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Research Article

The Effects of a *Quillaja/Yucca* Saponin Combination on Performance, *Clostridium perfringens* Counts and Percentage of *Salmonella* Positive Broiler Chickens

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Abstract

A series of four floor pen trials was carried out to determine the effects of a *Quillaja* and *Yucca* combination product (QY) on growth performance of broiler chickens reared in a high disease challenge environment. The trials also determined number of *Clostridium perfringens* in faeces and percentage of birds harboring *Salmonella* in their intestines. In each trial, used litter from farms known to have had outbreaks of necrotic enteritis and difficulties with *Salmonella* was used as bedding material. QY was administered in feed at 0, 250 and 500 ppm and fed for the duration of the 42-day tests. Data from the four studies were pooled and performance and total mortality were evaluated at 21 and 42 days of age. Intestinal and faecal samples were collected at the same intervals in order to quantify faecal *Clostridium perfringens* counts and the incidence of *Salmonella.* Results demonstrated that QY produced significant linear improvements ($P < 0.001$) in performance and mortality. At both 21 and 42 days, significant linear reductions ($P < 0.001$) in numbers of faecal Clostridia were observed and the percentage of birds harboring *Salmonella* was reduced. These results indicate that in addition to improving bird performance and mortality, QY may reduce the levels of important bacteria that adversely affect bird performance and influence the safety and acceptability of poultry meat products.

***Keywords**: Saponins; Quillaja/Yucca Combination; Broiler Performance; Clostridium perfringens; Salmonella*

Abbreviations

QY: *Quillaja Yucca* Combination; FCR: Feed Conversion Ratio; CFU: Colony Forming Unit; TSC: Tryptose Sulphite Cycloserine Agar; TT: Tetrathionate Broth; XLT4: Xylose Lysine Tergitol-4 Agar; BG Sulfa: Brilliant Green Sulfa Agar; PSEM: Pooled Standard Error of the Mean

Introduction

Saponins are natural compounds of plant origin that have been shown to produce a wide variety of biological effects [7]. Saponin-induced effects are associated with unique molecular structures that contain both water soluble and fat soluble moieties, which impart detergent-like characteristics to these molecules [11]. In plants, these properties are thought to prevent invasion by pathogens and to thwart insect attack [20]. As a result, saponin research has intensified in recent years due to their potential development as antiparasitic and anti-infective agents, and for their ability to influence animal growth [11].

Certain plants are known to be especially rich in saponin content. According to Cheeke [7], *Quillaja saponaria,* the Chilean soap bark tree and *Yucca schidigera*, a plant of the arid regions of the American southwest, have become major sources of commercially used saponins. Due to their chemical properties, saponins from these plants are used in detergents, cosmetics and soft drinks [7]. In addition, their pharmacologic properties have been examined and they are known to affect serum cholesterol, modify ammonia production and influence the integrity of biological membranes [2,11]. Fleck., *et al*. [10] have also indicated that *Quillaja* saponins stimulate the immune response and their use as adjuvants in human and veterinary vaccines is now common [15].

Of many anti-infective activities examined, those dealing with antiprotozoal effects are best-characterized. Activity against *Giardia, Leishmania* and *Plasmodium* has been reviewed by several authors [7,11] and supported with recent reports on *Trichomonas* [18] and *Eimeria* [5]. Overall, activity against protozoal species is thought to be widespread [11]. In contrast, comparatively few reports address the antimicrobial effects of *Quillaja* and *Yucca* saponins, and our understanding of activities, modes of action, and effective dosages is lacking. Reports are largely confined to a few *in vitro* studies of indicator bacteria such as *Staphylococcus aureus* and *Escherichia coli* [12,19]. Of significance is the fact that few of these studies address bacteria known to influence performance and disease incidence in production animals.

As part of a developmental program focusing on the feed application of a *Quillaja* and *Yucca* combination product (QY) in broiler chickens, a series of studies was carried out to quantify changes in bird performance brought about by QY feeding in a disease challenged environment. Because bacteria were part of this challenge, assessment of bacterial changes was also made.

Objective of the Study

The objectives of the current studies were to evaluate the effects of graded feeding levels of QY (0, 250 and 500 ppm) on performance and total mortality of broilers reared under disease challenge, and to determine whether QY feeding exerted any effects on the prevalence of *Clostridium perfringens* and *Salmonella*, bacteria deemed to be of critical importance in commercial broiler production.

Materials and Methods

Testing of the combination of *Quillaja* and *Yucca* (QY) used in these trials involved a commercially prepared product (Magni-Phi®, Phibro Animal Health Corp., Teaneck, New Jersey, USA). The product consists of 100% ground plant material derived from *Quillaja saponaria* trees and *Yucca schidigera* plants formulated in a proprietary ratio where *Quillaja* is the major component. No excipients or carriers are used in the product. Thus, unaltered, unextracted *Quillaja* saponins supplemented with the saponins naturally contained in *Yucca* comprise the active ingredients of the product. These substances constitute about 4% of the total product. All trials were conducted at AHPharma, Inc., Hebron, Maryland USA. The Animal Care and Use Committee of AHPharma, Inc. approved all procedures used in the trials. Birds were humanely euthanized by cervical dislocation using procedures approved by the committee noted above.

A series of four floor pen studies was carried out to evaluate graded levels of QY on performance and mortality, and to determine intestinal and fecal levels of *Salmonella* and *Clostridium perfringens*, respectively. In these trials QY was fed at 0, 250 and 500 ppm throughout each 42-day test. In one trial, only 0 and 250 ppm were evaluated. At the outset of each test, pens contained 55 Cobb 500 broilers. Completely randomized block designs were used in each test, where at least 10 blocks per trial were employed. Data pooled from these studies represent a total of 50 replicates of each treatment.

A feeding program utilizing 3 rations (starter, grower and finisher) was used in each test. All rations were maize-soy based and were designed to meet the nutritional requirements of growing broilers as determined by the National Research Council [16]. The tests were conducted in a high disease challenge environment employing used commercial broiler litter known to contain the spores of *Clostridium*

perfringens and a variety of *Salmonella* serotypes. This litter originated on commercial broiler farms known to have had difficulties with necrotic enteritis and *Salmonella* contamination. An independent laboratory confirmed the presence of clostridial spores and determined that 5 *Salmonella* serotypes (Enteritidis, Typhimurium, Kentucky, Indiana and Heidelberg) were present. The contaminated litter was distributed equally among the pens of the research facility. Prior to the start of each trial, additional sporulated oocysts of *Eimeria acervulina* and *E. maxima* (1×10^5 and 3.5×10^4 per bird, respectively) were added to each pen. Evaluation of coccidial and clostridial lesions at critical time points in the trials confirmed the pathogenic nature of this challenge. All birds in all trials were vaccinated for coccidiosis at the hatchery with Coccivac B52 (Merck Animal Health, New Jersey, USA).

Performance data were collected at 21 and 42 days of age. Bacterial assessments were carried out from faecal and intestinal samples that were collected on days 21 and 42 of the tests. Numbers of *C. perfringens* were determined using US Food and Drug Administration procedures adapted for fresh faecal samples [4]. Samples were collected from 4 and 10 birds at days 21 and 42, respectively. Each sample was separately diluted 1:10 in peptone dilution fluid; additional ten-fold dilutions were made by adding 1 ml into each dilution. Diluted samples were plated onto tryptose sulphite cycloserine (TSC) agar without egg yolk. Additional liquid TSC agar without egg yolk was then added to each plate so the plated inoculum was completely covered, thereby creating anaerobic growth conditions. After the TSC agar had solidified, each plate was incubated under anaerobic conditions at 35°C for 20 - 24 hours. Following incubation, the numbers of black colonies with an opaque white zone around the colony were counted and recorded. CFUs were determined by colony count and dilution rate. Data were converted to $\log_{10}$ by calculation and reported as $\log_{10}$ CFU per gram of faeces.

Identification of *Salmonella* positive broilers was determined using US Food and Drug Administration procedures adapted for live broiler chickens [3]. Four and 10 randomly selected birds per pen were evaluated on days 21 and 42, respectively; each was killed humanely by cervical dislocation. The procedure nonspecifically recognizes the presence of *Salmonella* organisms in intestinal contents; serotypes were not determined. From each bird selected, 1g of digesta was taken from the ileo-caecal region of the digestive tract and diluted 1:10 in tetrathionate (TT) broth, then homogenized using a vortex. Homogenized dilutions were incubated at 41°C for 24 hours, after which 10 µl of TT broth was plated onto xylose lysine tergitol-4 (XLT4) and brilliant green sulfa (BG Sulfa) agar and incubated at 35°C for 24 hrs. Positive samples were determined by the presence of black or red colonies after incubation on XLT4 media or when pink, opaque colonies were seen on BG Sulfa. Suspected negative plates were re-incubated for an additional 24 hours and rechecked for growth. The percentage of *Salmonella* positive broilers per pen (out of four or ten) was calculated.

Statistics

Data presented herein represent the results of 4 identical trials. Since the experimental designs, animals, pens, facilities, rations and study methods in each test were similar, data were pooled. The linear effects of graded QY levels on each metric were tested using linear regression analysis. The linear models can be written as $y_{metric} = \beta_0 + \beta_1 * QY\ level + \beta_2 * Block\ + \epsilon$ where y_{metric} represents the performance or bacterial metric tested and *QY level* is the level of QY dosage.

Statistical differences between individual graded level QY treatments were tested by applying the Holm-Bonferroni procedure to the Least Significant Difference methodology [14] and implemented using the R package *agricolae* [9]. 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 [14]. In all cases, statistical differences were established at $P < 0.05$. All statistical analyses were conducted in the R statistical language [17].

Results and Discussion

A primary objective of this series of trials was to determine the effects of QY on broiler performance in a high disease challenge environment. Table 1 shows that the overall mortality recorded for control birds in this series of tests was 10.5%. Since this figure is consider-

ably greater American industry averages, the general objective for rearing birds under high challenge was met. In this environment, QY significantly reduced mortality, and both 250 and 500 ppm significantly improved FCR compared to controls. Body weight gain responded in a similar manner. Significant linear effects were observed for each of the performance variables presented, indicating that in this environment, each dose of QY provided improvements compared to lower doses. These results are similar to previous reports [5,6] describing the effects of QY in broilers reared under intestinal challenge.

	Body Weight Gain (g)	FCR (g:g)	Percent Mortality	Body Weight Gain (g)	FCR (g:g)	Percent Mortality
	Day 21			Day 42		
QY Level ppm						
0	762[c]	1.384[a]	8.17[a]	2734[b]	1.911[a]	10.5[a]
250	802[b]	1.323[b]	2.18[b]	2808[a]	1.843[b]	3.1[b]
500	817[a]	1.304[b]	1.82[b]	2832[a]	1.808[c]	2.2[b]
PSEM[2]	7.392	0.017	1.339	30.107	0.022	1.589
Linear Coefficient[3]	0.113*	-0.002*	-0.014*	0.161*	-0.0002*	-0.017*

Table 1: *The effects of graded levels of a Quillaja-Yucca combination (QY) on 21 and 42 day performance and mortality of coccidiosis vaccinated broiler chickens reared under enteric disease challenge[1].*

*[1]Data are the results of four pooled pen trials representing a total of 50 replications per treatment. Means were separated by applying the Holm-Bonferroni procedure to the Least Significant Difference Test. Means within columns showing different superscripts are significant (P < 0.05). [2]Pooled standard error of the mean. [3]Linear coefficients represent the coefficient of QY level in linear models of the effects of QY feeding level on performance metrics and mortality. Coefficients with an * indicate a significant linear effect (P < 0.05).*

Since moderate anticoccidial activity has been associated with feeding the QY combination [5], improved coccidiosis control likely contributed to the performance improvements reported in the current trials. In fact, reports indicate that when combined with a live coccidiosis vaccine, QY reduced coccidial cycling and provided performance responses that exceeded the effects of the coccidial vaccine alone [6]. Thus, improved body weight gain and FCR recorded in the current trials are likely associated with this effect. However, it is well-recognized that even moderate coccidial exposure is frequently followed by bacterial complications, and research has clearly shown the association between coccidial infection and necrotic enteritis [8,21]. In addition, coccidia-induced damage to the intestine is often the portal for systemic *Salmonella* infections in broilers [1]. Thus, bacterial infections following coccidial exposure are often a greater threat to efficient broiler production and food safety than the initial coccidial challenge.

It is therefore important to note that in an environment containing coccidia, clostridia and salmonellae, QY reduced the numbers of *C. perfringens* in fresh faeces and diminished the percentage of birds harboring salmonellae in their intestines (Table 2). In fact, the lower levels of mortality observed in both QY treatments correlate well with reductions in faecal clostridial counts. Both findings are significant because these bacteria pose a meaningful risk to optimal bird performance and the safety of poultry meat products.

	Faecal *Clostridium perfringens* CFU per gram (Log_{10})		*Salmonella* Positive Broilers (Percent)	
	Day 21	Day 42	Day 21	Day 42
QY Level ppm				
0	4.05[a]	4.02[a]	77.4[a]	69.4[a]
250	3.59[b]	3.59[b]	51.4[b]	44.6[b]
500	3.19[c]	3.45[C]	29.4[c]	36.0[c]
PSEM[2]	0.197	0.123	7.393	4.935
Linear Coefficient[3]	-0.002*	-0.001*	-0.097*	-0.068*

Table 2: *The effects of graded levels of a Quillaja-Yucca combination (QY) on 21 and 42 day Clostridium perfringens counts and percent Salmonella positive broilers reared under enteric disease challenge[1].*

[1]Data are the results of four pooled pen trials representing a total of 50 replications per treatment; pen means represent 4 and 10 birds evaluated at days 21 and 42, respectively. Means were separated by applying the Holm-Bonferroni procedure to the Least Significant Difference Test. Means within columns showing different superscripts are significant (P < 0.05).

[2]Pooled standard error of the mean.

*[3]Linear coefficients represent the coefficient of QY level in linear models of the effects of QY feeding level on bacterial measurements. Coefficients with an * indicate a significant linear effect (P < 0.05).*

The *Quillaja* saponins contained in QY are known to exert antiprotozoal effects [2,11,18]. These effects are associated with adhesion of *Quillaja* saponins to the sterol components of the protozoal cell membrane, which is then followed by pore formation and cell lysis [2]. While this effect has been shown in several cell types, evidence of this nature has not been found for bacterial organisms, and to our knowledge, a defined mode of action for *Quillaja* saponins in any bacterial species has not been proposed [13]. Even though several studies have reported activity of *Quillaja* and *Yucca* saponins on *Staphylococcus aureus* and *Escherichia coli* [12,19], studies on the antibacterial effects of saponins are far from conclusive. To our knowledge, only one trial has evaluated activity against *Salmonella typhimurium* [13] and efficacy against *Clostridium perfringens* has not been reported. Moreover, assessment of antimicrobial effects in animals has not been common.

Thus, the bacterial changes demonstrated in our broiler trials are challenging to explain and clearly warrant verification and further investigation. It seems reasonable to suggest that *Quillaja* and *Yucca* saponins exerted an antimicrobial effect on these organisms, but our tests were designed to examine no more than bacterial numbers or incidence in broiler chickens. Since QY also produced significant linear reductions in coccidial lesion scores measured at day 21 (1.71, 1.12 and 0.47 respectively), it may be possible that reduced coccidial exposure affected the numbers of clostridia measured in faeces [8]. However, it is uncertain whether lower lesion scores would affect salmonellae in a similar manner. It may also be possible that the changes recorded in our trials resulted from modification of the intestinal environment since saponins are known to affect several digestive and physiologic processes in the gut [7,11]. Further, the ability of *Quillaja* saponins to promote the development of mucosal immunity is well recognized and is an active area of research [10]. Indeed, *Quillaja* saponins are widely used as adjuvants in both human and veterinary vaccines and are known for their ability to stimulate immunity [10,15].

Conclusion

Consistent with previous reports, data contained herein illustrate that under conditions of intestinal disease challenge, QY at both 250 and 500 ppm improved broiler performance and reduced total mortality. In all cases, significant linear effects occurred. These improvements were coincident with significant reductions in numbers of *C. perfringens* contained in faeces and the percentage of broilers harboring salmonellae in their intestinal tracts. Because the antimicrobial activity of *Quillaja* and *Yucca* saponins is poorly understood, changes in these important poultry bacteria may result from direct antimicrobial effects, from QY-induced changes in the intestinal microenvironment, through promotion of mucosal immunity, or by a combination of these factors. Future research trials will hopefully elucidate the intestinal effects associated with these bacterial changes and provide explanations for these responses. It is hoped they will serve as a stimulus for further saponin research.

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