Effects of Dietary *Echinacea purpurea* on PRRSV-infected Nursery Pigs

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Summary and Implications

The effect of dietary additions of *Echinacea purpurea* on the rate of rate of growth, viremia, and ontogeny of the humoral antibody response against porcine reproductive and respiratory syndrome virus (PRRSV) infection was evaluated in weaned pigs. In three replicates, weaned pigs (18 ± 1 day of age) from a PRRSV-naïve herd were randomly allotted to one of four pens (diets) in two rooms, each pen containing five pigs. Each pen of pigs (pens) began one of four dietary treatments 1 week before inoculation with PRRSV: 1) basal ration plus carbadox (0.055 g/kg); 2) basal ration plus Echinacea I (2% of the total ration); 3) basal ration plus Echinacea II (4% of the total ration); and 4) basal ration composed of corn, soybean meal, whey, and supplemented essential vitamins and minerals. *E. purpurea* was purchased in powder form and determined by chemical analysis to contain 1.35% cichoric acid. Seven days after starting the diets (day 7), all pigs in one room were intranasally inoculated with PRRSV isolate ATCC VR-2332 at a concentration of 10^4 $TCID_{50}$/ml. To monitor the effects of diet and PRRSV infection, body weight and blood samples were collected from all pigs at 7-day intervals (day 0 to 42). Serum samples were analyzed for the presence of PRRSV and PRRSV-specific antibodies. All inoculated pigs become infected with PRRSV and all uninoculated pigs remained free of infection. PRRSV-infected pigs had a lower percentage increase in body weight between day 7 and 42 compared with uninfected animals ($P<0.06$). There were no differences in body weight, average daily gain (ADG), average daily feed intake (ADFI), or gain:feed ratio (G:F) in PRRSV-infected compared with uninfected animals. Animals receiving diets supplemented with Echinacea (treatments 2 and 3), no differences were observed in percentage increase in body weight, ADG, ADFI, and G:F ratio in either the PRRSV-infected and the uninfected pigs. Among PRRSV-infected animals, dietary Echinacea did not affect the rate or level of the ELISA-detectable antibody response day 7 to 42 or the level and duration of PRRSV in serum. Under the conditions of this study, dietary Echinacea did not reverse the growth-inhibiting effects of PRRSV, did not exhibit antiviral effects and did not show any evidence of immunostimulatory properties.

Introduction

Antimicrobial agents are given to food animals as therapy for an infection or, in the absence of disease, for the subtherapeutic purpose of increased rate of gain and improved feed efficiency (1, 2). Increasing interest to curb the use of antibiotics has led to a growing interest in alternatives. Botanicals may be used in swine diets because of their natural stimulation of the immune system and/or enhanced growth performance. Extracts from Echinacea have been shown to have nonspecific immunostimulatory properties in vitro (3). Nonspecific effects include phagocytosis (4), cytokine production (5), and natural killer cell activity (6). Rehman et al. (7) showed an increase in primary and secondary IgG response in mice treated with Echinacea. These antiviral properties of Echinacea could provide resistance to viral swine diseases, such as PRRSV and diminish opportunistic secondary infections. Few scientific studies have assessed the efficacy of Echinacea in vivo with varying results (8–10). We know of no studies involving pigs as an animal model in assessing the efficacy of Echinacea as an immunostimulant. Work with Echinacea as a possible growth promotant is limited (11). Therefore, our objectives were to determine the effects of *E. purpurea* on growth and viremia of nursery pigs when challenged with PRRSV.

Materials and Methods

Animals

Three replicate trials involving a total of 120 pigs (average initial body weight of 8.46 kg) were conducted at the Iowa State University Livestock Infectious Disease Isolation Facility (LIDIF), Ames, IA. All pigs were farrowed and reared at the Iowa State University Lauren Christian Swine Research and Demonstration Farm, Atlantic, IA. The protocol was approved by the Iowa State University Committee on Animal Care (Log No. 6-1-4861-S). In each trial, 40 crossbred piglets from a herd naïve for PRRSV were weaned between 17 to 19 days of age and allotted to one of eight pens in two separate rooms (four pens per room). Pigs were allotted to balance for pig weight and litter origin. At birth, the pigs were tail docked, ear notched, teeth clipped, males castrated, and injected subcutaneously with 2 ml of iron dextran and 0.5 ml of ceftiofur sodium). On day 7, all pigs received a second injection of 1 ml of ceftiofur sodium. At weaning (day 17 to 19), all pigs received injections of 0.5 ml of ivermectin and

1 ml of penicillin, were weighed, allotted, and moved to their treatment. Two pigs were removed before inoculation. Feed disappearance, body weights, and blood were collected at 7-day intervals until the completion of each 42-day trial. The ADG, ADFI, G:F, and percentage gain were calculated. The blood samples were centrifuged at 2000 × g for 10 min at 4°C. The serum was stored at –20°C until tested.

Housing

LIDIF is a biosafety level 2 building. The environment in each room is strictly controlled (humidity 70%, temperature 26.6˚C). The pigs were housed in an infected room or a control (uninfected) room. Five pigs were allotted per pen on nursery decks (1.22 × 2.43 m) with plastic slatted floor. Each pen had one nipple waterer and a 4-hole Kane polyethylene nursery feeder.

Experimental diets

Four dietary treatments were fed (one per pen) in each room during each trial. The diets were identical except for the treatment additives containing carbadox (0.055 g/kg), Echinacea I (2% of total ration), Echinacea II (4% of total ration), and control (no additive). Four phases of each diet were fed to coincide with stages of growth to meet or exceed nutritional requirements (12). All diets were in meal form and animals were given *ad libitum* access to feed.

The gross energy of Echinacea was determined to be 3344 kcal/kg (adiabatic bomb calorimeter; Parr Instrument Company Inc., Moline, IL). Using the gross energy value of Echinacea and values from the NRC (12) and Ewan (13), the diets were adjusted to compensate for the low energy value of Echinacea. The diets were formulated to be isolysinic. Feeding mats (0.42 × 0.77 m) were placed in front of the feeders. Waste feed was minimal and not recorded.

Certified organic *E. purpurea* root was used for the presumed immunostimulatory activity of the caffeic acid derivative, cichoric acid (14). Three-year-old plants were harvested in September and the root was dried with forced air to a moisture content of 9% (Nature's Cathedral Inc., Blairstown, IA). The root was ground and sifted to a powder then mixed with basal diets and fed. The treatment levels of 2 and 4% Echinacea were chosen based on preliminary work at Iowa State University (11). Determination of phenolics in Echinacea was performed by Alpha Laboratories Division (Petaluma, CA). High-pressure liquid chromatography was used to determine the content of caftaric acid, chlorogenic acid, cichoric acid, and echinacoside in the dried powdered *E. purpurea* root. The final results are expressed as a percentage of the total components in the material analyzed. The Echinacea contained 0.39% caftaric acid, 0.01% chlorogenic acid, less than 0.01% echinacoside, and 1.35% cichoric acid.

Virus preparation

A clone of North American prototype PRRSV isolate ATCC VR-2332 (15, 16) was used in the study. The concentration of 10^4 $TCID_{50}$/ml of the cloned virus was adjusted for the challenge virus. Pigs exposed to PRRSV were intranasally inoculated on day 7 with 2 ml (1 ml/naris) of clarified virus supernatant.

Enzyme-linked immunosorbent assay

A commercial ELISA kit (Herdchek Porcine Reproductive and Respiratory Syndrome Virus Antibody kit, IDEXX Laboratories, Westbrook, ME) was used to detect PRRSV-specific antibody in serum samples. A sample was classified as positive for PRRSV antibody if the sample-to-positive (S/P) ratio was equal to or greater than 0.4.

Data analysis

Data were analyzed as a complete randomized block design by analysis of variance techniques using general linear model (GLM) of SAS (SAS Inc., Cary, NC). The pig was considered the experimental unit for absolute weights, ADG, PRRSV-specific antibodies, and serum virus concentrations. The pen was used as the experimental unit for ADFI and G:F ratio. Pig weight at day 0 was used as a covariate when analyzing pig performance. Data were reported as least-square means.

Results and Discussion

Growth

Feeding Echinacea to PRRSV-infected pigs had no effect on individual pig body weight, percentage gain, ADG, ADFI, or G:F ratio compared with carbadox and control treatments (Table 2). Feeding Echinacea to PRRSV uninfected pigs had no effect on individual pig body weight, percentage gain, ADG, ADFI, or G:F ratio compared with carbadox and control treatments (Table 3).

PRRSV-infected pigs had a lower percentage increase in body weight between day 7 and 42 compared with uninfected animals ($P<0.06$) (Table 4). No differences were seen between PRRSV-infected compared with uninfected animals in body weight, ADG, ADFI, or G:F. According to these data, PRRSV inhibited growth of infected animals compared with healthy animals over time.

Viremia

E. purpurea was fed before inoculation with PRRSV until the end of the 42-day trial to ensure possible antiviral and immunostimulatory properties time to be observed. *E. purpurea* has been shown to increase IgG levels and reduce virus titer in rats (7). However, in the current study feeding dietary Echinacea to the PRRSV-infected pigs did not stimulate an ELISA-detectable antibody response (Table 5). Dietary Echinacea did not affect the level or duration of viremia. Specifically animals fed diets supplemented with Echinacea were the lasts to reach a zero serum PRRSV titer (Table 6). No differences were detected for the dietary treatments in number of pigs positive for PRRSV antibody or from which virus was recovered from serum (Tables 7 and 8). Based on this study, *E. purpurea* is not an effective

alternative as a growth promotant in nursery pigs. *E. purpurea* with this defined chemical profile did not stimulate antigen specific antibodies to PRRSV, inhibit virus replication, or improve elimination of virus from nursery pigs. Continued work is needed to establish the chemical constituents of *E. purpurea* that exhibit antiviral and immunostimulatory properties and their mechanisms in pigs.

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Table 1. Basal diet composition (as-fed basis).

Ingredient	Phase 1	Phase 2	Phase 3	Phase 4
		% Diet		
Corn	36.65	48.20	62.55	67.45
Dehulled soybean meal	29.00	37.00	32.50	28.00
Dried whey	25.00	10.00	0	0
Dicalcium phosphate	1.45	2.0	1.7	1.45
Fat	1.0	1.0	1.0	1.0
Calcium carbonate	.90	.70	.90	.90
Vitamin premix	.60[a]	.50[b]	.45[c]	.45[c]
Lysine	.20	.20	.20	.10
Mineral premix[d]	.10	.10	.10	.10
D,L-Methionine	.10	.10	.10	.10
Salt	0	.20	.50	.45
Spray dried plasma	5.0	0	0	0
Total	100	100	100	100
Calculated Analysis				
CP, %	24.2	23.0	20.7	18.9
Lysine, %	1.71	1.50	1.31	1.10
ME, kcal/kg	3264	3269	3291	3304

[a] Contributed per kilogram of diet: 13,200 IU of vitamin A; 3,300 IU of vitamin D_3; 66 IU of vitamin E; 19.8 g of riboflavin; 52 mg of d-pantothenic acid; 100 mg of niacin; 60 µg of vitamin B_{12}.
[b] Contributed per kilogram of diet: 11,000 IU of vitamin A; 2,750 IU of vitamin D_3; 55 IU of vitamin E; 16.5 g of riboflavin; 43.3 mg of d-pantothenic acid; 83.3 mg of niacin; 55 µg of vitamin B_{12}.
[c] Contributed per kilogram of diet: 9,900 IU of vitamin A; 2,475 IU of vitamin D_3; 49.5 IU of vitamin E; 14.9 g of riboflavin; 39 mg of d-pantothenic acid; 75 mg of niacin; 49.5 µg of vitamin B_{12}.
[d] Contributed in part per million of diet: Zn, 150.0; Fe, 175.0; Mn, 60.0; Cu, 17.6; and I, 2.0.

Table 2. Effects of feeding *E. purpurea* on growth performance in nursery pigs infected with PRRSV.

Item	Dietary treatment				
	Carbadox	Control	Echinacea I	Echinacea II	SEM
Total ADG, kg	489	513	528	522	0.02
Total ADFI, g	850	810	843	879	0.04
Total G:F, g/kg	634	719	696	641	0.08

ADG, average daily growth; ADFI, average daily feed intake.

Table 3. Effects of feeding *E. purpurea* on growth performance in nursery pigs.

Item	Dietary treatment				
	Carbadox	Control	Echinacea I	Echinacea II	SEM
Total ADG, g	524	525	527	547	0.02
Total ADFI, g	910	824	861	884	0.04
Total G:F, g/kg	632	824	674	667	0.08

ADG, average daily growth; ADFI, average daily feed intake.

Table 4. Analysis of summary for growth data for PRRSV infected and uninfected pigs.[a,b]

Day of experiment	Treatment		
	PRRSV Negative	PRRSV Positive	SEM
0–42	270.8	261.5	6.5
7–42	211.3[c]	199.2[d]	4.5
21–42	84.5	82.8	1.8

[a]Values are least-square means of the percent gain of individual pigs.
[b]Inoculation with PRRS virus at day 7 of the trial.
[c,d]Mean values within rows with a different superscript differ (P<0.06).

Table 5. Enzyme-linked immunosorbent assay serum-to-positive ratios for PRRSV-infected pigs.[a,b]

Day of experiment	Treatment				
	Carbadox	Control	Echinacea I	Echinacea II	SEM
0	0.00	0.02	0.01	0.00	0.006
7	0.00	0.00	0.00	0.01	0.004
14	0.01	0.04	0.02	0.02	0.008
21	0.67	0.79	0.46	0.47	0.157
28	1.03	1.06	0.78	0.71	0.122
35	1.47	1.49	1.25	1.19	0.109
42	1.49	1.44	1.29	1.39	0.114

[a]Values are least-square means of the serum-to-positive ratios for individual pig samples.
[b]Inoculation with PRRS virus at day 7.

Table 6. Serum PRRSV titer for PRRSV-infected pigs.[a,b]

Day of experiment	Treatment				
	Carbadox	Control	Echinacea I	Echinacea II	SEM
14	3.11	2.85	2.50	2.50	0.33
21	2.15	2.24	2.20	2.18	0.26
28	0.97	0.88	0.80	1.11	0.26
d 35	0.05	0.00	0.39	0.29	0.14
d 42	0.00	0.02	0.17	0.02	0.06

[a]Values are least square means of the $\log_{10}$ ($TCID_{50}$/mL) for individual pig samples.
[b]Inoculation with PRRS virus at day 7.

Table 7. Number of pigs within treatment groups positive for PRRSV antibody.[a]

Day of experiment	Dietary treatment[b]			
	Carbadox	Control	Echinacea I	Echinacea II
0	0	0	0	0
7	0	0	0	0
14	0	0	0	0
21	10	9	6	6
28	15	14	11	10
35	15	15	15	14
42	15	15	15	15

[a]n=15 pigs for each dietary treatment.
[b]Inoculation with PRRS virus at day 7.

Table 8. Number of pigs within treatment groups from which PRRSV was recovered from serum samples.[a,b]

Day of experiment	Dietary treatment			
	Carbadox	Control	Echinacea I	Echinacea II
14	15	14	11	11
21	14	14	14	12
28	11	1	6	9
35	2	0	3	3
42	0	1	2	1

[a]n = 15 pigs for each dietary treatment.
[b]Inoculation with PRRS virus at day 7.