on the effects of oregano essential oils on growth performance show variable results, and differences in oregano sources, manufacturing practices, and final delivery concentrations may explain these inconsistencies. The objective of this study was to evaluate the effects of Orego-Stim provided through water or feed on growth performance, digestibility of nitrogen and dry matter (DM), and fecal dry matter in nursery pigs.

Procedures

The protocol used in this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee. This study was conducted at the Kansas State University Swine Teaching and Research nursery facility in Manhattan, KS. Each

pen (5 × 5 ft) contained a 4-hole, dry self-feeder and a nipple waterer to provide *ad libitum* access to feed and water.

Animals and diets

A total of 375 weanling pigs (DNA 600 × 241; initially 12.8 ± 0.03 lb) were used in a 42-d study. Pigs were weaned at approximately 18 d of age and divided into three BW categories of light (initially 9.8 ± 0.02 lb), medium (initially 12.7 ± 0.02 lb), and heavy (initially 15.8 ± 0.02 lb) weight pens, with equal distribution of gilts and barrows across treatments. Pens of mixed-sex pigs were then randomly allotted to one of five dietary treatments in a generalized randomized block design with five pigs per pen and 15 pens per treatment (five replications per BW category).

Diets were corn-soybean meal-based and arranged in a 2 × 2 + 1 factorial. Main effects were no added or added pharmacological levels of Zn (ZnO added to achieve Zn levels of 3,000 and 2,000 ppm in phases 1 and 2, respectively) and no added Orego-Stim Plus Liquid 2X (OSPL; Anpario, Worksop, United Kingdom) or added Orego-Stim Plus Liquid 2X provided through the drinking water system (delivered at a final dilution of approximately 1:8,000). The additional treatment included 0.03% of Orego-Stim Plus Powder 2X (OSPP; Anpario, Worksop, United Kingdom) in the feed without pharmacological Zn. Thus, the five treatments consisted of 1) positive control (PC) with pharmacological levels of Zn; 2) negative control (NC) without pharmacological levels of Zn; 3) PC + OSPL; 4) NC + OSPL; and 5) NC + OSPP. Diets were analyzed for proximate and mineral analyses in duplicate at Midwest Labs (Omaha, NE). Treatment diets were fed in three phases with phase 1 fed from d 0 to 10 (Table 1), phase 2 from d 10 to 24 (Table 2), and phase 3 from d 24 to 42. All diets contained 110 ppm of Zn from zinc sulfate provided by the trace mineral premix. Treatment diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS, and fed in pellet form in phase 1 and meal form in phases 2 and 3. Titanium dioxide was included at 0.5% in phase 2 treatment diets as an indigestible marker to determine ATTD of DM and CP.

Measurements and sampling

Pig weights and feed disappearance were measured on d 0, 10, 17, 24, 35, and 42 to determine ADG, ADFI, and F/G. On d 0 (weaning) and d 42, the same gilt per pen was bled and serum separated for the determination of *M. hyopneumoniae* S/P ratios. Pigs were vaccinated with Circumvent PCV-M G2 (Merck Animal Health, Madison, NJ) at 7 d of age (1 mL/pig) and 25 d after weaning (1 mL/pig). Serum samples were analyzed at the Iowa State University Veterinary Diagnostic Laboratory using an *M. hyopneumoniae* ELISA. The sample-to-positive (S/P) ratio represents the optical density of each sample relative to the assay's positive control and is used to estimate the relative antibody response to *M. hyopneumoniae* vaccination or exposure. Fecal samples were collected from the same three randomly selected pigs per pen on d 10 and 21 to determine percentage DM. Samples were dried at 131°F in a forced-air oven for 48 h, and the ratio of dried-to-wet fecal weight determined the fecal dry matter. Fecal samples were analyzed separately for each pig and the average of the three samples from each pen was then used for statistical analysis.

Digestibility analysis

Following fecal DM on d 21, both ground feed and pen-level pooled fecal samples were dried in a 275°F oven for 2 h to determine percentage DM of the samples used for titanium analysis. Samples were analyzed for N using a LECO nitrogen analyzer at the Kansas State University Swine Laboratory and converted to CP by using a 6.25 conversion factor. Titanium dioxide concentration in both dried feed and fecal samples was determined and ATTD of DM and CP was determined using procedures outlined by Leone[3]. The ATTD of DM and CP was calculated using the index method described by Adeola[4] using the following equation:

Statistical analysis

Data were analyzed as a generalized randomized block design. The lmer function was used from the lme4 package in RStudio (Version 4.3.1, R Core Team, Vienna, Austria) with pen serving as the experimental unit. For performance data, the model used treatment and BW block as fixed effects. For fecal dry matter, data were analyzed as repeated measures, considering the two sampling days. In addition, the model incorporated pen as a random intercept to account for the subsampling associated with multiple individual pigs measured within each pen. *M. hyopneumoniae* blood titers were analyzed in a similar manner to fecal DM, with statistical analysis for both responses performed using the GLIMMIX procedure of SAS OnDemand for Academics (SAS Institute, Inc., Cary, NC).

Contrast coefficients were set to evaluate the main effects of ZnO and OSPL, and their interaction. Additionally, NC + OSPP was analyzed by pairwise comparisons to three treatments: 1) PC, 2) NC, and 3) NC + OSPL. When treatment was a significant source of variation, differences were determined by pairwise comparison using the Tukey-Kramer multiplicity adjustment to control for type I error. Results were considered significant with $P \leq 0.05$ and were considered marginally significant with $0.05 < P \leq 0.10$.

Results and Discussion

Growth performance

From d 0 to 10 (phase 1), main effects of pharmacological levels of Zn were observed, where pigs receiving ZnO had increased ($P = 0.046$) ADG and a tendency for increased ADFI ($P = 0.068$) compared with the pigs not receiving pharmacological levels of Zn. Additionally, pigs fed diets containing additional ZnO tended to have increased ($P = 0.080$) d 10 BW (Table 3).

From d 10 to 24 (phase 2), main effects of pharmacological levels of Zn were observed, where pigs receiving ZnO had increased ($P \leq 0.001$) ADG, ADFI, and d 24 BW compared with pigs not receiving additional ZnO. Additionally, pigs fed the PC had increased ($P < 0.05$) ADG, ADFI, and d 24 BW compared with pigs fed NC + OSPP.

From d 24 to 42 (phase 3), a tendency for an interaction was observed ($P = 0.099$) for ADG, where pigs previously fed pharmacological levels of Zn from ZnO with OSPL had lower ADG than those without ZnO ($P = 0.009$) with no differences in ADG

[3] Leone, J. L. 1973. Collaborative study of the quantitative determination of titanium dioxide in cheese. AOAC. 56(3):535.

[4] Adeola, O. 2001. Digestion and balance techniques in pigs. pp. 903. Swine Nutrition, 2nd ed. A. J. Lewis and L. L. Southern ed. CRC Press, Washington, DC.

within the treatments without OSPL. Additionally, main effects of pharmacological levels of Zn were observed, where pigs fed diets previously containing pharmacological levels of ZnO had decreased ADG and worsened feed efficiency ($P \leq 0.041$). Furthermore, pigs previously fed the PC tended to have decreased ($P < 0.10$) ADG compared with pigs fed the NC + OSPP.

Overall (d 0 to 42), no treatment differences were observed in ADG, ADFI, F/G due to oregano essential oil in feed or water or ZnO inclusion.

Fecal dry matter analysis

There were observational concerns with scouring as treatment means were above 17% DM on d 10 and 21. On d 10, a ZnO × OSPL interaction was observed ($P = 0.016$), where within the no OSPL treatment, pigs fed ZnO had greater fecal DM ($P = 0.008$) than those without added ZnO. In contrast, no added ZnO differences were observed when OSPL was provided. A tendency for a NC + OSPL versus NC + OSPP × day interaction was observed ($P = 0.068$), where pigs fed OSPP had greater fecal DM on d 10 ($P = 0.024$), with no difference observed on d 21. Furthermore, a NC versus NC + OSPP × day interaction was observed ($P = 0.010$), where pigs fed OSPP had greater fecal DM on d 10 ($P < 0.001$), with no differences observed on d 21.

DM and N digestibility

Main effects of pharmacological levels of Zn were observed for the ATTD of DM and CP ($P < 0.001$), where pigs fed diets containing added ZnO had greater digestibility than pigs fed diets without ZnO. Additionally, pigs receiving OSPL tended to have lower DM digestibility ($P = 0.090$) and had lower CP digestibility ($P = 0.015$) than pigs not receiving OSPL.

Pigs fed NC + OSPP had greater DM and CP digestibility than pigs receiving NC and NC + OSPL ($P < 0.001$). In contrast, pigs fed NC + OSPP tended to have lower CP digestibility than pigs fed PC ($P = 0.087$).

M. hyopneumoniae S/P ratios

Barn health was stable with no clinical health issues and minimal mortalities/removals. No treatment differences were observed for *Mycoplasma hyopneumoniae* S/P ratios on d 0 or 42. Titer concentrations were near zero for all treatments on d 0, whereas concentrations increased similarly across treatments by d 42 ($P < 0.001$).

In conclusion, pharmacological Zn from ZnO improved early nursery growth performance and nutrient digestibility, although OSPL elicited no beneficial response. Additionally, NC + OSPP resulted in greater fecal DM during the early nursery and improved digestibility compared with NC + OSPL. However, these early responses did not translate into overall growth differences.

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Table 1. Phase 1 diet composition (as-fed basis)[1]

	Positive control (PC)	Negative control (NC)	NC + OSPP[2]
Ingredient, %			
Corn	49.93	50.35	50.32
Soybean meal, 47.7% CP	15.23	15.20	15.20
Whey powder	20.85	20.85	20.85
Microbially enhanced soybean meal[3]	6.25	6.25	6.25
Spray-dried bovine plasma	2.50	2.50	2.50
Soybean oil	2.00	2.00	2.00
Calcium carbonate	0.40	0.41	0.41
Monocalcium P, 21.5% P	0.80	0.80	0.80
Salt	0.30	0.30	0.30
L-Lys-HCl	0.40	0.40	0.40
DL-Met	0.20	0.20	0.20
L-Thr	0.17	0.17	0.17
L-Trp	0.03	0.03	0.03
L-Val	0.09	0.09	0.09
Trace mineral premix	0.15	0.15	0.15
Vitamin premix	0.25	0.25	0.25
Phytase[4]	0.06	0.06	0.06
Zinc oxide	0.40	---	---
Orego-Stim Plus Powder 2X[2]	---	---	0.03
Total	100	100	100
Calculated analysis			
SID AA, %			
Lys, %	1.36	1.36	1.36
Ile:Lys	58	58	58
Leu:Lys	118	118	118
Met:Lys	34	34	34
Met and Cys:Lys	58	58	58
Thr:Lys	65	65	65
Trp:Lys	20.2	20.2	20.2
Val:Lys	70	70	70
His:Lys	35	35	35
NE, kcal/lb	1,179	1,184	1,184
SID Lys:NE, g/Mcal	5.23	5.21	5.21
CP, %	20.9	20.9	20.9
Ca, %	0.61	0.61	0.61
STTD P, %	0.56	0.56	0.56
Ca:P	1.00	1.00	1.00
Analyzed CP, %[5]	19.9	19.4	20.0
Analyzed Zn, mg/kg[5]	2,785	409	287

[1] Phase 1 diets were fed from d 0 to 10.

[2] Orego-Stim Plus Powder 2X (Anpario, Worksop, United Kingdom).

[3] MEPRO, Prairie Aquatech, Brookings, SD.

[4] HiPhorius 2400 (Novonesis, Kongens Lyngby, Denmark) provided an estimated release of 0.12% STTD P with 1,500 FYT/kg.

[5] Diet samples were analyzed in duplicate at Midwest Labs (Omaha, NE).

Table 2. Phases 2 and 3 diet composition (as-fed basis)[1]

	Phase 2			Phase 3	
	Positive control (PC)	Negative control (NC)	NC + OSPP[2]	NC	NC + OSPP[2]
Ingredient, %					
Corn	58.55	58.83	58.80	66.52	66.50
Soybean meal, 47.7% CP	22.24	22.22	22.22	29.42	29.42
Whey powder	10.00	10.00	10.00	---	---
Microbially enhanced soybean meal[3]	5.00	5.00	5.00	---	---
Calcium carbonate	0.44	0.44	0.44	0.90	0.90
Monocalcium P, 21.5% P	0.87	0.87	0.87	0.95	0.95
Salt	0.58	0.58	0.58	0.60	0.60
L-Lys-HCl	0.50	0.50	0.50	0.51	0.51
DL-Met	0.22	0.22	0.22	0.21	0.21
L-Thr	0.23	0.23	0.23	0.23	0.23
L-Trp	0.05	0.05	0.05	0.06	0.06
L-Val	0.13	0.13	0.13	0.14	0.14
Trace mineral premix	0.15	0.15	0.15	0.15	0.15
Vitamin premix	0.25	0.25	0.25	0.25	0.25
Phytase[4]	0.06	0.06	0.06	0.06	0.06
Titanium dioxide	0.50	0.50	0.50	---	---
Zinc oxide	0.26	---	---	---	---
Orego-Stim Plus Powder 2X[2]	---	---	0.03	---	0.03
Total	100	100	100	100	100
Calculated analysis					
SID AA, %					
Lys, %	1.35	1.35	1.35	1.30	1.30
Ile:Lys	58	58	58	55	55
Leu:Lys	115	115	115	115	115
Met:Lys	37	37	37	37	37
Met and Cys:Lys	58	58	58	58	58
Thr:Lys	65	65	65	64	64
Trp:Lys	20.1	20.1	20.1	20.2	20.2
Val:Lys	70	70	70	70	70

His:Lys	35	35	35	36	36
NE, kcal/lb	1,111	1,114	1,114	1,104	1,103
SID Lys:NE, g/Mcal	5.52	5.50	5.50	5.34	5.35
CP, %	21.0	21.0	21.0	20.4	20.4
Ca, %	0.59	0.59	0.59	0.74	0.74
STTD P, %	0.50	0.50	0.50	0.46	0.46
Ca:P	1.01	1.01	1.01	1.26	1.26
Analyzed CP, %[5]	19.4	20.0	19.8	19.1	19.2
Analyzed Zn, mg/kg[5]	2,190	237	236	134	117

[1] Phase 2 diets were fed from d 10 to 24. Phase 3 diets were fed from d 24 to 42.

[2] Orego-Stim Plus Powder 2X (Anpario, Worksop, United Kingdom).

[3] MEPRO, Prairie Aquatech, Brookings, SD.

[4] HiPhorius 2400 (Novonesis, Kongens Lyngby, Denmark) provided an estimated release of 0.12% STTD P with 1,500 FYT/kg.

[5] Diet samples were analyzed at Midwest Labs (Omaha, NE).

Table 3. Effects of Orego-Stim and zinc oxide (ZnO) on growth performance, fecal dry matter (DM), apparent total tract digestibility of DM and crude protein, and *M. hyopneumoniae* blood titers of nursery pigs[1]

							P^3 =		
	PC	NC	PC + OSPL	NC + OSPL	NC + OSPP	SEM	ZnO × OSPL	ZnO	OSPL
BW, lb									
d 0	12.8	12.8	12.8	12.8	12.8	0.03	0.848	0.834	0.949
d 10	15.7	15.3	15.9	15.5	15.7	0.23	0.954	0.080	0.463
d 17[4]	21.8	20.5	21.8	20.7	20.8	0.34	0.842	0.001	0.777
d 24[4]	29.7	28.0	29.8	27.8	28.3	0.46	0.771	< 0.001	0.897
d 35	43.4	41.8	42.7	42.6	42.8	0.62	0.232	0.149	0.901
d 42	56.4	54.9	56.0	55.2	56.1	0.70	0.602	0.101	0.948
Phase 1 (d 0 to 10)									
ADG, lb	0.30	0.25	0.31	0.26	0.29	0.022	0.895	0.046	0.595
ADFI, lb	0.41	0.36	0.42	0.38	0.39	0.021	0.851	0.068	0.471
F/G	1.39	1.71	1.12	1.47	1.40	0.147	0.453	0.597	0.710
Phase 2 (d 10 to 24)									
ADG, lb[4]	1.00	0.89	0.99	0.88	0.88	0.027	0.943	< 0.001	0.642
ADFI, lb[4]	1.33	1.21	1.31	1.19	1.19	0.035	0.985	0.001	0.598
F/G	1.33	1.35	1.34	1.36	1.35	0.018	0.845	0.314	0.769
Phase 3 (d 24 to 42)									
ADG, lb[5,6]	1.48	1.49	1.44	1.52	1.54	0.023	0.099	0.038	0.687
ADFI, lb	2.35	2.34	2.31	2.38	2.40	0.037	0.328	0.446	0.995
F/G	1.58	1.57	1.61	1.56	1.56	0.015	0.299	0.041	0.505
Overall (d 0 to 42)									
ADG, lb	1.04	0.99	1.01	1.01	1.02	0.018	0.286	0.176	0.795
ADFI, lb	1.54	1.48	1.52	1.50	1.51	0.028	0.415	0.170	0.959
F/G	1.49	1.50	1.50	1.49	1.48	0.012	0.537	0.948	0.593
Fecal DM, %[7]									
d 10[8]	21.3	17.2	18.4	19.5	23.0	1.08	0.016	0.179	0.751
d 21	21.6	21.7	21.5	22.4	21.9	1.08	0.748	0.650	0.799
Apparent total tract digestibility									
DM[9]	88.5	85.6	87.7	84.7	89.2	0.51	0.995	< 0.001	0.090
CP[5,9]	83.5	77.4	82.2	74.7	81.5	0.82	0.386	< 0.001	0.015
M. hyopneumoniae S/P ratio									
d 0	-0.02	-0.02	-0.02	0.00	-0.01	0.012	0.287	0.526	0.473
d 42	1.71	1.81	1.94	1.92	1.85	0.132	0.646	0.760	0.216

[1] A total of 375 pigs (initially 12.8 ± 0.03 lb) were used in a 42-d growth study with five pigs per pen and 15 replicates per treatment. Diets were corn-soybean meal-based and arranged in a 2 × 2 + 1 factorial. Main effects were no added or added pharmacological levels of Zn (ZnO added to achieve Zn levels of 3,000 and 2,000 ppm in phases 1 and 2, respectively) and no added Orego-Stim Plus Liquid 2X (OSPL; Anpario, Worksop, United Kingdom) or added Orego-Stim Plus Liquid 2X provided through the drinking water system (delivered at a final dilution of approximately 1:8,000). The additional treatment included 0.03% of Orego-Stim Plus Powder 2X (OSPP; Anpario, Worksop, United Kingdom) in the feed without pharmacological Zn. Thus, the five treatments consisted of 1) positive control (PC) with pharmacological levels of Zn; 2) negative control (NC) without pharmacological levels of Zn; 3) PC + OSPL; 4) NC + OSPL; and 5) NC + OSPP.

[2] Anpario, Worksop, Nottinghamshire, UK.

[3] Three pairwise comparisons were made: (1) PC to NC + OSPP, (2) NC to NC + OSPP, (3) NC + OSPL to NC + OSPP. Results were $P > 0.10$ unless otherwise indicated.

[4,5] Comparison of PC to NC + OSPP, 4$P < 0.05$. 5$P < 0.10$.

[6] Simple effects of ZnO in no OSPL ($P = 0.753$), ZnO in OSPL ($P = 0.009$).

[7] Comparison of NC + OSPL to NC + OSPP × day ($P = 0.068$). Effect on d 10 ($P = 0.024$), no effect on d 21 ($P = 0.744$). Comparison of NC to NC + OSPP × day ($P = 0.010$). Effect on d 10 ($P < 0.001$), no effect on d 21 ($P = 0.935$).

[8] Simple effects of ZnO in no OSPL ($P = 0.008$), ZnO in OSPL ($P = 0.448$), OSPL in ZnO ($P = 0.054$), OSPL in no ZnO ($P = 0.138$).

[9] Comparison of NC + OSPL to NC + OSPP ($P < 0.001$). Comparison of NC to NC + OSPP ($P < 0.001$).