EFFECTS OF A *QUILLAJA SAPONARIA* EXTRACT ON WEANLING PIG GROWTH PERFORMANCE AND IMMUNE FUNCTION DURING AN ACUTE ENTERIC DISEASE CHALLENGE[1]

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Summary

A total of 96 pigs (initially 19 lb and 17 d of age) was used in a 28 d growth trial to determine the effects of *Quillaja saponaria* (QS) extract on weanling pig growth performance and immune function in response to enteric disease challenge with *Salmonella typhimurium* (ST). Experimental treatments were arranged in a 2 × 4 factorial with main effects of disease challenge (control vs. ST challenge) and dietary addition of QS (0, 4, 8, or 16 oz/ton). The results suggest little beneficial effect of QS on growth performance or immune response in the presence or absence of ST challenge.

(Key Words: Weanling Pigs, Disease Challenge, *Salmonella, Quillaja.*)

Introduction

The popular press and empirical evidence have suggested that many plant extracts offer benefits in terms of boosting the immune system and preventing disease. Furthermore, there is growing sentiment among scientists and the general public to find alternatives for feed-grade antibiotics to promote growth and prevent disease in food animal production systems.

The extract of the South American tree, *Quillaja saponaria* (QS), has been widely used over the past three decades as a vaccine adjuvant (Quil A). The active ingredient appears to be the saponin fraction. Recent studies have shown that saponins can inhibit *in vitro* growth of *E. coli* and alter the rumen microflora *in vivo*. The objective of the present study was to determine the effects of dietary supplementation with a crude QS extract on growth performance and immune function of weanling pigs challenged with ST.

Procedures

The experimental protocol used in this study was approved by the KSU Institutional Animal Care and Use Committee. The 96 pigs (initially 19 lb and 17 d of age) were blocked by initial weight, equalized for sex, and allotted randomly to one of eight treatments in a 28 d growth assay. Each treatment had six replicates (pens) with two pigs per pen.

The eight treatments were arranged in a 2 × 4 factorial with main effects of disease challenge (control or ST) and dietary treatment (Table 1; 0, 4, 8, or 16 oz/ton of added QS). The QS extract used in this study was obtained from Desert King, Inc., Chula Vista, California.

All pigs were housed in two similar environmentally controlled rooms according to disease challenge. Pens contained one self-feeder and one nipple waterer to provide ad libitum access to feed and water. Prior to the start of the study, fecal samples were

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taken to ensure that all pigs were free of *Salmonella*. Pigs were weighed and feed disappearance was measured on d 0, 7, 14, 21, and 28 to determine ADG, ADFI, and F/G. On d 14, each pig housed in the ST room (n=48) were orally gavaged with approximately 10.5×10^9 CFU of *S. typhimurium* in 10 ml of growth medium. Each pig housed in the control room (n=48) received a similar volume of sterile growth medium. Rectal temperature was measured on one pig per pen through 7 d after challenge. Daily feed intake also was monitored through 7 d after challenge. On d 0, 7, and 14 with respect to challenge, serum samples were obtained from one pig per pen and analyzed for haptoglobin. On d 7 and 14 after challenge, fecal samples were obtained from all pigs and cultured for *S. typhimurium*.

Data were analyzed as a 2 × 4 factorial in a randomized complete block design replicated over time using the mixed model procedure of SAS. All means presented are least-square means.

Table 1. Diet Composition (As-Fed Basis)

Ingredient	% of Diet
Corn	51.72
Soybean meal (46.5% CP)	27.86
Spray-dried whey	10.00
Select menhaden fish meal	4.50
Choice white grease	3.00
Monocalcium phosphate	1.20
Limestone	0.68
Salt	0.35
Vitamin premix	0.25
Trace mineral premix	0.15
L-Lysine HCl	0.15
Cornstarch[a]	0.10
DL-Methionine	0.05

[a]*Quillaja saponaria* extract replaced cornstarch to provide the experimental treatments.
Diet was formulated to contain 1.40% lysine, 0.90% Ca, and 0.79% P.

Results and Discussion

No differences (P>.10) in ADG, ADFI, or F/G occurred between dietary treatments (Table 2). However, a challenge by time interaction (P<.0001) was observed (Table 2). Prior to challenge, ADG, ADFI, and F/G were similar between control and ST-challenged pigs. However, ST challenge resulted in reduced ADG (P<.0001), ADFI (P<.0001), and F/G (P<.0001) as compared to controls during wk 3 of the study. The negative impact of ST challenge on growth performance was resolved quickly, and ADG, ADFI, and F/G did not differ between control and ST-challenged pigs during wk 4.

During wk 1 after challenge, a challenge by day interaction (P<.0001) affected daily feed intake and rectal temperature (Table 3). Daily feed intake for the ST-challenged pigs dropped dramatically between 24 to 48 h after challenge, remained depressed through 5 d after challenge, and returned to levels comparable to controls by d 6 after challenge. Rectal temperature of control pigs remained constant during the 7 d after challenge, but ST challenge resulted in a febrile response. Rectal temperature of ST-challenged pigs was higher (P<.05) on d 1 to 4 after challenge, but returned to levels similar to controls by d 5 after challenge.

A challenge by day interaction (P<.0001) affected serum insulin-like growth factor-1 (IGF-1) concentrations (Table 4). IGF-1 did not differ between control and ST-challenged pigs prior to challenge. *Salmonella typhimurium* challenge resulted in a reduction in circulating IGF-1 on d 2 and 4 after challenge. Although IGF-1 in ST-challenged pigs began to increase by d 6, it was still lower (P<.05) than that of controls.

A challenge by time interaction (P<.002) affected serum haptoglobin concentration (Table 5). *Salmonella typhimurium* challenge produced a rise (P<.0001) in serum haptoglobin on d 7 after challenge, but levels were comparable to those of controls by d 14 after challenge.

On 7 d after challenge, one control pig cultured positive for *Salmonella*. However, the rectal temperature of this pig remained constant through 7 d after challenge, and serum haptoglobin levels on d 7 and 14 after challenge were actually lower than the pre-challenge level. Therefore, we were satisfied that biosecurity was maintained and attribute the positive culture to laboratory error. On d 7 after challenge, 68.75% (33/48) of the ST-challenged pigs had a positive culture for *Salmonella*. At 14 d after challenge, the percentage of ST-challenged pigs shedding *Salmonella* had dropped to 20.83% (10/48), and no control pigs had positive culture results.

No differences in total white blood cell count, red blood cell count, hemoglobin, hematocrit, or phagocytic function of peripheral white blood cells isolated from ST-challenged pigs occurred on d 6 versus d 13 after challenge (data not shown). A diet effect ($P<.05$) was observed. The higher inclusion levels of QS (8 and 16 oz/ton) appeared to depress phagocytic function of peripheral white blood cells (Table 6).

The results of this trial agree with previous studies using this disease challenge model to document the detrimental effects of enteric infection on growth performance, feed intake, and IGF-1. In contrast to a similar study by our laboratory using this same model of disease challenge, compensatory gain and improved F/G for ST-challenged pigs during the second wk after challenge were not observed. However, growth performance of ST-challenged pigs was comparable to that of controls during wk 4 of the study. This was accompanied by a decrease in serum haptoglobin and the return of IGF-1 to prechallenge levels. This further supports the concept that an acute enteric disease challenge in weanling pigs results in only transient alterations in growth performance.

Quillaja saponaria extract appears to influence phagocytic cell function in a quadratic fashion (4 oz vs. 8 or 16 oz/ton). From a physiological perspective, this impact of QS supplementation seems marginal. Thus, in summary, inclusion of QS, at the levels reported herein, apparently has little benefit on growth performance or immune function in unchallenged controls or ST-challenged pigs.

Table 2. Effects of *Salmonella* Challenge and Dietary QS on Growth Performance of Weanling Pigs

	Salmonella			Dietary Level of QS (oz/ton)				
Item	Control	Challenge	SEM	0	4	8	16	SEM
Day 0 to 7								
ADG, lb	.88[a]	.90[a]	.050	.91	.85	.93	.88	.067
ADFI, lb	1.12[a]	1.13[a]	.060	1.08	1.04	1.28	1.11	.077
F/G	1.29[a]	1.27[a]	.067	1.20	1.24	1.38	1.29	.095
Day 8 to 14								
ADG, lb	1.21[bd]	1.33[d]	.050	1.21	1.31	1.24	1.32	.067
ADFI, lb	1.81[e]	1.96[b]	.060	1.87	1.85	1.90	1.92	.077
F/G	1.53[b]	1.48[b]	.067	1.60	1.42	1.55	1.46	.095
Day 15 to 21								
ADG, lb	1.55[c]	.90[a]	.050	1.21	1.21	1.21	1.26	.067
ADFI, lb	2.32[c]	1.71[e]	.060	2.01	1.94	2.10	2.02	.077
F/G	1.52[b]	2.14[c]	.067	1.86	1.84	1.83	1.78	.095
Day 22 to 28								
ADG, lb	1.67[ce]	1.69[e]	.050	1.61	1.76	1.68	1.66	.067
ADFI, lb	2.72[d]	2.73[d]	.060	2.64	2.74	2.85	2.67	.077
F/G	1.64[b]	1.64[b]	.067	1.69	1.56	1.69	1.61	.095

[a,b,c,d,e]Means within rows or columns without common superscripts differ ($P<.05$).

Table 3. Effects of *Salmonella* Challenge on Daily Feed Intake and Rectal Temperature of Weanling Pigs

	Feed Intake, lb		Rectal Temperature, °F	
Day after Challenge	Control	Challenge	Control	Challenge
0	- -	- -	103.6[ad]	103.6[ad]
1	2.23[a]	2.12[ah]	103.5[ad]	104.4[cf]
2	2.15[ah]	.87[e]	103.3[ab]	104.6[c]
3	2.28[ac]	1.16[f]	103.3[ab]	104.1[fg]
4	2.30[adi]	1.53[g]	103.1[b]	103.9[dg]
5	2.59[b]	1.90[h]	103.4[abe]	103.6[ad]
6	2.33[ab]	2.08[ah]	103.2[ab]	103.8[deg]
7	2.50[bci]	2.34[ab]	103.6[ad]	103.8[deg]

a,b,c,d,e,f,g,hMeans within rows or columns without common superscripts differ (P<.05); feed intake SEM = ±0.113; rectal temperature SEM = ±0.170.

Table 4. Effect of *Salmonella* Challenge on IGF-1 Concentration (ng/ml) of Weanling Pigs

Day after Challenge	Control	Challenge
0	149.18[a]	180.92[ab]
2	161.94[a]	46.87[c]
4	154.93[a]	50.81[c]
6	206.09[b]	149.87[a]

[a,b,c]Means within rows or columns without common superscripts differ (P<.05); SEM = ± 19.351.

Table 5. Effect of *Salmonella* Challenge on Haptoglobin Concentration (mg/dl) of Weanling Pigs

Day after Challenge	Control	Challenge
0	23.83[a]	22.92[a]
7	21.25[a]	35.96b
14	19.50[a]	22.25[a]

[a,b]Means within rows or columns without common superscripts differ (P<.05); SEM = ± 2.599.

Table 6. Effect of Dietary *Quillaja saponaria* (QS) on Phagocytic Function and Oxidative Burst Activity of Phagocytic Cells from Weanling Pigs Challenged with *Salmonella typhimurium*

Diet (oz/ton QS)	% of Cells Reacting
0	37.25[ab]
4	40.46[b]
8	31.78[a]
16	34.09[a]

[a,b]Means without common superscripts differ (P<.05); SEM = ± 2.539.