Magni-Phi® in High Disease Challenge Environments: 1. Alteration of Selected Bacterial Populations

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Abstract

A series of four floor pen trials was carried out to determine the effects of Magni-Phi® (MP) in high disease challenge environments. In each trial, used litter from farms known to have had outbreaks of necrotic enteritis and difficulties with *Salmonella* was utilized. Additions of coccidial oocysts at the start of each test increased the challenge. MP was fed at 0, 250 or 500 ppm and fed throughout the duration of the 42-day tests. At 42 days of age, performance and mortality were determined. Coccidial lesions were scored at day 21, and selected intestinal and/or fecal samples were collected at 21 and 42 days in order to quantify *Clostridium perfringens* and the incidence of *Salmonella*. Results demonstrated that birds fed MP showed significant linear improvements ($P < 0.001$) in performance, mortality and coccidial lesion scores. At both 21 and 42 days, significant linear reductions ($P < 0.001$) in numbers of fecal Clostridia were observed and the number of birds harboring *Salmonella* was significantly reduced. These results indicate that feeding MP may also help reduce important bacterial populations known to cause adverse effects on performance and product acceptability.

Introduction

Saponins are natural products of plant origin. Previous work (1 – 4) with saponins derived from yucca plants (*Yucca schidigera*) and quillaja trees (*Quillaja saponaria*), has been shown in broiler chickens and turkeys, to potentiate the anticoccidial properties of existing anticoccidials.

As broiler producers have moved to the antibiotic free (ABF) and no antibiotics ever (NAE) environments, the unique combination of yucca and quillaja found in Magni-Phi has played a significant role in the maintenance that is needed in these challenging environments. Additionally, field reports often indicate that birds fed MP show a reduced incidence of mortality and improved intestinal health when reared in high disease challenge situations. Consequently, the purpose of studies reported herein was to examine the effects of feeding birds MP in high challenge situations by evaluating coccidial lesions, incidence of *Salmonella* positive broilers and by enumerating *Clostridium perfringens* in the lower intestinal tract.

Materials and Methods

A series of four floor pen studies was carried out in a major broiler producing area of the USA. All tests were conducted in a high disease challenge environment employing used litter and supplementing that bedding material with litter known to contain *C. perfringens* and a variety of *Salmonella* serotypes. This supplemental litter was taken from commercial farms known to have had difficulties with necrotic enteritis and *Salmonella*. An independent outside laboratory determined the *Salmonella* serotypes present in this litter were *Enteritidis*, *Typhimurium*, *Kentucky*, *Indiana* and *Heidelberg*. Prior to the start of each trial, additional coccidial oocysts (primarily *Eimeria acervulina* and *E. maxima*) were added to each pen.

All birds in all trials were vaccinated for coccidiosis with Coccivac B52®, a widely used live coccidiosis vaccine, applied at the hatchery. Since the objective of these trials was to determine the effects of increasing feeding rates of MP on selected bacterial pathogens, MP was fed at 0, 250 or 500 ppm throughout each 42-day trial. In one test, only 0 and 250 ppm were evaluated. Treatments were replicated at least 10 times in each trial, and a total of 50 replicates of each treatment were compiled in the pooled data analysis. At the outset of each test, pens contained 55 Cobb 500 broilers.

Performance data were collected at 21 and 42 days of age; birds were evaluated for coccidial lesions at day 21 (5). Bacterial assessments were carried out from fecal and intestinal samples that were collected from four birds per pen on days 21 and 42. The procedures used for Clostridial enumeration and determining the percentage of *Salmonella* positive broilers are described below:

Clostridium perfringens counts in fecal samples:

> Four birds per pen were removed from the pen environment and fresh fecal samples were collected from each bird. Each sample was separately diluted to 1:10 in peptone dilution fluid. Ten-fold dilutions were made by adding 1 ml into each dilution up to the maximal level. Diluted samples were plated onto 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 log10 by calculation, and reported as log10 units.

Incidence of *Salmonella* in pen-raised broilers:

> The procedure is intended to determine the presence of any *Salmonella* organism in intestinal contents; it does not determine serotypes. Four birds were selected at random from each pen and killed at the appropriate time (21 or 42 days of age). One cc samples of digesta were taken from the lower digestive tract from each bird and then subjected to a double enrichment process in TT broth. After incubation for 24 hours, 10 µl of TT broth was plated onto XTL4 and BG Sulfa plates and incubated at 35 °C for 18 - 24 hrs. Positives were determined when black or red colonies were observed after incubation on XTL4 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. Percentage of *Salmonella* positive broilers per pen (out of four) was calculated.

Data Analysis

For all variables, the pen mean was considered the observational unit. Data presented herein represent the pooled results of four trials. All data were analyzed by ANOVA procedures and the linear effects of MP feeding rates were determined for each variable. For all pooled data, treatment means were separated by Tukey's HSD, where $P < 0.05$ was considered significant.

Results and Discussion

A primary objective of this series of trials was to determine the effects of feeding MP 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 considerably above American industry averages, the general objective for rearing birds under high challenge was met. In this environment, MP fed birds were shown to have significantly reduced mortality, and birds fed the 250 or the 500 ppm MP levels both had significantly improved FCR compared to controls. Likewise, pooled body weight gain responded in a similar manner. Significant linear effects were also observed for each of the variables presented, indicating that in this environment, each level of MP fed to birds provided improvements compared to lower levels.

Previous work (2, 3, 4) has demonstrated the ability of MP, when fed to birds, to help reduce coccidial challenge and improve performance in conditions where *Eimeria spp*. are known to be problematic. The coccidial lesion scores recorded in the present studies (Table 2) are consistent with these observations, and when birds were fed each level of MP the severity of the lesions presented was significantly reduced. A significant linear reduction in coccidial lesions was observed ($P < 0.001$).

The results of the *C. perfringens* enumeration (Table 2) were consistent with mortality and coccidial lesion scores presented above. At both intervals where *Clostridium* was logarithmically enumerated, birds fed MP exhibited significant level-related reductions in the numbers of organisms present (linear effect $P < 0.001$). When these same data are expressed in a natural form (not converted to logarithms), responses indicate at least three-fold reductions in fecal Clostridial counts produced by the addition of MP to the diet. Taken in concert, these combined results (coccidial lesions and Clostridial enumeration) help to explain the effects seen in birds fed MP, who's overall mortality at day 42 was reduced.

As with the Clostridial data presented above, Table 3 indicates the effects of MP in the diet on the incidence of birds with *Salmonella* in the lower intestine. At both intervals where these measurements were made, MP significantly reduced the percentage of broilers harboring *Salmonella*. In addition, linear reductions ($P < 0.001$) in these percentages were evident over the treatment range tested.

Data contained herein clearly show that the benefits of feeding MP extend beyond additive improvements in coccidiosis control reported in previous studies (1 – 4). Perhaps of greater significance are the results reported when birds fed MP had reduced numbers of Clostridial organisms in feces and there was a lower incidence of birds with *Salmonella*. These results are likely the first documented evidence of these effects, and seem to be consistent with field observations made during commercial usage where lower mortality and better intestinal health have been observed. Both findings are significant because these bacteria pose a great risk to optimal bird performance and the safety and acceptability of poultry products.

Although the ability of feeding MP to potentiate antiprotozoal and anticoccidial properties is reasonably well understood, the effects of feeding MP saponins on antimicrobial effects in poultry appear to be limited. While some activity of quillaja and yucca saponins has been reported for Gram-positive bacteria (6), other reports found few antibacterial effects on Gram-negative organisms (7). In either case, antibacterial activity is not considered a prominent feature of these products (8, 9), and our own studies on bacterial inhibition with MP support this conclusion. Consequently, it seems unlikely that the effects reported herein are associated with antimicrobial activity directed specifically against *Clostridium* and *Salmonella*. Conversely, Cheeke (9) described a variety of different physiological processes in the gut that are influenced by yucca and quillaja saponins. Among these are increased permeability of the intestine, increased micelle formation and fat absorption, protection of bile acids from bacterial degradation and improved vitamin and mineral absorption. These, of course, are in addition to the well-characterized enhancements of immunity (9) and reduced coccidial exposure (1 – 4). As a result, it seems more likely that the effects of feeding MP are associated with a modification of the intestinal environment, which may allow bacterial populations containing fewer pathogens to become established. Current studies, designed to determine whether feeding MP induces changes in intestinal bacterial populations, should provide the information needed to support or refute this hypothesis.

Literature Cited

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Table 1. Forty-two day pooled performance and total mortality recorded for Cobb broilers fed graded levels of Magni-Phi in four floor pen trials.

	FCR (g:g)	BWG (kg)	Mortality (%) at 42 d
Magni-Phi (ppm)*			
0	1.920[a]	2.784[a]	10.5[a]
250	1.844[b]	2.881[b]	3.1[b]
500	1.810[c]	2.894[b]	2.2[b]

Results represent pooled data from four trials involving graded levels of Magni-Phi, where each treatment was replicated at least 10 times per trial. In total, 50 replications per treatment comprised the pooled analysis.
*Magni-Phi linear effect was significant ($P < 0.001$) for all variables presented. [a-c] Different superscripts in each column denote statistical differences ($P < 0.05$) determined by Tukey's HSD.

Table 2. Pooled lesion scores and *Clostridium* counts determined at selected intervals in birds fed graded levels of Magni-Phi in four floor pen trials.

	Coccidial Lesion Scores Day 21	*Clostridium perfringens* Enumeration (log_{10})	
		Day 21	Day 42
Magni-Phi (ppm)*			
0	1.71[a]	4.09[a]	4.06[a]
250	1.12[b]	3.56[b]	3.57[b]
500	0.47[c]	3.13[b]	3.42[b]

Results represent pooled data from four trials involving graded levels of Magni-Phi, where each treatment was replicated at least 10 times per trial. In total, 50 replications per treatment comprised the pooled analysis.
Four birds per pen were evaluated at each interval in every trial.
*Magni-Phi linear effect was significant ($P < 0.001$) for all variables presented. [a-c] Different superscripts in each column denote statistical differences ($P < 0.05$) determined by Tukey's HSD.

Table 3. Pooled incidence of *Salmonella* determined at two intervals in Cobb broilers fed graded levels of Magni-Phi in four floor pen trials.

	Salmonella Incidence (Percent)	
	Day 21	Day 42
Magni-Phi (ppm)*		
0	78.1[a]	70.0[a]
250	54.3[b]	44.7[b]
500	33.2[c]	37.7[b]

Results represent pooled data from four trials involving graded levels of Magni-Phi, where each treatment was replicated at least 10 times per trial. In total, 50 replications per treatment comprised the pooled analysis.
*Magni-Phi linear effect was significant ($P < 0.001$) for both 21- and 42-day data.
a-c Different superscripts in each column denote statistical differences ($P < 0.05$) determined by Tukey's HSD.