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Effects of Multiple Mycotoxins and a Hydrated Sodium Calcium Aluminosilicate in Poultry¹

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Abstract: Effects of feeding a combination of mycotoxins at naturally occurring levels in broiler chicks and turkey poults were evaluated. The efficacy of a hydrated sodium calcium aluminosilicate (HSCAS) to ameliorate the effects of the combination of mycotoxins was also determined. Day-old chicks and poults were randomly assigned to each of four dietary treatments for 21 days. A 2 x 2 factorial arrangement was used with treatments containing either no mycotoxins or multiple mycotoxins (MM) with 0 or 1% HSCAS. The MM consisted of 1 mg deoxynivalenol, 5 mg moniliformin, 5 mg fumonisin B_1 , 100 μ g aflatoxin B_1 , 1 mg zearalenone, and 0.5 mg ochratoxin A per kg diet. In experiment 1, feed intake and BW gain were decreased (P < 0.05) in chicks fed MM. Relative heart and gizzard weights were higher (P < 0.05) in chicks fed diets containing MM and HSCAS. In experiment 2, performance of poults was not affected (P > 0.05) by dietary treatments. Relative spleen weights, serum albumin, protein, globulin, and calcium levels were decreased (P < 0.05) in poults fed MM. Mean cell volume was decreased (P < 0.05) in poults fed diets containing HSCAS. Data indicate that a combination of low levels of mycotoxins decreased chick performance and altered several hematological and serum biochemical values in poults. Addition of HSCAS to diets containing MM did not prevent the negative effects observed in chicks and poults.

Key words: Poults, chicks, multiple mycotoxins, adsorbent

Introduction

Mycotoxins are a group of structurally diverse secondary metabolites of fungi that occur as contaminants of grains worldwide. Aspergillus, Alternaria, Claviceps, Fusarium, and Penicillium species of fungi are ubiquitous in nature and under ideal conditions often infect economically important crops and forages in the field, during storage, shipment, and processing. Many of the secondary metabolites produced by these fungi can cause serious health problems in animals and their presence in agricultural commodities may result in serious economic losses. Effects of mycotoxin contamination, in livestock, may include reduction in performance such as feed intake, body weight gain, and feed efficiency (Charmley et al., 1994) as well as increased susceptibility to disease. Over 300 mycotoxins have been reported in the literature and the target sites and mechanisms of toxicity are varied for each one, as are their chemical structures. In nature, mycotoxins rarely occur as a single contaminant since many fungal species that produce mycotoxins grow and produce their toxic metabolites under similar conditions. Furthermore a typical poultry ration is made up of several grain sources; each of which may be contaminated with a different mycotoxin or more than one mycotoxin. When more than one mycotoxin is present in a diet, the effects can either be additive, synergistic, or antagonistic

(Casarett, 1986). A number of studies have been conducted to evaluate the effects of multiple mycotoxins in livestock. However, these studies primarily focused on the acute effects of these mycotoxins. The levels of mycotoxins used in these acute studies were relatively high and are seldom seen in commercial poultry rations. In routine animal feed screening, mycotoxins are usually found at relatively low levels. Limited information exists regarding the effects of low levels of multiple mycotoxins in livestock. It has been suggested that combinations of mycotoxins at low concentrations may have negative effects on livestock, even though the concentrations of individual mycotoxins are well below concentrations reported to cause negative effects.

A number of approaches have been evaluated to deal with mycotoxin contaminated animal feeds. One of these approaches is the addition of adsorbent materials to the diet. Adsorbents have been used to alleviate the effects of aflatoxin in poultry diets (Huff et al., 1992; Kubena et al., 1993; Phillips et al., 1988). Anecdotal reports claim that the addition of similar adsorbents to poultry diets does, in fact, lessen the negative effects of low levels of multiple mycotoxins. A number of aluminosilicate adsorbents have been shown to bind to aflatoxin in vivo and in vitro (Phillips et al., 1988; Huff et al., 1992; Kubena et al., 1990; Ledoux et al., 1999) as well as smaller amounts of other mycotoxins in vitro (Kubena et

al., 1992). The objectives of this study were to determine the effects of feeding low levels of a combination of multiple mycotoxins to poultry, and to determine if the addition of a typical hydrated sodium calcium aluminosilicate (HSCAS) adsorbent would ameliorate the effects of multiple mycotoxins.

Materials and Methods

Experimental Design and Birds: Two experiments were conducted in this study using 144 day-old male broiler chicks² and 144 day-old female poults³. Birds were weighed, wing-banded, and allotted randomly to pens in stainless steel chick batteries. Birds were maintained on a 24 hour constant light schedule and allowed to consume feed and water ad libitum. A 2 X 2 factorial arrangement of treatments was used with six pen replicates of six chicks or poults each, assigned to one of four dietary treatments, from day 1 to 21. Birds were monitored daily for signs of disease and mortality. The animal care and use protocol was reviewed and approved by the University of Missouri-Columbia Animal Care and Use Committee.

Diet Preparation: Dietary treatments consisted of the diet (corn-soybean meal based diet) supplemented with either no mycotoxins or a combination of multiple mycotoxins (MM) and 0 or 1% HSCAS. The combination of mycotoxins consisted of 1 mg deoxynivalenol/kg, 5 mg moniliformin/kg, 5 mg fumonisin B_1/kg , 100 μg aflatoxin B_1/kg , 1 mgzearalenone/kg, and 0.5 mg ochratoxin A/kg diet. Dietary treatments containing mycotoxins and/or HSCAS were prepared by substituting mycotoxins and/or HSCAS for ground corn in a typical corn-soybean meal basal diet. Diets were fed as a mash and were formulated to be isocaloric and isonitrogenous, and either met or exceeded the nutrient requirements of broiler chicks or turkey poults as recommended by the National Research Council (1994). Prior to the addition of mycotoxins to the diets, diets were screened for the presence of mycotoxins by previously reported methods, and were found to be free of aflatoxin, citrinin, deoxynivalenol, sterigmatocystin, zearalenone. ochratoxin A (Rottinghaus et al., 1982), the fumonisins (Weibking et al., 1993) and moniliformin (Ledoux et al., 1995).

Sample Collection: At the end of the experiments, birds were individually weighed, and feed consumption and feed conversion determined for each pen. Birds were then euthanized with CO₂ and blood was collected,

via cardiac puncture, for hematology and serum chemistry analysis. Analysis of serum included glucose, sodium, potassium, albumin, protein, globulin, calcium, phosphorous, cholesterol, aspartate aminotransferase (AST), alkaline phosphatase (ALKP), gamma glutamyl transferase (GGT) and uric acid. Serum values were determined using an auto analyzer⁴. Hemoglobin (HB) was measured as cyanmethemoglobin⁵. Red blood cell counts (RBC), mean cell volume (MCV), and hematocrits (HCT) were determined with a coulter counter⁶ using instrument settings described by Steel et al. (1977). Mean cell hemoglobin (MCH) and mean cell hemoglobin concentrations (MCHC) were calculated. Following blood sampling, chicks were necropsied and heart, kidney, liver, spleen, gizzard, and proventriculus were collected and organ weights determined.

Histopathology: Post-mortem examinations were performed on five chicks from each treatment group at three weeks of age. Chicks were euthanized with CO_2 and samples of liver, kidney, bursa, thymus, spleen, and heart were collected and fixed in 10% neutral buffered formalin. Fixed tissues were trimmed, embedded in paraffin, sectioned at 4 μ m, and stained with hematoxylin and eosin stain. Tissue samples from all treatment groups were examined microscopically.

Statistical Analysis: Data were analyzed as a 2 X 2 factorial by the General Linear Model (GLM) procedure of SAS® software (SAS Institute, 1996). Mean differences were determined using Fisher's protected least significant difference test. All statements of significance were based on the 0.05 level of probability, unless otherwise indicated.

Results

Experiment 1: Broiler Study: The individual and combined effects of MM and HSCAS on chick performance are summarized in Table 1. Feed intake and BW gain in chicks fed diets containing MM were significantly lower ($P \le 0.05$) compared with chicks fed diets without mycotoxins. Feed conversion was not affected by dietary treatments. The addition of 1% HSCAS to the basal diet did not affect (P > 0.05) chick performance, nor did the addition of 1% HSCAS to the MM diet prevent the decrease in performance observed in chicks fed MM. No MM by HSCAS interaction was observed for chick performance.

The individual and combined effects of MM and HSCAS on chick relative organ weights are summarized in Table

²Stover Hatchery, P.O. Box 216, Stover, MO 65078. ³Hudson Farms, Inc., Monett, MO 65708. ⁴Kodak Ektachem Analyzer, Eastman Kodak Co., Rochester, NY 14650. ⁵Sigma Diagnostics, Sigma Chemical Co., St. Louis, MO 63178-9916. ⁶Coulter Counter Model ZB1, Coulter Electronics, Hialeah, FL 33010.

Table 1: Effects of multiple mycotoxins and a hydrated sodium calcium aluminosilicate (HSCAS) on chick performance¹

Mycotoxins ²	HSCAS	Feed	Weight	Feed:			
-	(%)	intake	gain	Gain			
		(g)	(g)	(g:g)			
-	0	1024	758	1.35			
-	1	1028	775	1.33			
+	0	971	743	1.31			
+	1	925	722	1.28			
Pooled SEM		25	16	0.03			
Source of vari	ation		P				
Mycotoxins		0.0057	0.0505	0.1288			
HSCAS		0.4045	0.8993	0.3706			
Mycotoxins x	HSCAS	0.3291	0.2453	0.9390			
Main Effect Means							
Mycotoxins	-	1026°	767°	1.34			
	+	948 ^b	733⁵	1.30			
HSCAS	0	998	751	1.33			
	1	977	749	1.31			

¹Data are means of six pen replicates of six chicks each. ²Combination of 1 mg deoxynivalenol, 5 mg moniliformin, 5 mg fumonisin B_1 , 100 μg aflatoxin B_1 , 1 mg zearalenone, and 0.5 mg ochratoxin A/kg diet. ^{a,b}Means within columns and within main effects with no common superscripts are significantly different (P ≤ 0.05).

2. Chicks fed diets containing MM had significantly higher (P < 0.05) heart and gizzard weights compared with chicks fed no mycotoxins. With the exception of the relative heart and gizzard weights, MM did not affect (P > 0.05) other organ weights, including kidney, liver, spleen, and proventriculus weights (data not shown). The addition of 1% HSCAS to the basal diet did not affect (P > 0.05) the relative organ weights, nor did the addition of 1% HSCAS to the MM diet prevent the increased heart and gizzard weights observed in chicks fed MM. No MM by HSCAS interaction was observed for chick relative organ weights.

Effects of dietary treatments on serum chemistry are summarized in Table 2. Serum albumin levels were significantly decreased (P < 0.05) in chicks fed multiple MM and by the addition of 1% HSCAS. However, statistical differences in serum albumin appeared to be primarily due to the significant decrease in albumin in chicks fed the diet containing both MM and HSCAS. A significant (P < 0.05) MM by HSCAS interaction was observed for serum Ca. The interaction occurred because the addition of HSCAS to the control diet reduced serum Ca, whereas the addition of HSCAS to the MM diet caused an increase in serum Ca. In addition to the MM by HSCAS interaction, there was also a significant MM effect with serum Ca levels in chicks fed MM significantly decreased (P < 0.05) compared with chicks fed no MM. The addition of 1% HSCAS to the diet containing MM did not prevent the decrease in serum

albumin but did reduce the negative effects of MM on serum Ca. No significant differences (P > 0.05) were observed for serum total protein, globulin, phosphorous, uric acid, cholesterol, AST, or ALKP in chicks fed MM (data not shown).

Dietary treatments did not affect (P > 0.05) any hematological variables in chicks (data not shown). Histopathology results also indicated no significant microscopic lesions in sections of liver, kidney, bursa, thymus, spleen, or heart from chicks fed dietary treatments.

Experiment 2: Poult Study: The effects of MM and HSCAS on poult performance are presented in Table 3. Addition of MM, HSCAS or a combination of both to diets did not have any significant effect (P > 0.05) on poult feed intake, BW gain or feed conversion which averaged 793 g, 461 g, and 1.73 g:g, respectively.

Table 4 summarizes the effects of dietary treatments on relative organ weights and selected serum chemistries. The addition of MM to the basal diet resulted in a reduction (P < 0.05) in relative spleen weights. The addition of 1% HSCAS to the basal diet did not have an effect on organ weights (P > 0.05), nor did the addition of 1% HSCAS to the MM diet prevent the decrease in spleen weights. Relative weights of the heart, kidney, liver, gizzard, and proventriculus were not affected (P > 0.05) by MM or HSCAS (data not shown). Serum albumin, total protein, globulin, and calcium levels were significantly lower (P < 0.05) in poults fed diets containing MM. The addition of 1% HSCAS to the MM diet did not prevent the reductions in serum albumin, total protein, globulin, and Ca levels. No significant differences were observed among treatments for serum P, uric acid, cholesterol, AST, and ALKP (data not shown).

The effects of dietary treatments on hematology of poults are summarized in Table 5. Mean cell volume was reduced (P < 0.05) in poults fed diets containing HSCAS compared with those not fed HSCAS. Significant (P < 0.05) interactions between MM and HSCAS were observed for HB, MCH, and MCHC. The addition of HSCAS to the basal diet caused an increase in all three response variables, whereas the addition of HSCAS to the diet containing MM caused a decrease in all three response variables. In addition to these interactions, poults fed diets containing MM had higher (P < 0.05) HB, MCH, and MCHC compared with poults fed no mycotoxins.

Histopathology results indicated no significant microscopic lesions in sections of liver, kidney, bursa, thymus, spleen, or heart from poults fed dietary treatments.

Discussion

Results of the current study indicate chicks fed diets containing MM had decreased feed intake and BW gain,

Table 2: Effects of multiple mycotoxins and a hydrated sodium calcium aluminosilicate (HSCAS) on selected relative organ weights and selected serum chemistries of chicks¹

Mycotoxins ²	HSCAS	Heart	Gizzard	ALB^3	GLOB ³	Protein ³	Ca³
	(%)	(g/100g body weight)			(g/dL)		(mg/dL)
-	0	0.71	2.05	1.32	1.35	2.62	8.93
-	1	0.68	2.15	1.28	1.29	2.57	8.08
+	0	0.74	2.22	1.27	1.33	2.59	7.87
+	1	0.76	2.23	1.18	1.21	2.38	8.14
Pooled SEM		0.02	0.06	0.03	0.05	0.08	0.23
Source of variat	ion			P			
Mycotoxins		0.0297	0.0242	0.0103	0.2737	0.1663	0.0423
HSCAS		0.9216	0.3346	0.0205	0.0840	0.0923	0.2203
Mycotoxins x HS	SCAS	0.2792	0.3772	0.3564	0.5513	0.2823	0.0232
Main Effect Mea	ins						
Mycotoxins	-	0.70 ^b	2.10 ^b	1.29°	1.32	2.59	8.50°
	+	0.75°	2.23°	1.22 ^b	1.26	2.48	8.00 ^b
HSCAS	0	0.73	2.14	1.29°	1.33	2.60	8.40
	1	0.72	2.19	1.22 ^b	1.25	2.47	8.10

¹Data are means of six pen replicates of six chicks each. ²Combination of 1 mg deoxynivalenol, 5 mg moniliformin, 5 mg fumonisin B_1 , 100 μ g aflatoxin B_1 , 1 mg zearalenone and 0.5 mg ochratoxin A/kg diet. ³ALB = albumin; PROT = protein; GLOB = globulin; Ca = calcium. ^{a,b}Means within columns and within main effects with no common superscripts are significantly different (P < 0.05).

Table 3: Effects of multiple mycotoxins and a hydrated sodium calcium aluminosilicate (HSCAS) on poult performance¹

	репоппаг	ice					
Mycotoxins ²	HSCAS	Feed	Weight	Feed:			
	(%)	intake	gain	Gain			
		(g)	(g)	(g:g)			
-	0	804	467	1.72			
-	1	756	427	1.77			
+	0	817	476	1.73			
+	1	794	473	1.69			
Pooled SEM		24	18	0.05			
Source of vari	ation	P					
Mycotoxins		0.2820	0.1441	0.4372			
HSCAS		0.1491	0.2366	0.9078			
Mycotoxins x HSCAS		0.6105	0.3141	0.3439			
Main Effect Means							
Mycotoxins	-	780	447	1.74			
	+	806	474	1.70			
HSCAS	0	810	472	1.72			
	1	775	450	1.73			

 1 Data are means of six pen replicates of six poults each. 2 Combination of 1 mg deoxynivalenol, 5 mg moniliformin, 5 mg fumonisin B₁, 100 µg aflatoxin B₁, 1 mg zearalenone, and 0.5 mg ochratoxin A/kg diet.

even though, the mycotoxin levels used were below levels reported to individually cause problems, suggesting an additive effect (Casaret, 1986).

Levels of these mycotoxins reported to cause toxic effects in poultry are: > 16 mg/kg deoxynivalenol (Leeson *et al.*, 1995); 64 mg/kg moniliformin (Leeson *et al.*, 1995); 75 mg/kg fumonisin B₁ (Weibking *et al.*, 1993); 0.2 mg/kg aflatoxin B₁ (Giambrone *et al.*, 1985); > 800

mg/kg zearalenone (Chi *et al.*, 1980); 1 mg/kg ochratoxin A (Huff *et al.*, 1974). These concentrations are higher than the levels used in the current study.

These results are consistent with two recent reports indicating chick performance was depressed in birds fed naturally contaminated feed containing combinations of low concentrations of mycotoxins. Swamy et al. (2002) fed chicks diets containing a combination of deoxynivalenol (8.2 mg/kg), fusaric acid (20.3 mg/kg), and zearalenone (0.56 mg/kg) from naturally contaminated corn and wheat, and observed a reduction in body weight gain during the finishing phase (d 42-56). Aravind et al. (2003) fed chicks diets containing a combination of aflatoxin (168 µg/kg), ochratoxin A (8.4 μg/kg), zearalenone (54 μg/kg), and T-2 toxin (32 μg/kg) from naturally contaminated corn, and observed reductions in feed intake and body weight gain, and poorer feed conversions in birds fed diets from hatch to day 35.

In contrast to chicks, the combination of low levels of mycotoxins did not have any significant effect on performance of poults. It is possible that chicks may have lower tolerance to the combined effects of these mycotoxins compared with poults. However, it is much more likely that chicks because of their higher feed intake consumed a much larger dose of the mycotoxins resulting in the observed decrease in performance.

Although poult performance was not affected by the combination of MM, several serum biochemical values were affected. Serum albumin, total protein, globulin, and serum Ca levels were reduced by 7, 13, 15, and 19%, respectively, in poults fed diets containing MM. The addition of HSCAS to the MM diet was not effective in

Table 4: Effects of multiple mycotoxins and a hydrated sodium calcium aluminosilicate (HSCAS) on selected relative

organ	i weights and s	selected serum	chemistries of	poults			
Mycotoxins ²	HSCAS	Heart	Spleen	ALB^3	GLOB ³	Protein ³	CA ³
	(%)	(g/100g body weight)			(g/dL)	(g/dL)	
-	0	0.57	0.11	1.37	1.85	3.25	10.13
-	1	0.55	0.12	1.43	1.80	3.22	10.06
+	0	0.59	0.10	1.27	1.58	2.83	8.19
+	1	0.60	0.09	1.33	1.60	2.94	9.56
Pooled SEM		0.02	0.01	0.03	0.05	0.07	0.36
Source of varia	ation				P		
Mycotoxins		0.1416	0.0078	0.0024	0.0008	0.0012	0.0031
HSCAS		0.6544	0.8159	0.0560	0.8315	0.6467	0.0877
Mycotoxins x F	HSCAS	0.6134	0.2716	1.0000	0.5256	0.4128	0.0617
Main Effect Me	eans						
Mycotoxins	-	0.55	0.11 ^a	1.39°	1.82°	3.23°	10.09 ^a
•	+	0.59	0.09 ^b	1.29 ^b	1.58⁵	2.88 ^b	8.87 ^b
HSCAS	0	0.58	0.10	1.31	1.71	3.03	9.15
	1	0.57	0.10	1.37	1.70	3.07	9.80

¹Data are means of six pen replicates of six poults each. ²Combination of 1 mg deoxynivalenol, 5 mg moniliformin, 5 mg fumonisin B_1 , 100 μ g aflatoxin B_1 , 1 mg zearalenone and 0.5 mg ochratoxin A/kg diet. ³ALB = albumin; PROT = protein; GLOB = globulin; CA = calcium. ^{a,b}Means within columns and within main effects with no common superscripts are significantly different (P < 0.05).

Table 5: Effects of multiple mycotoxins and a hydrated sodium calcium aluminosilicate (HSCAS) on hematology of poults¹

Mycotoxins ²	HSCAS	MCV ³	MCH ³	MCHC ³	HCT ³	RBC ³	HB ³
	(%)	(µM³)	(pg)	(%)	(millions/µL)	(g/dL)
-	0	179	49.2	27.5	36.9	2.06	9.96
-	1	176	57.4	32.7	34.4	1.95	11.03
+	0	180	60.8	33.8	34.1	2.02	11.53
+	1	175	57.4	32.7	34.8	1.95	11.26
Pooled SEM	1	2.53	1.39	1.28	0.07	0.31	
Source of vari	ation				- P		
Mycotoxins		0.7366	0.0319	0.0341	0.3719	0.3782	0.0084
HSCAS		0.0035	0.3465	0.1638	0.5086	0.9659	0.2038
Mycotoxins x I	HSCAS	0.5820	0.0320	0.0364	0.2215	0.2084	0.0411
Main Effect Me	eans						
Mycotoxins	-	177	53.3⁵	30.1⁵	35.7	2.01	10.5⁵
-	+	178	59.1°	33.3°	34.5	1.94	11.4ª
HSCAS	0	179°	55.0	30.7	35.5	1.98	10.7
	1	176⁵	57.4	32.7	34.7	1.97	11.1

¹Data are means of six pen replicates of six poults each. ²Combination of 1 mg deoxynivalenol, 5 mg moniliformin, 5 mg fumonisin B_1 , 100 μ g aflatoxin B_1 , 1 mg zearalenone, and 0.5 mg ochratoxin A/kg diet. ³MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; HCT = hematocrit; RBC = red blood cell; HB = hemoglobin. ^{a,b} Means within columns and within main effects with no common superscripts are significantly different (P < 0.05).

preventing the depression in any of the serum biochemical values. Similar findings were also observed in chicks fed MM. Compared with control chicks, serum albumin and Ca levels were reduced by 20 and 12%, respectively, in chicks fed the diet containing MM only. The addition of HSCAS to the MM diets was not effective in preventing the depression in either serum albumin or Ca.

Results of the current study indicated that MM at these

low concentrations negatively affected chicks and poults, however the addition of HSCAS to the diets containing MM did not prevent or ameliorate the negative effects observed in chicks and poults. The mode of action for HSCAS adsorbents binding to mycotoxins such as AFB $_{\rm l}$ involves the sequestration of AFB $_{\rm l}$ in the gastrointestinal tract and tight binding to HSCAS (Grant and Phillips, 1998) rendering the AFB $_{\rm l}$ unavailable for absorption from the gastrointestinal tract. The hypothesized

mechanism for the efficacy of HSCAS against aflatoxin may well explain the lack of efficacy of HSCAS against other mycotoxins. The chemisorption of aflatoxins to HSCAS involves the formation of a complex by the β -keto-lactone of aflatoxin with uncoordinated metal ions in HSCAS (Phillips *et al.*, 1988). Unless these mycotoxins have some other type of structure systems that would facilitate chemisorption, or the adsorbent was capable of binding mycotoxins by some other mechanism, it is not surprising that HSCAS was not effective in preventing the toxic effects of these mycotoxins. At the present time, aflatoxin B_1 is the only mycotoxin that is known to be bound by adsorbents of this type in poultry (Grant and Phillips, 1998; Phillips *et al.*, 1988; Kubena *et al.*, 1993).

In contrast to the present study, an adsorbent consisting of esterified glucomannan (EGM) has been reported to be effective in ameliorating the toxic effects of multiple mycotoxins present in naturally contaminated feed (Swamy et al., 2002; Aravind et al., 2003). Although the mode of action of the EGM in ameliorating the toxic effects of the mycotoxins is not known, it has been hypothesized that the mycotoxin(s) is trapped in the glucomannan matrix preventing its absorption from the intestine (Raju and Devegowda, 2000).

In conclusion, the current study demonstrated that a combination of low levels of mycotoxins decreased performance, organ weights, and serum chemistry in chicks and altered several hematological and serum biochemical values in poults, and the addition of HSCAS to diets containing MM did not prevent the negative effects observed in chicks and poults fed diets containing MM.

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