The effects of a quillaja and yucca combination on performance and carcass traits of coccidia-vaccinated broilers exposed to an enteric disease challenge

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ABSTRACT A series of 6 floor pen trials was conducted to determine the effects of a quillaja and yucca combination product on the performance and carcass traits of growing broiler chickens vaccinated for coccidiosis at the hatchery. In each of the trials graded levels (0, 250, and 500 ppm) of a quillaja and yucca combination (**QY**) were fed to Ross 708 broilers for the duration of each 42 d test. Trials were arranged in completely randomized block designs involving a minimum of 11 blocks per trial. At the start of each trial, pens contained 55 broilers. In order to provide each bird with an enteric disease challenge, 5 kg commercial broiler litter containing 10^4 CFU *Clostridium perfringens* per gram was placed in each pen. In addition, the sporulated oocysts of *Eimeria acervulina* and *E. maxima* were added to each pen at the outset of each test. At d 21 of the trials, coccidial lesion scores, mortality and performance were determined; final performance and total mortality were assessed at 42 d. At the completion of each test, 10 birds of average body weight per pen were selected for carcass evaluations; whole and chilled carcass yield were determined, and pre- and post-chill breast measurements were made. A combined analysis of the results of the 6 trials (75 replications per treatment) was used to determine treatment effects and each variable was assessed by linear regression analysis. Results indicated that QY significantly reduced mortality and coccidial lesions scores at d 21 ($P < 0.05$). Performance was significantly improved by both levels of QY at 21 and 42 d, and significant linear effects were observed for these variables ($P < 0.05$). All carcass characteristics were significantly improved by QY administration and significant linear responses were observed for each carcass variable ($P < 0.05$). These results indicate that by reducing intestinal disease challenge, QY provided linear improvements in performance. In addition, QY positively affected carcass parameters as each variable responded linearly to QY feeding ($P < 0.05$).

Key words: quillaja and yucca saponins, performance, carcass yield, coccidiosis, necrotic enteritis

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INTRODUCTION

Saponins are natural products of plant origin that are known to display many biological activities. The chemical structures of these molecules contain both fat soluble and water soluble moieties, and as a result, they have the ability to influence many biological processes and affect many disease organisms (Francis et al., 2002). It is believed that their natural role in plants is to prevent invasion by pathogens (Sparg et al., 2004) and this in turn, has led to study of these molecules as potential antimicrobials and antiparasitics. Reviews of the subject indicate that antiprotozoal effects are widely recognized and appear to be related to pore formation and instability of the parasitic cell membrane (Augustin et al. 2011; Fleck et al., 2019). Saponin-induced lytic effects on bacteria have also been described, but modes of action in bacterial species are not well-understood (Hassan et al., 2010). Cheeke (2000) noted that the effects described above lend themselves well for use as feed additives in animal production, and it is noteworthy that since the reduction in antibiotic use in animal production, specific saponins have be used precisely as Cheeke (2000) intended.

Commercially available saponins are most commonly sourced from plants that thrive in arid environments. *Quillaja saponaria*, the Chilean soap bark tree and *Yucca schidigera* plants are common to the deserts of South and North America, respectively, and are raised commercially because of their high saponin content (Cheeke, 2000; Sparg et al., 2004). Saponins from these

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sources are used in many animal-related applications, and in poultry production are often used to promote intestinal health and immunity (Oelschlager et al., 2019). Reports describing their effects on *Eimeria* spp. and improved performance of coccidia-vaccinated broilers are recent evidence of these responses (Bafundo et al., 2020; 2021a).

Necrotic enteritis (**NE**) and coccidiosis are among the most economically significant diseases affecting broiler chickens (Skinner et al., 2010 and Blake et al., 2020). Although coccidiosis has long been considered a disease of primary importance, reduced usage of antibiotics in recent years has raised the profile of NE and today, it is among the diseases of greatest importance in broiler production (Hofacre et al., 2018). Coccidial infections in broilers are ubiquitous and the damage to the intestinal tract caused by their development is the primary predisposing factor to the onset of NE (Collier et al., 2008; Timbermont et al., 2011). Together, these conditions are known to affect performance and increase mortality (Hofacre et al., 2018). However, damage to the intestinal mucosa also impairs nutrient absorption (Rochell et al., 2016; M'Sadeq et al., 2015a), and as a result, carcass and parts yield are also affected (Oikeh et al, 2019; Ahiwe et al, 2019). Because recent research has shown that a saponin combination of quillaja and yucca (**QY**) impaired the development of *Eimeria* and reduced mortality associated with NE infections in broilers (Bafundo et al., 2020; 2021b), further investigations into the effects of QY on carcass yield were warranted. Thus, the objectives of the current research were to determine whether graded levels of QY (0, 250, and 500 ppm) would improve performance and carcass yield of coccidia-vaccinated broilers reared under an intestinal disease challenge produced by *Eimeria* spp. and *Clostridium perfringens.*

MATERIALS AND METHODS

The product tested in this series of trials was a combination of quillaja and yucca saponins that was sourced from *Quillaja saponaria* trees and *Yucca schidigera* plants. The quillaja and yucca combination was a commercially prepared product (Magni-Phi, Phibro Animal Health Corp., Teaneck, NJ) that consists of 100% ground plant material that is formulated in a proprietary ratio where quillaja is the major component. No excipients, carriers or extracted materials are used in the product. Appropriate amounts of the finished product were added to each ton of feed to achieve the intended dosages used in the tests.

The experiments carried out in this trial series were conducted at AHPharma, Inc., Hebron, MD. All birds used in these tests were reared under the animal welfare guidelines specified by the Animal Care and Use Committee of AHPharma, Inc. Birds were humanely euthanized by cervical dislocation using procedures approved by the committee noted above. In the analysis of carcass parameters, commercial welfare practices were applied in all situations, and these procedures were approved and monitored by the same committee.

A series of 6 floor pen studies was carried out to evaluate graded levels of QY (0, 250, and 500 ppm) on performance and mortality, and to determine whether these levels of supplementation influenced carcass yield. All birds in all studies were vaccinated for coccidiosis at the hatchery with Coccivac B52 (Merck Animal Health, Madison, NJ) using procedures recommended by the manufacturer. Each of the 6 trials contained the following three treatments: 1) a control group in which birds were vaccinated for coccidiosis and fed a standard feed devoid of anticoccidials and QY, 2) birds vaccinated for coccidiosis as above and fed QY (250 ppm), or 3) birds vaccinated for coccidiosis and fed QY (500 ppm). QY was fed for the duration of each 42-d growth period. The individual pen results for these treatments in each of the 6 studies were combined so that comparisons between the treatments could be made. At the outset of each test, pens contained 55 Ross 708 broilers. Completely randomized block designs were used in each experiment, where a minimum of 11 blocks per trial were employed. The combined analyses carried out from these studies represent a total of 75 replicates of each treatment.

Diets used in the tests were standard corn-soy based commercial formulations intended to meet the nutrient requirements of growing broilers (National Research Council, 1994). Nutrient profiles and proximate analyses

Table 1. Dietary formulas and proximate analyses used in the evaluation of a quillaja and yucca combination on performance and carcass yield of broilers reared for 42 d.

Ingredient	Starter[1] %	Grower[1] %	Finisher[1] %
Corn	57.80	62.90	65.28
Soybean meal (47.6% CP)	35.75	31.07	28.95
Animal and vegetable fat	0.45	0.66	1.15
Limestone	1.36	1.25	1.18
Dicalcium phosphate	2.29	1.94	1.46
NaCl	0.60	0.55	0.50
Choline chloride 70%	0.023	0.022	0.012
D,L-methionine	0.83	0.75	0.65
L-lysine HCl	0.40	0.36	0.30
Vitamin premix[2]	0.05	0.05	0.05
Mineral premix[3]	0.075	0.075	0.075
	Proximate nutrient analysis		
Protein %	22.56	20.11	18.78
Gross energy (kcal/kg)	4,354	4,372	4,450
Metabolizable energy (kcal/kg)	3,128	3,142	3,197
Fiber %	2.26	2.09	2.17
Ash %	5.28	4.80	4.67
Calcium %	0.97	0.91	0.86
Phosphorus %	0.83	0.80	0.70

[1]QY (Magni-Phi) was added at the appropriate level (0, 227, or 454 g per ton) to achieve the intended doses of 0, 250, or 500 ppm of product in each feed.

[2]Each kg of vitamin premix contained: 17,600,000 I.U. Vit. A; 66,00,000 I.U. Vit. D3; 110,000 IU Vit. E; 137.8 mg 25-hydroxy vit.D_3; 2,910 mg menadione; 4,400 mg Vit. B1; 15,400 mg riboflavin; 7,000 mg Vit. B6; 26.5 mg Vit. B12; 88,000 mg niacin; 397 mg biotin; 1,764 mg folic acid; 24,000 mg d-pantothenic acid.

[3]Each kg of mineral premix contained: 30 mg ferrous iron; 2 mg ferric iron; 85 mg zinc; 115 mg manganese; 5 mg copper; 1.5 mg iodine; 0.3 mg selenium; 0.18 mg magnesium.

for starter, grower, and finisher feeds are presented in Table 1. Starter feeds were fed from placement until d 14, and grower feeds were administered to d 28. Finisher diets were provided from d 29 to 42. All feeds were assayed for saponin content using an assay developed specifically for the saponins contained in QY. All assay results were within acceptable ranges of the intended dosages. Antibiotics were not administered during these tests and management procedures used in all the trials were consistent with methods used in US broiler production.

The litter conditions used in each of the individual trials were designed to induce an enteric disease challenge often experienced in commercial operations where use of Gram positive antibiotics and anticoccidial ionophores had ceased. In addition to the use of live coccidiosis vaccines, all birds in these trials were reared on used litter that was pooled and homogeneously mixed after previous usage. Each pen was then supplemented with approximately 5 kg of litter that was collected from commercial broiler farms in the Delmarva region of the United States. Even though an independent laboratory confirmed that spores of *C. perfringens* were present in this litter, our intent was for this supplemental litter to contain 10^4 CFU *C. perfringens* per gram. As a result, each 5 kg aliquot of litter distributed to the individual pens was fortified with a culture of *C. perfringens* needed to achieve this final concentration. The supplemental *C. perfringens* added to this material was confirmed to be *netB*-positive, which, when combined with a coccidial infection, is known to induce NE (Yang et al., 2019). The supplemental contaminated litter was evenly raked into the existing litter in each pen. Prior to the start of each trial, an aqueous solution of sporulated oocysts of *Eimeria acervulina* and *E. maxima* (1×10^5 and 3.5×10^4 per bird, respectively) was sprayed onto the litter of each pen. The pathogenic nature of this challenge was confirmed through scoring of coccidial and clostridial lesions at several points in each trial. As an indicator of intestinal challenge, the mean mortality of the control treatment was 2 to 3 times greater than American industry standards. The majority of the mortality occurred during the first 21 days of testing.

Body weight gain and feed conversion values were determined at both 21 and 42 d; all performance data were corrected for mortality. Bird mortality was evaluated daily, but is expressed as percent mortality from all causes recorded from d 0 to 21 and percent total mortality throughout the test period (d 0−42). Coccidial lesion scores were evaluated in 3 birds per pen on d 21 using the procedures described by Johnson and Reid (1970). Following the determination of final body weights, 10 birds of average weight were selected from each pen for carcass yield and further processing evaluations. These birds were reared for an additional day, consuming the diets to which they were originally assigned. Broilers designated for carcass yield evaluation were fasted for 8 h prior to electrical stunning and exsanguination; they were then mechanically picked and eviscerated. Carcasses were chilled in an ice cold water bath for approximately 1 h, after which post-chilling measurements were made.

Statistics

The statistical analyses presented herein represent data from 6 trials collected over a 2-yr period. Since experimental treatments, animals, pens, facilities, rations, and study methods in each test were the same, data from the 6 trials were combined (75 replicate pens per treatment). The linear effects of graded QY levels on each metric were tested using linear regression analysis. The linear models can be written as $y_{metric} = \beta_0 + \beta_1 * QY\ level + \beta_2 * Block + \beta_3 * Study + \epsilon$ where y_{metric} represents the performance, mortality or carcass yield metric tested, *Study* and *Block* represent the study and block respectively, of the observed data, and *QY level* is the level of QY dosage.

Statistical differences between individual graded level QY treatments were tested by the least significant difference (**LSD**) method. The LSD models incorporated block and study effects and the underlying model can be written as $y_{metric} = \beta_0 + \beta_1 * Treatment\ Group + \beta_2 * Block + \beta_3 * Study + \epsilon$. The LSD tests were implemented using the R package *agricolae* (di Mendiburu, 2020). Multiple comparisons were controlled by incorporating the Holm-Bonferroni procedure into the LSD tests. Compared to the Bonferroni test, the Holm-Bonferroni procedure offers improved statistical power while still controlling family wise error rates. It is valid under the same conditions as the Bonferroni method (Holm, 1979). In all cases, statistical differences were established at $P < 0.05$. All statistical analyses were conducted in the R statistical language (R Core Team, 2020).

RESULTS AND DISCUSSION

Recent assessments by Hofacre et al. (2018) indicate that NE is one of the major diseases affecting commercial broiler production. In recent years, diminished use of growth promoting antibiotics has certainly been major contributor to this development. However, with the implementation of antibiotic free marketing programs and a decrease in the usage of ionophore anticoccidials, the significance of coccidial infection and its influence on the occurrence of NE has also increased. As shown by Collier et al. (2008) and Timbermont et al. (2011), coccidial infection serves as the primary predisposing factor to the onset of NE. Together, these entities are considered a multifactorial disease complex, where *E. acervulina* and *E. maxima* promote the clostridial proliferation that affects bird performance and increases flock mortality (Hofacre et al., 2018).

The trials presented in the current report were designed to simulate the commercial conditions under which NE develops in young broiler chickens. That is,

Table 2. The effects of graded levels of quillaja and yucca combination (QY) on coccidiosis lesion scores and interim and final mortality of floor pen-raised broilers exposed to an enteric disease challenge[1].

QY level ppm	Coccidial lesion scores[2] d 21	Total mortality[3] d 21	Total mortality[3] d 42
0	1.35[a]	7.30[a]	9.06[a]
250	0.88[b]	2.12[b]	2.76[b]
500	0.27[c]	1.60[b]	1.65[b]
PSEM[4]	0.048	0.269	0.298
Linear coefficient[5]	−0.002*	−0.015*	−0.018*

[1]Data are the results of 6 pooled pen trials representing a total of 75 replications per treatment. Means were separated by applying the Holm-Bonferroni procedure to the least significant difference test.

[2]Coccidial lesion scores are the average scores for *E. acervulina* and *E. maxima* lesions.

[3]Percent mortality from all causes.

[4]Pooled standard error of the mean.

[5]Linear coefficients are the coefficients of QY in linear models of the effects of QY level on coccidial lesion scores and mortality. Coefficients designated with * (asterisk) indicate significant linear effects ($P < 0.001$).

[abc]Means within columns showing different superscripts are significant ($P < 0.05$).

broilers were reared on litter containing both *C. perfringens* and the intestinal *Eimeria* species known to play a major role in the induction of NE (Hofacre et al., 2018). The 21 d coccidial lesion scores determined for control treatments (Table 2) verified the presence of *E. acervulina* and *E. maxima* in the environment. Additionally, the severity of the coccidial lesions produced in these trials was appropriate for maximizing the incidence of NE (Yang et al. (2019). Table 2 also demonstrates that during the initial 21 days of the trials, more than 7% mortality occurred in the control treatments. While this figure is 2 to 3 times greater than commercial industry averages, it is typical for cases where acute NE has occurred (M'Sadeq et al., 2015b; Hofacre et al., 2018), and is a reasonable representation of commercial mortality following the elimination of growth promoting antibiotics and anticoccidial ionophores (Hofacre et al., 2018). Table 2 also illustrates that both coccidial lesions and total mortality were significantly reduced by QY administration ($P < 0.05$), an observation that has been made in several previous reports (Bafundo et al., 2021a,b).

The pooled performance results of these trials are presented in Table 3 and indicate that under the challenge conditions described above, QY significantly improved performance at d 21. Both body weight gain and feed conversion values were improved by each graded level of QY, and significant linear responses were recorded for these variables. Combined results show that similar effects were recorded at d 42. Responses of this type have been reported previously and are consistent with effects of QY measured during coccidial (Bafundo et al., 2020) and bacterial challenges (Bafundo et al., 2021b). Because studies have shown that quillaja saponins can affect the integrity of the parasitic cell membrane (Augustin et al, 2011; Fleck et al., 2019), this effect could be involved in reducing the reproductive capacity of *Eimeria* spp. shown to occur in both current and previous trials (Bafundo et al., 2020). However, the effects of QY on bacterial populations are not as well understood, and the exact means by which bacterial populations are affected is not known (Hassan et al., 2010). Despite this gap in understanding, it seems likely that diminished exposure to these pathogens was a contributor to performance improvements recorded during these experiments.

Consistent with these responses were improvements in whole carcass and chilled carcass yield (Table 4). Analysis of breast meat, whether as a percentage of live weight

Table 3. The effects of graded levels of quillaja and yucca combination (QY) on interim performance, final performance and feed consumption of coccidiosis vaccinated broilers reared under enteric disease challenge[1].

	Body weight gain (g)	Feed intake (g)	FCR (g:g)	Body weight gain (g)	Feed intake (g)	FCR (g:g)
QY level ppm		D 21			D 42	
0	759[c]	1,073	1.387[a]	2,549[c]	5,019	1.900[a]
250	805[b]	1,076	1.330[b]	2,641[b]	5,032	1.836[b]
500	835[a]	1,084	1.293[c]	2,672[a]	5,000	1.810[c]
PSEM[2]	2.526	3.417	0.004	6.834	13.868	0.005
Linear coefficient[3]	0.139*	−0.001	−0.0002*	0.367*	−0.002	−0.0002*

[1]Data are the results of 6 pooled pen trials representing a total of 75 replications per treatment. Performance data were corrected for mortality. Used commercial broiler litter supplemented with *Eimeria* oocysts was used to induce the enteric challenge. Means were separated by applying the Holm-Bonferroni procedure to the least significant difference test.

[2]Pooled standard error of the mean.

[3]Linear coefficients are the coefficients of QY in linear models of the effects of QY level on performance variables. Coefficients designated with * (asterisk) indicate significant linear effects ($P < 0.001$); those without * (asterisk) are not significant ($P > 0.05$).

[abc]Means within columns showing different superscripts are significant ($P < 0.05$).

Table 4. The effect of graded levels of quillaja and yucca combination (QY) on whole carcass and breast yield variables determined at 42-d in 6 pooled floor pen trials[1].

QY level ppm	Percent Whole carcass yield	Percent Chilled carcass yield	Breast yield as a percent of live weight	Breast yield as a percent of carcass weight	Breast yield as a percent of chilled carcass weight
0	67.582[c]	69.164[c]	15.618[c]	23.176[c]	22.635[c]
250	69.205[b]	70.793[b]	16.598[b]	24.042[b]	23.493[b]
500	71.151[a]	72.844[a]	17.686[a]	24.956[a]	24.360[a]
PSEM[2]	0.141	0.141	0.045	0.053	0.051
Linear coefficient[3]	0.007*	0.007*	0.003*	0.002*	0.002*

[1]Treatment means are the results of 6 pooled pen trials representing a total of 75 replications per treatment where 10 birds per pen were assessed for carcass traits. Means were separated by applying the Holm-Bonferroni procedure to the least significant difference test.
[2]Pooled standard error of the mean.
[3]Linear coefficients are the coefficients of QY in linear models of the effects of QY level on carcass variables. Coefficients designated with * (asterisk) indicate significant linear effects ($P < 0.001$).
[abc]Means within columns showing different superscripts are significant ($P < 0.05$).

or before or following chilling, showed that graded levels of QY linearly improved breast meat yield (Table 4). At face value, carcass yield is often highly correlated with final body weight and examination of the QY-induced changes body weight gain (Table 3) suggests that the carcass yield responses shown in Table 4 were a function of the effect of QY on body size.

However, many recent studies have shown that the apparent ileal digestibility of energy, nitrogen, and amino acids is impaired during intestinal coccidiosis (Adedokun et al., 2016; Rochell et al., 2017). These effects result from damage to the absorptive surface during parasite development, and are known to influence carcass yield (Oikeh et al., 2019). Recent work also shows that effects of this nature occur in mild coccidial infections, such as those following coccidial vaccination (Lehman et al., 2009; Cloft et al., 2019). NE also influences nutrient digestibility (M'Sadeq et al., 2015a; Hilliar et al., 2020), but its effects on the absorptive surface are the result of bacterial toxins generated at the site of infection that induce epithelial cell death (Timbermont et al., 2011). Therefore, both coccidial infection and NE influence carcass and parts yield as a result of the structural changes to the intestine that affect the absorptive efficiency of the epithelium (Oikeh et al., 2019; Ahiwe et al., 2019).

In the experiments conducted in the current study, QY significantly reduced coccidial lesion scores and total mortality, and these observations underscore the ability of QY to improve intestinal health in the face of a multifactorial intestinal disease challenge. Since a healthier intestinal tract absorbs nutrients more efficiently, the ultimate effect of these improvements is manifested in the analysis of carcass traits where QY increased total carcass yield, chilled carcass yield, and all breast yield measurements.

Live coccidial vaccination was used in these experiments as a commonly-applied technique for managing coccidiosis. It is well-recognized however, that live vaccines applied at day-of-age act as intestinal stressors that are capable of reducing growth rate and impairing feed conversion (Chapman et al., 2002; Lee et al., 2011). Lehman et al. (2009) and Cloft et al. (2019) have noted that coccidial vaccination can also affect carcass yield. These effects occur as a result of damage to the intestinal epithelium brought about by parasite development required for immune stimulation. As mentioned above, nutrient digestibility is affected in this process, and feed intake is also impaired (Cloft et al., 2019). While it is clear that these processes may influence the uptake of many different nutrients (Rochell et al., 2017), protein and amino acid digestibility appear to be very sensitive to these changes (Lee et al., 2011; Cloft et al., 2019). One outcome of a decrease in protein availability is a reduction in muscle accretion. Indeed, protein and amino acid supplementation has been shown to offset these coccidia-induced effects, resulting in greater body weights and improved carcass responses of coccidia-vaccinated broilers (Lee et al., 2011; Mussini et al., 2012; Cloft et al., 2019).

In the current study, the coccidia-vaccinated, disease challenged broilers receiving QY showed consistent growth responses and there was no indication of changes in feed intake at any point in our testing. Moreover, all carcass variables responded positively to QY. These facts lend support to both field and research observations showing that the untoward effects of coccidiosis vaccination described above are offset in the presence of QY. Whether this response is an effect of diminished coccidial cycling (Bafundo et al., 2021a), better nutrient utilization or a combination of both factors has not been determined, but further experimentation holds promise for a reasonable explanation of these effects. Along these same lines, QY has been shown to improve the apparent total tract digestibility of dry matter, organic matter, fat, and ash, and to increase nitrogen retention of growing broilers (Bafundo et al., 2021c). These findings help to explain the improvements in performance and carcass yield of the disease-challenged coccidial vaccinates shown in the current study and add additional support to the intestinal benefits of QY in the growing broiler.

In summary, the results presented herein indicate that under conditions of a multifaceted intestinal disease challenge, QY improved the growth performance of coccidial-vaccinated broilers. Coincident with these effects were improvements in all carcass variables that were measured. While a portion of these responses is attributable to a reduction in the enteric pathogen load and its subsequent effects on improved growth, previous work has shown that QY positively affected nutrient digestibility. The combination of these effects produced a healthier intestine where better growth and improved carcass characteristics result.

DISCLOSURES

There are no conflicts of interest associated with this work.

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