K-STATE Research and Extension

SWINE DAY 2019

KANSAS STATE UNIVERSITY SWINE INDUSTRY DAY 2019

Evaluating the Interactive Effects of *Cordyceps* Mushroom Powder and Carbadox to Pharmacological Copper and Zinc for Nursery Pigs

Jacob A. Richert, Morgan Thayer,[1] Clayton Chastain,[1] Alan Duttlinger,[1] Julie Feldpausch,[1] Ricardo Garcia,[1] Brian Richert,[1] and Jim Nelssen

Summary

The objective of this study was to evaluate the independent and additive effects of *Cordyceps* mushroom powder (MP) and carbadox to pharmacological levels of copper and zinc in nursery pig diets. Two hundred and ten crossbred weanling pigs (Duroc × (York × Landrace)) average of 19 d of age and 12.8 lb were used in a 33-day growth trial. Pigs were allotted by weight, sex, ancestry, and assigned to body weight (BW) blocks. Within BW blocks, sex ratios were constant in each pen. Pen was the experimental unit, and growth performance was analyzed using BW, average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F:G). There were 7 pigs/pen and 6 pens/treatment. Treatments were: 1) a negative control diet (NC); 2) positive control (PC; carbadox, 50 g/ton); 3) NC+ 300 ppm *Cordyceps* mushroom powder (NC+MP); 4) PC + 300 ppm mushroom powder (PC+MP); 5) supplemental copper sulfate (125 ppm) and zinc oxide (3000 ppm d 0 to 7, 2000 ppm d 7 to 35), CuZn. Dietary treatments were fed in a four-phase feeding program (d 0 to 7, d 7 to 14, d 14 to 21, and d 21 to 33). Pigs fed the PC, PC+MP, and CuZn diets had increased BW ($P < 0.05$), ADG ($P < 0.05$), and ADFI ($P < 0.10$) over those fed the NC at the end of phases 1, 2, and 3, with no main effect of MP treatment. During phase 4, pigs fed MP, PC, and CuZn diets all had increased ADG ($P < 0.05$; 0.95, 1.05, 1.00, 1.11, 1.07 lb/d, diet 1–5, respectively) and ADFI ($P < 0.05$) over the NC fed pigs. Overall, d 0 to 33, pigs fed PC diets and CuZn had increased ADG ($P < 0.05$) and ADFI ($P < 0.05$), with pigs fed MP tending to have increased ADFI ($P < 0.08$) over NC-fed pigs. Plasma TNF-α concentrations at d 14 postweaning showed a trend for a carbadox main effect, as well as a mushroom by carbadox interaction ($P < 0.10$) for plasma TNF-α, with the 300 ppm MP having the numerically highest value, while the combination of carbadox and 300 ppm MP had the lowest concentration of TNF-α. Feeding nursery pigs pharmacological levels of Cu+Zn and carbadox have economical value to increase nursery pig performance, while MP may increase pig ADFI and final BW through potentially complementary modes of action to carbadox.

[1] Department of Animal Sciences, College of Agriculture, Purdue University, West Lafayette, IN.

Introduction

Antibiotics are an important aspect of swine production for disease prevention and treatment. Antimicrobials used in feed post-weaning, such as carbadox, are under heavy scrutiny due to the growing concerns of antibiotic-resistant pathogens. Because of these concerns and changes in antibiotic use regulations, swine producers are reducing antibiotic use. The effects of feeding carbadox to nursery pigs has traditionally shown improved growth performance and feed efficiency (F/G) compared to pigs fed diets without antimicrobial agents. Industry concern over antibiotic resistance and potential monetary losses has led to research into alternatives to antibiotics. One of these possible alternatives is a Chinese herbal mushroom blend of *Cordyceps militaris* and *Cordyceps sinensis*.[2]These mushrooms have long been used by the Chinese as a human health-promoting additive. The particular compound, cordycepin, found in these mushrooms is currently being studied as a possible anti-cancer agent by many research institutes. The mushroom itself has antimicrobial and antiviral characteristics.[3] Based on a previous study utilizing this mushroom[4] with positive results, as well as our own research, a study was conducted to determine whether there is an additive effect when combining the mushroom and carbadox in the nursery phase in a true negative control type diet.

Procedures

Two hundred and ten gilts and barrows (18.4 d of age) weighing an average of 12.8 lb (Duroc × (York × Landrace)) were put on test for a 33-day growth trial. Growth performance was analyzed using BW, ADG, ADFI, and feed conversion as F:G ratios. There were 5 dietary treatments fed throughout the nursery period. Treatments were: 1) a negative control diet (NC); 2) positive control (PC; carbadox, 50 g/ton); 3) NC+ 300 ppm *Cordyceps* mushroom powder (NC+MP); 4) PC + 300 ppm *Cordyceps* mushroom powder (PC+MP); 5) NC+ supplemental copper sulfate (125 ppm) and zinc oxide (3000 ppm d 0 to 7, 2000 ppm d 7–35), CuZn. Pigs were divided by weight, sex, litter, and assigned to BW blocks with 7 pigs per pen. Within BW blocks, sex ratios were constant in each pen. Each pen within a BW block was then randomly assigned a dietary treatment. Pigs and feeders were weighed on day 0, 7, 14, 21, 27, and 33. The studies were performed at Purdue University's Animal Sciences Research and Education Center (ASREC). Purdue University's Animal Care and Use Committee approved the protocol used in this experiment. Feed was made at the ASREC feed mill.

The pigs were fed four dietary phases over a 33-day period. Phase 1 was d 0 to 7, phase 2 was d 7 to 14, phase 3 was d 14 to 21, and phase 4 was d 21 to 33 (Table 1). Phases 1 and 2 were made with a basal diet, which was split then remixed with the treatment premix. Phases 3 and 4 were made as individual diet treatment batches.

Pigs had *ad libitum* access to feed and water within each pen having a 5-hole nursery feeder and 1-cup waterer. Feeders and waterers were checked daily, with the target of

[2] Shen, H.S., S. Shao, J. C. Chen, T. Zhou. 2017. Antimicrobials from Mushrooms for Assuring Food Safety. Comprehensive Reviews in Food Science and Food Safety. Vol.16:316-329.
[3] Zhou, X. L. Luo, W. Dressel, G. Shadier, D. Krumbiegel, P. Schmidtke, F. Zepp, C. U. Meyer. 2008. Cordycepin is an immunoregulatory active ingredient of Cordyceps sinesis. Am J Chin Med 36:967-980.
[4] Cheng, Y. H., C. M. Wen, A. Dybus, W.S. Proskura. 2016. South African Journal of Animal Science 2016, 46 (No. 2):121-128.

having partial pan coverage (40–50% coverage) while also minimizing feed wastage. Daily checks consisted of checking and cleaning feeders, waterers, observations of the pigs, adding feed if needed, treating pigs with antibiotics when signs of disease were detected, and completing treatment paperwork.

Feed samples for each phase were collected and stored at -4°F for analysis at the Purdue University's Swine Nutrition Laboratory, Purdue University, West Lafayette, IN. Diet crude protein, energy, dry matter, ash, and phosphorus concentrations were analyzed. All samples were analyzed in duplicate and adjusted for standards. If the values exceeded a 5% difference the samples were repeated until values were within 5%.

Blood samples were collected from 1 median-weight barrow and gilt in each pen in one EDTA vacutainer tube using an 18 gauge, 1.5-in needle on d 14. Following blood collection, EDTA blood tubes were gently inverted 3 to 4 times to distribute EDTA, and placed immediately on ice. Blood was spun down in a centrifuge at 39°F for 15 minutes at 2000 × g, with plasma aliquoted into 3 samples and stored in the -4°F freezer. Plasma TNF-α concentrations were determined using solid-phase sandwich ELISA kits.[5] All the recommendations of the manufacturing company were followed (R&D Systems Inc, McKinley Place NE, Minneapolis, MN). The optical density (OD) value was read at 450 nm within 30 minutes by an ELISA plate reader (Tecan Spark 10M; Tecan Group Ltd., Seestrasse 103, 8708 Männedorf, Switzerland). A standard curve of OD value versus TNF-α concentration was generated, and the plasma TNF-α concentration was then determined according to the standard curve.

On day 32 of the study, fecal sampling occurred, by rectal stimulation, for fecal volatile fatty acid (VFA) analysis and future microbiome analysis of 1 median-weight barrow and gilt of each pen. Volatile fatty acid concentrations in fecal samples were determined by a gas chromatographic method.[6] Briefly, fecal samples were thawed and 4 ± 0.1 g samples were taken, diluted with 4 mL distilled water and 2 mL of 25% metaphosphoric acid, mixed (VWR Mini Vortexer MV1, IKA Works, Inc., Wilmington, NC), and centrifuged at 15,000 × g, 39°F, for 10 min (Beckman J-21C, Beckman Instruments, Inc., Palo Alto, CA). After centrifugation, the supernatant was transferred into a 2-dram vial. The sample was re-centrifuged (15,000 × g, 39°F, for 15 min) and the supernatant was filtered through a polyethersulphone membrane filter (0.25 mm, Whatman, UK) and 1.5 mL transferred into a DPID vial. The concentrations of VFA were determined by gas chromatography (Varian 3900, Varian, Inc., Walnut Creek, CA 94598). The least detectable limit for all VFA was 0.1 mmol/L.

The individual pig body weights and pen feed intake were recorded to determine pen ADG, ADFI, and F:G. Pen was the experimental unit, and the growth parameters analyzed were BW, ADG, ADFI, and F/G every week and summarized by dietary phase and overall. Blood and fecal sample data were also analyzed as a pen mean of the two pigs that were sampled. All data were analyzed as a randomized complete block using

[5] Chaytor, A.C., M.T. See, J. A. Hansen, A. L. P. de Souza, T. F. Middleton, S.W. Kim. 2011. Effects of chronic exposure of diets with reduced concentrations of aflatoxin and deoxynivalenol on growth and immune status of pigs. J Anim Sci 89:124-135.

[6] Erwin, E.S., G. J. Marco, E. M. Emery. 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. J of Dairy Science 44:1768-1771.

the GLM procedure in SAS (v. 9.4, SAS Institute, Inc., Cary, NC) using single degree of freedom preplanned contrasts to test for differences among dietary treatments.

Results and Discussion

During phase 1 of the trial (d 0 to 7), there were significant increases in ADG, ADFI, d 7 BW, and improved F/G ($P \leq 0.01$) for CuZn compared to the NC, and carbadox-fed pigs had increases in ADG, d 7 BW, improved F/G ($P < 0.01$), and a tendency for an increase in ADFI ($P < 0.08$) over diets without carbadox. There was a tendency for a MP × carbadox interaction in phase 1 ADFI ($P < 0.10$), with the MP treatment increasing ADFI over the NC treatment; however, the pigs fed the combination of MP and carbadox consumed less feed than the pigs fed the carbadox alone.

For phases 2 and 3, CuZn-fed pigs had greater ADG, ADFI, BW, and improved F:G ($P < 0.03$) compared to the NC. Carbadox improved ADG, ADFI, F:G, and BW in phase 2 ($P < 0.02$), and in phase 3 carbadox increased ADG, ADFI, and BW ($P < 0.04$) compared to diets without carbadox. In phase 2, the MP diets had poorer F:G ratio ($P < 0.02$), primarily when fed alone and this was not evident when pigs were fed with carbadox (interaction, $P < 0.03$). Phase 3 had no statistical differences for MP or MP × carbadox interaction.

In phase 4, MP ($P < 0.02$) and carbadox ($P < 0.01$) both increased ADG and ADFI. In phase 4, CuZn-fed pigs had improved ADG and ADFI ($P < 0.01$) but poorer F:G ($P < 0.01$) than pigs fed the NC. Overall, pigs fed carbadox had greater ADG and ADFI and improved F:G ($P < 0.01$). While feeding CuZn dramatically increased overall ADG and ADFI ($P < 0.01$), it did not improve F/G over the NC treatment. For the overall nursery period, pigs fed MP tended to have increased ADFI ($P < 0.08$) over those without MP in the diets.

Pigs fed the CuZn diet averaged 0.20 lb/d better ADG over the negative control, resulting in the final BW for the CuZn treatment being 6.4 lb greater than the negative control ($P < 0.01$). Carbadox has well-documented positive growth performance effects in the nursery. In this study, these results were reproduced with pigs fed carbadox having significantly improved performance in every category over the NC, adding 3.8 lb of BW ($P < 0.01$) by the end of the study. Pigs fed the mushroom powder treatment appear to have a delayed effect. The pigs were slightly numerically improved over the negative control pigs throughout the study in ADG and ADFI, but in the final week of phase 4, matched carbadox performance in ADG and tended to increase overall ADFI ($P < 0.08$). Additionally the treatment of carbadox + MP had similar ADG throughout the study compared to carbadox until the second week of phase 4, where the pigs in this treatment were gaining an additional 0.15 lb/d compared to the carbadox alone treatment.

There were no differences in fecal acetic acid concentrations among treatments (Table 3; $P > 0.1$). However there was a significant ($P < 0.05$) or tendency ($P < 0.10$) for all other VFAs and total VFAs with both MP and carbadox treatments to have elevated levels compared to the NC, but reduced levels when the treatments were combined; VFAs were reduced to levels similar or lower than the NC. There was also a main carbadox effect to decrease valeric acid concentrations ($P < 0.03$). When fecal

VFAs are calculated as a percentage of total VFAs (Table 4) to show the pattern of VFAs, MP and carbadox decrease acetic acid; however, when combined these products increase acetic acid ($P < 0.05$). Fecal isovalareic acid ($P < 0.10$) and valeric acid ($P < 0.02$), as a percentage of total VFAs, increase when pigs are fed MP but decrease when MP is fed in combination with carbadox. The CuZn treatment decreased propionic acid ($P < 0.09$) and increased butyric acid ($P < 0.04$), as a percentage of total VFAs, over the NC.

Plasma concentrations of TNF-α at d 14 postweaning had a trend ($P < 0.06$) for a carbadox main effect, as well as an MP by carbadox interaction ($P < 0.08$). The MP treatment had the numerically greatest value on the study, while the combination of carbadox and MP had the lowest TNF-α concentrations.

Previous studies with MP indicated some potential as an alternative to in-feed antimicrobials, averaging approximately 75% of the carbadox response. However, those diets contained pharmacological levels of copper and zinc, and were not a true negative control.

Mushroom powder improved performance slightly early, but primarily had a delayed response. During the final week MP matched carbadox in ADG, and had an additive effect in the final phase when added into the carbadox treatment. The Cu and Zn treatment outperformed every treatment other than in the final week, when the combination of carbadox and MP were the best treatment for gain. It also appears MP may not be as effective when combined with a true negative control. It appeared to perform better with Zn and Cu included in the diet in the previous studies.

Interestingly MP had the greatest total VFA concentrations out of any treatment, but the combination of carbadox and MP had lowest total VFA concentrations. This is the opposite of what the concentrations looked like in a previous titration study for the 300 ppm level of MP, warranting further investigation as to what impact this level of mushroom powder has on fecal VFA concentrations.

Plasma TNF-α concentrations were greatest in the MP treatment, which is again conflicting with the previous titration study. There was a tendency for reduced TNF-α concentrations in carbadox-fed pigs. However, with the combination of carbadox and MP, the plasma TNF-α levels decreased in concentration further than with carbadox alone.

The MP treatment could provide some benefit to pigs to stimulate feed intake. However the response is primarily late in the nursery phase. Based on previous results it is possible the level of the mushroom powder needs to be adjusted to different levels at certain time points, and a refined titration study may be warranted. Further research is needed to investigate whether there is a possible carryover effect into the grow-finish stage of production. Further research is also needed on the composition of this mushroom product, to determine why the effect is delayed. It may take time for the effect to be expressed if it is primarily changing the gut microbiome or gut immune function.

In conclusion, carbadox and pharmacological copper and zinc are effective at promoting nursery pig growth performance, showing 12 and 20% increases in final BW, respectively. The mushroom powder at 300 ppm appears to offer a delayed response in nursery pigs. It is worth exploring further the mechanisms of the MP and carbadox additive effects in the last 2 weeks of the nursery period, suggesting there are different mechanisms at work between the MP and carbadox.

Brand names appearing in this publication are for product identification purposes only. No endorsement is intended, nor is criticism implied of similar products not mentioned. Persons using such products assume responsibility for their use in accordance with current label directions of the manufacturer.

Table 1. Basal diet composition

Ingredient, %	Phase 1	Phase 2	Phase 3	Phase 4
Corn	36.265	42.415	46.420	53.885
Soybean meal, CP 48%	14.000	16.950	26.500	28.925
DDGS, 7% fat	---	5.000	7.500	10.000
Choice white grease	---	---	---	3.000
Soybean oil	5.000	4.000	3.000	---
Limestone	0.650	0.810	0.890	1.415
MonoCal P	0.480	0.530	0.180	0.560
Vitamin premix[1]	0.250	0.250	0.250	0.250
TM premix[2]	0.125	0.125	0.125	0.125
Se premix[3]	0.050	0.050	0.050	0.050
Phytase premix[4]	0.100	0.100	0.100	0.100
Salt	0.250	0.250	0.300	0.350
Plasma protein	5.000	2.500	---	---
Spray-dried blood meal	1.500	1.000	---	---
Soy concentrate	5.000	3.250	---	---
Fish meal	4.650	4.500	5.000	---
Dried whey	25.750	17.150	8.600	---
Lysine-HCL	0.130	0.275	0.300	0.435
DL-Methionine	0.230	0.210	0.160	0.150
L-Threonine	0.060	0.110	0.110	0.140
L-Tryptophan	0.010	0.025	0.015	0.015
Banminth-48	---	---	---	0.100
Treatment premix	**0.500**	**0.500**	**0.500**	**0.500**
Total	100.00	100.00	100.00	100.00

continued

Table 1. Basal diet composition

Ingredient, %	Phase 1	Phase 2	Phase 3	Phase 4
Calculated nutrients				
Metabolizable energy, Kcal/lb	1606.3	1580.8	1558.6	1543.9
Net energy, Kcal/lb	1245.6	1207.1	1164.7	1139.4
CP, %	24.49	23.25	23.15	21.53
Total Lys, %	1.73	1.63	1.53	1.43
SID Lys, %	1.55	1.45	1.35	1.25
SID Met, %	0.55	0.53	0.50	0.44
SID M+C, %	0.91	0.85	0.79	0.73
SID Thr, %	0.97	0.91	0.84	0.78
SID Tryp, %	0.28	0.26	0.24	0.23
SID Iso, %	0.86	0.81	0.82	0.75
SID Val, %	1.08	0.98	0.91	0.83
Ca, %	0.85	0.85	0.80	0.75
P, %	0.76	0.72	0.62	0.55
Available P, %	0.55	0.50	0.45	0.35

[1]Provided per lb of diet available minerals: iron, 55.0 mg; zinc, 55.0 mg; manganese, 6.8 mg; copper, 5.1 mg; and iodine, 0.21 mg.
[2]Provided per lb of diet: vitamin A, 3000 IU; vitamin D3, 300 IU; vitamin E, 20 IU; vitamin K, 1.0 mg; riboflavin, 4.1 mg; pantothenic acid, 11 mg; niacin, 15.0 mg; and B12, 17.5 mg.
[3]Provided 0.136 mg Se per lb of diet.
[4]Provided 272 FTU per lb of the diet.
CP = crude protein. DDGS = dried distillers grains with solubles. SID = standard ileal digestible.

Table 2. Effect of *Cordyceps* mushroom powder (MP), carbadox (Carb.), and pharmacological copper and zinc on nursery pig growth performance

							Probability,[2] *P* <			
Diet[1]	NC	MP	PC-Carb.	Carb.+MP	CuZn	SE	MP effect	Carb. effect	MP × Carb.	NC vs. CuZn
Pens/diet	6	6	6	6	6					
d 0 BW, lb	12.81	12.82	12.82	12.89	12.85	0.027	0.20	0.16	0.27	0.40
d 0 to 7										
ADG, lb	0.17	0.23	0.30	0.28	0.35	0.028	0.47	<0.01	0.20	<0.01
ADFI, lb	0.25	0.32	0.34	0.32	0.41	0.025	0.35	0.08	0.10	<0.01
F:G	1.56	1.46	1.19	1.15	1.21	0.081	0.40	<0.01	0.71	0.01
d 7 BW, lb	13.99	14.40	14.90	14.85	15.30	0.210	0.39	<0.01	0.28	<0.01
d 7 to 14										
ADG, lb	0.24	0.21	0.36	0.36	0.54	0.033	0.76	<0.01	0.63	<0.01
ADFI, lb	0.43	0.47	0.52	0.54	0.76	0.032	0.41	0.02	0.82	<0.01
F:G	1.87	2.42	1.52	1.54	1.40	0.114	0.02	<0.01	0.03	0.01
d 14 BW, lb	15.64	15.87	17.40	17.39	19.10	0.355	0.76	<0.01	0.74	<0.01
d 14 to 21										
ADG, lb	0.62	0.66	0.74	0.71	0.83	0.040	0.96	0.04	0.41	<0.01
ADFI, lb	0.80	0.85	0.96	0.92	1.19	0.048	0.99	0.02	0.41	<0.01
F:G	1.30	1.29	1.29	1.30	1.45	0.046	0.97	0.98	0.84	0.03
d 21 BW, lb	19.98	20.46	22.61	22.37	24.90	0.544	0.82	<0.01	0.52	<0.01
d 21 to 33 (phase 4)										
ADG, lb	0.95	1.00	1.05	1.11	1.07	0.022	0.02	<0.01	0.85	<0.01
ADFI, lb	1.41	1.50	1.56	1.64	1.74	0.033	0.02	<0.01	0.93	<0.01
F:G	1.49	1.50	1.48	1.48	1.62	0.027	0.88	0.71	0.79	<0.01
d 33 BW, lb	31.38	32.49	35.21	35.71	37.79	0.601	0.19	<0.01	0.62	<0.01
d 0 to 33										
ADG, lb	0.56	0.60	0.68	0.69	0.76	0.018	0.21	<0.01	0.58	<0.01
ADFI, lb	0.96	1.02	1.08	1.12	1.26	0.027	0.08	<0.01	0.54	<0.01
F:G	1.70	1.72	1.60	1.62	1.67	0.019	0.34	<0.01	0.95	0.35

[1]Diets: Negative control (NC) = no feed antimicrobial and no pharmacological zinc oxide or copper sulfate; MP = NC + mushroom powder at 300 ppm; positive control (PC)-Carb = NC + carbadox at 50 g/ton; Carb+MP = NC + carbadox at 50 g/ton and 300 ppm mushroom powder; and CuZn = 125 ppm Cu d 0 to 33 from copper sulfate and 3000 ppm Zn from d 0 to 7 declining to 2000 ppm from d 7 to 33 from zinc oxide.

[2]Contrasts: MP effect tested NC and PC vs. MP and PC+MP; Carb. effect tested NC and MP vs. PC and PC+MP; MP × Carb. Tests for the interaction between with or without carbadox and with or without MP treatments; and NC vs. CuZn contrasts the NC diet to the CuZn treatment.

ADG = average daily gain. ADFI = average daily feed intake. F:G = feed to gain ratio.

Table 3. Effect of *Cordyceps* mushroom powder, carbadox, pharmacological copper, and zinc on day 32 fecal volatile fatty acids

							Probability,[2] *P* <			
Diet[1]	NC	MP	PC-Carb.	Carb.+MP	CuZn	SE	MP effect	Carb. effect	MP × Carb.	NC vs. CuZn
A[3]	75.64	78.93	77.57	74.11	71.30	3.715	0.98	0.70	0.36	0.40
P	31.97	36.64	35.23	31.56	27.71	1.914	0.79	0.63	0.04	0.12
iB	2.21	2.75	2.52	2.06	1.98	0.281	0.88	0.50	0.08	0.56
B	14.73	16.05	17.32	13.22	17.19	1.587	0.38	0.94	0.09	0.27
iV	2.58	3.60	3.34	2.45	2.36	0.438	0.88	0.65	0.04	0.72
V	3.40	4.69	3.56	2.56	2.69	0.420	0.73	0.03	0.01	0.23
Total	130.52	142.66	139.54	125.94	123.24	6.373	0.91	0.54	0.05	0.41

[1]Diets: Negative control (NC) = no feed antimicrobial and no pharmacological zinc oxide or copper sulfate; MP = NC + mushroom powder at 300 ppm; PC-Carb = NC + carbadox at 50 g/ton; Carb+MP = NC + carbadox at 50 g/ton and 300 ppm mushroom powder; CuZn = 125 ppm Cu d 0 to 33 from copper sulfate and 3000 ppm Zn from d 0 to 7 declining to 2000 ppm from d 7 to 33 from zinc oxide.
[2]Contrasts: MP effect tested NC and PC vs. MP and PC+MP; Carb. effect tested NC and MP vs. PC and PC+MP; MP × Carb. Tests for the interaction between with or without carbadox and with or without MP treatments; NC vs. CuZn contrasts the NC diet to the CuZn treatment.
[3]Volatile fatty acids: A = acetic acid; P = propionic acid; iB = isobutyric acid; B = butyric acid; iV = isovalareic acid; V = valeric acid; Total = total VFA concentrations.

Table 4. Effect of *Cordyceps* mushroom powder, carbadox (Carb.), and pharmacological copper and zinc on day 32 volatile fatty acids (VFAs) as a percentage of total VFAs

							Probability,[2] *P* <			
Diet[1]	NC	MP	PC-Carb.	Carb.+MP	CuZn	SE	MP effect	Carb. effect	MP × Carb.	NC vs. CuZn
A,[3] %	58.21	55.22	55.71	58.86	57.96	1.466	0.96	0.70	0.05	0.91
P, %	24.45	25.78	25.26	25.04	22.48	0.804	0.49	0.97	0.33	0.09
iB, %	1.70	1.91	1.79	1.64	1.60	0.169	0.84	0.60	0.29	0.67
B, %	11.11	11.32	12.37	10.47	13.94	0.965	0.38	0.83	0.28	0.04
iV, %	1.98	2.49	2.35	1.95	1.90	0.266	0.84	0.74	0.10	0.82
V, %	2.55	3.29	2.54	2.04	2.13	0.246	0.62	0.02	0.02	0.22

[1]Diets: Negative control (NC) = no feed antimicrobial and no pharmacological zinc oxide or copper sulfate; MP = NC + mushroom powder at 300 ppm; positive control (PC)-Carb = NC + carbadox at 50 g/ton; Carb+MP = NC + carbadox at 50 g/ton and 300 ppm mushroom powder; and CuZn = 125 ppm Cu d 0 to 33 from copper sulfate and 3000 ppm Zn from d 0 to 7 declining to 2000 ppm from d 7 to 33 from zinc oxide.
[2]Contrasts: MP effect tested NC and PC vs. MP and PC+MP; Carb. effect tested NC and MP vs. PC and PC+MP; MP × Carb. tests for the interaction between with or without carbadox and with or without MP treatments; and NC vs. CuZn contrasts the NC diet to the CuZn treatment.
[3]Volatile Fatty Acids: A = acetic acid; P = propionic acid; iB = isobutyric acid; B = butyric acid; iV = isovalareic acid; V = valeric acid; Total = total VFA concentrations.

Table 5. Effect of *Cordyceps* mushroom powder, carbadox (Carb.), and pharmacological copper and zinc on day 14 plasma TNF-α

							Probability, P <			
Diet	NC	MP	PC-Carb.	Carb.+MP	CuZn	SE	MP effect	Carb. effect	MP × Carb.	NC vs. CuZn
TNF-α, pg/mL	67.4	82.7	65.9	59.5	70.1	6.59	0.48	0.06	0.08	0.76

[1]Diets: Negative control (NC) = no feed antimicrobial and no pharmacological zinc oxide or copper sulfate; MP = NC + mushroom powder at 300 ppm; positive control (PC)-Carb = NC + carbadox at 50 g/ton; Carb+MP = NC + carbadox at 50 g/ton and 300 ppm mushroom powder; and CuZn = 125 ppm Cu d 0 to 33 from copper sulfate and 3000 ppm Zn from d 0 to 7 declining to 2000 ppm from d 7 to 33 from zinc oxide.

[2]Contrasts: MP effect tested NC and PC vs. MP and PC+MP; Carb. effect tested NC and MP vs. PC and PC+MP; MP × Carb. tests for the interaction between with or without carbadox and with or without MP treatments; NC vs. CuZn contrasts the NC diet to the CuZn treatment.