The Effect of Added Complex Carbohydrates or Added Dietary Fiber on Necrotic Enteritis Lesions in Broiler Chickens[1]

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ABSTRACT Two trials utilizing two corn diets and four wheat diets were conducted. In Trial 2, all chicks were crop-infused at 9 d of age with *Eimeria acervulina.* In both trials, a broth culture of *Clostridium perfringens* was mixed with the diets for 3 consecutive d. Necrotic enteritis lesion scores were lowest in chickens consuming the corn diet with no *C. perfringens* and highest in chickens fed the wheat diets with *C. perfringens.* Chickens consuming a wheat diet with no added complex carbohydrates or added fiber exhibited the highest lesion score. Chickens on wheat diets with 4% new, ground, pine shavings had intestinal lesion scores intermediate to those of chickens that consumed the wheat or corn diets. Chickens consuming corn diets yielded the lowest lesion scores. Chickens provided diets containing either guar gum or pectin were not fully consumed and thus probably reduced the number of challenge organisms ingested.

(*Key words*: wheat, intestinal motility, coccidia, guar gum, pectin)

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INTRODUCTION

Necrotic enteritis was first reported from a flock of 6- to 7-wk-old cockerels in England by Parish (1961). Necrotic enteritis is an acute, infectious, noncontagious disease (Schwartz, 1988) affecting chickens 2 wk to 6 mo of age (Ficken, 1991). Long (1973) reported the disease to be most common at 3 wk of age and to appear most commonly in July, August, September and October. The causative organism, *Clostridium perfringens*, has been isolated from contaminated feed and litter, dust, feces, intestinal contents, and soil (Kohler *et al.*, 1974; Komnenov *et al.*, 1981). *Clostridium perfringens* has been reported to be the principal obligate anaerobe of the normal chicken intestine (Johansson and Sarles, 1948; and Shapiro and Sarles, 1949). Smith (1965) reported that the first organisms to colonize the alimentary tract of most animals, including the chick, were *Escherichia coli, Clostridium welchii* (*C. perfringens*), and streptococci.

Diet composition has been reported (Smith, 1965) to have a marked effect on the flora that develops in the alimentary tract of the chick. Nairn and Bamford (1967) subsequently postulated that *C. perfringens* intestinal tract numbers and onset of clostridial intestinal disease of chickens may be precipitated by the nature of the ration. High levels of fish meal in the diet have been reported to predispose animals to or exacerbate outbreaks of NE (Johnson and Pinedo, 1971; and Truscott and Al-Sheikhly, 1977). Further, broilers fed wheat-based diets are more susceptible to NE than broilers fed corn-based diets (Branton *et al.*, 1987; and Riddell and Kong, 1992), although the reason(s) for the increased susceptibility has not been determined.

This study was conducted to determine whether the addition of either complex carbohydrates or added fiber in the form of new, ground, pine shavings in wheat-based diets would ameliorate lesions of necrotic enteritis in broiler chickens.

MATERIALS AND METHODS

Chickens

In each of two trials, 180 day-old, straight-run broiler chicks were purchased from a commercial hatchery. Chicks were placed in a Petersime starter battery and consumed a basal corn-soybean broiler starter diet (Table 1) *ad libitum* through 20 (Trials 1) or 13 (Trial 2) d of age. Ten chicks were housed in each compartment. Each treatment was randomly assigned to three compartments (i.e., replicates).

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[1]Trade names in this article are used solely to provide specific information. Use of trade names does not constitute a guarantee or warranty by USDA and does not signify that the product is approved to the exclusion of other comparable products.

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TABLE 1. Composition of diets used in experiments

Ingredients	Starter diet	Grower diets	
		Corn	Wheat
	(%)		
Yellow corn	58.85	68.65	. . .
Soft wheat	. . .	. . .	65.55
Soybean meal, 48% CP	33.30	26.10	25.40
Animal fat (7,718 kcal/kg ME)	4.30	1.80	5.70
Dicalcium phosphate (22, 18.5)	1.64	1.68	1.55
Ground limestone (38)	0.92	0.91	0.94
Salt	0.44	0.42	0.39
Trace element premix[1]	0.25	0.25	0.25
MHA-Ca, 93%	0.30	0.19	0.22
Calculated analysis			
CP	21.1	18.4	18.7
ME, kcal/kg	3,140	3,110	3,110
Lysine	1.15	0.96	0.96
Methionine plus cystine	0.92	0.77	0.77
Calcium	0.80	0.80	0.80
Available phosphorus	0.42	0.42	0.42

[1]Broiler premix furnished the following per kilogram of feed: vitamin A, retinyl acetate (gelatin coated) 6,614 IU; cholecalciferol 1,654 IU; vitamin E, DL-α-tocopherol acetate, 2.2 IU; riboflavin, 4.4 mg; niacin, 27.6 mg; d-pantothenic acid, 8.8 mg; ethoxyquin, 55 mg; menadione sodium bisulfite complex, 2.8 mg or menadione sodium bisulfite, 1.7 mg; pyridoxine, 0.55 mg; manganese (oxide form), 66.25 mg; zinc (oxide form), 44 mg; iodine, 1.25 mg; iron (sulfate form), 20 mg; and copper (sulfate form), 2 mg.

Experimental Diets

Diets were based on those previously described (Branton *et al.*, 1987). No attempt was made to formulate isonitrogenous diets. A grower diet (Table 1) was consumed *ad libitum* by chickens from 20 d of age in Trial 1 and from 13 d of age in Trial 2. The same grower diet (Table 1) was used at 14 and 21 d of age (Trials 2 and 1, respectively) with the major cereal ingredients (corn and wheat), added complex carbohydrates (pectin and guar gum), or ground, pine shavings substituted for one another on an equal-weight basis. In each trial, treatment diets consisted of: 1) corn, 2) corn + *C. perfringens*, 3) wheat + *C. perfringens*, 4) wheat + *C. perfringens* + pectin,[3] 5) wheat + *C. perfringens* + guar gum,[3] and 6) wheat + *C. perfringens* + ground pine shavings. Guar gum, pectin, and pine shavings were each used at a level of 4%, replacing an equivalent weight of wheat. Wheat, corn, and pine shavings were ground through a 3.2-mm hammer mill screen with each ground in a similar manner and through a single pass.

Organism

Chickens were challenged with *C. perfringens* isolated from clinical necrotic enteritis and previously reported (Branton *et al.*, 1987). The challenge procedure followed the method outlined by Long and Truscott (1976) and used by Riddell and Kong (1992). Briefly, after recovering the isolate organism from the original meat infusion broth storage media, a stock culture was produced in thioglycolate[4] broth media (TBG). From the stock culture, an aliquot (5 mL) was used to inoculate 800-mL flasks of TBG. The flasks were placed in an anaerobic incubator[5] for 12 h at 37 C. The culture was subsequently mixed with weighed amounts of feed at a ratio of 800 mL of culture to 1,000 g of feed. The culture from one flask was mixed with one replicate of a feed treatment, and this procedure was repeated with each flask. Fresh cultures and mixtures of culture and feed were prepared daily. Chickens were fed the mixtures for periods of 3 d, starting at 14 d of age (Trial 2) or at 21 d of age (Trial 1) such that the chickens had continual access to the feed mixtures during the challenge period. Water was consumed *ad libitum*.

Bacterial counts were performed on two flasks of each batch of culture daily just prior to mixing with the feed. Ten-fold dilutions of the culture were made in sterile PBS and cultured anaerobically on prereduced blood agar plates at 37 C for 24 h. Bacterial culture counts varied from 1.0×10^6 to 2.0×10^7 per milliliter.

Necropsy

All chickens that died during the course of the experiment were necropsied and all surviving chickens were euthanatized by cervical dislocation and necropsied at the termination of the experiment (17 and 24 d of age, Trials 2 and 1, respectively). The intestine of each chicken was incised longitudinally from the *pars pyloris gastris* to Meckels' diverticulum (MD) and examined for gross evidence of necrotic enteritis lesions. In Trial 1, no categorical lesion description was formulated prior to necropsy. Rather, all observed intestinal abnormalities were noted for each of the dietary treatments. A

[3]Sigma Chemical Co., St. Louis, MO 63178-9916.
[4]Difco Laboratories, Detroit, MI 48232.
[5]Bactron IV, Sheldon Laboratories, Inc., Cornelius, OR 97113.

TABLE 2. Percentage of necrotic enteritis intestinal lesions in 24-d-old broiler chickens on wheat-based or corn-based diets, Trial 1[1]

Variable	Corn	Corn + CP	Wheat + CP	Wheat + Wood + CP	Wheat + Pectin + CP	Wheat + GG + CP
Hyperemia	6.6[b]	10[b]	10[b]	10[b]	40[a]	10[b]
Thickened gut	0[b]	0[b]	0[b]	0[b]	100[a]	100[a]
Sloughing	0[c]	6.6[c]	40[a]	20[b]	0[c]	0[c]

[a–c]Percentages within a row with no common superscript differ significantly ($P < 0.05$).

[1]CP = *Clostridium perfringens,* GG = guar gum.

categorical lesion description was recorded for each chicken in Trial 2. The lesions were scored such that: 0 was apparently normal, no lesions; 1 comprised a pale, mucoid intestinal surface; 2 evidenced thickened areas or sloughing of the mucous membrane; and 3 was characterized by a diphtheritic membrane lining the intestine.

Statistical Analysis

Differences in lesion scores were analyzed using one-way analysis of variance for a completely randomized experimental design. This allowed testing for the effects of marginal differences between treatments. When significant differences were found, means were separated by Fisher's Protected Least Significant Difference (Steel and Torrie, 1980). All data were analyzed using the General Linear Models procedure of SAS® (SAS Institute, 1994). Statements of significance were based on $P < 0.05$ unless otherwise noted.

Coccidia Challenge

In Trial 2 only, all chicks were crop-infused at 9 d of age with 1 mL of distilled water containing 25,000 oocysts per milliliter of *Eimeria acervulina.* Ten birds were randomly selected at 14 d of age (5 d after coccidial challenge), euthanatized by cervical dislocation, and evaluated for coccidial lesion scores using the method of Johnson and Reid (1970).

Histopathology

Three blocks of tissue were trimmed transversely from each duodenal loop yielding a total of six transverse sections of duodenum from each of four 17-d-old broilers randomly selected from each of the six treatments in Trial 2. The tissue was processed routinely for paraffin embedding, sectioned at 6 μm, and stained with hematoxylin and eosin. The number of necrotic foci associated with large bacterial rods was recorded for each section, and the total number of sections in which coccidia were seen in each bird was also noted.

RESULTS

In Trial 1, chickens in all treatments exhibited hyperemia of the proximal small intestine. However, chickens that consumed the wheat diet with added pectin and *C. perfringens* exhibited a significantly higher incidence of intestinal hyperemia than chickens that consumed any of the other treatments (Table 2). No significant difference was observed in the incidence of intestinal hyperemia among the other treatments at necropsy. A grossly visible, thickened intestinal wall was noted in all chickens that consumed either the wheat + pectin + *C. perfringens* diet or the wheat + guar gum + *C. perfringens* diet (Table 2). A grossly visible, thickened intestinal wall was not observed in any other treatment. No significant difference was observed in the incidence of thickened intestinal wall between the wheat + pectin + *C. perfringens* and the wheat + guar gum + *C. perfringens* diets. However, sloughing of the intestinal epithelial cells was observed in three treatments. There were significant differences observed among the three treatments such that the wheat + *C. perfringens* diet exhibited the highest incidence followed by wheat + ground pine shavings + *C. perfringens* diet and finally corn + *C. perfringens* diet (Table 3).

TABLE 3. Broiler (14-d-old) coccidial lesion scores prior to placement on dietary treatments

Chicken	Sex	Weight	*Eimeria acervulina*	*Eimeria maxima*	*Eimeria tenella*	Other
		(g)				
1	Male	355	2	0	0	0
2	Male	276	3	0	0	0
3	Male	317	2	0	0	0
4	Female	311	2	0	0	0
5	Female	318	3	0	0	0
6	Male	354	2	0	0	0
7	Female	331	3	0	0	0
8	Female	308	2	0	0	0
9	Female	306	4	0	0	0
10	Male	321	3	0	0	0
Mean		319.7	2.6	0	0	0

TABLE 4. Incidence of necrotic enteritis intestinal lesions in 17-d-old broiler chickens on wheat-based or corn-based diets, Trial 2[1]

	Diets					
	Corn	Corn + CP	Wheat + CP	Wheat + GG + CP	Wheat + Pectin + CP	Wheat + Wood + CP
Intestinal lesion score	0.267[d]	0.533[cd]	1.833[a]	1.267[b]	0.793[c]	1.133[b]

[a–d]Means with no common superscript differ significantly ($P < 0.05$).

[1]CP = *Clostridium perfringens*, GG = guar gum.

In Trial 2, the mean coccidial lesion score in the duodenal loop at 14 d of age was 2.6 (Table 3). In Trial 2, chickens that consumed the wheat diet with added *C. perfringens* exhibited the greatest mean intestinal lesion score (1.83) at 17 d of age, which was significantly different from the score from all other treatments (Table 4). Chickens that consumed the diets with either wheat with added *C. perfringens* and guar gum or wheat with added *C. perfringens* and ground pine shavings exhibited a mean intestinal lesion score that was not significantly different between the two treatments; however, the mean intestinal lesion scores for the two treatments were significantly less than scores for chickens that consumed the wheat diet with added *C. perfringens*. The mean intestinal lesion score for these two treatments was significantly greater than the mean intestinal lesion score of all other treatments. Chickens that consumed wheat with added *C. perfringens* and pectin exhibited a mean intestinal lesion score that was less than that of chickens that consumed the wheat diet with added *C. perfringens*, chickens that consumed the wheat diet with added *C. perfringens* and guar gum, or chickens that consumed the wheat diet with added *C. perfringens* and ground pine shavings. However, chickens that consumed the wheat diet with added *C. perfringens* and pectin were not significantly different in mean intestinal lesion score from chickens that consumed corn with added *C. perfringens*, but were significantly different in mean lesion score from chickens that consumed the corn diet with no added *C. perfringens*. Chickens that consumed the corn diet with no added *C. perfringens* exhibited the lowest mean intestinal lesion score; however, mean intestinal lesion scores of this treatment were not significantly different from scores of chickens that consumed the corn diet with added *C. perfringens* (Table 4). Intestinal lesions occurred distally to the *pars pyloris gastris* but were predominately observed in the anterior duodenum.

Histological examination of fixed duodenal loop from 17-d-old broilers euthanatized in Trial 2 revealed foci of Clostridial infection that tended to be small and located at or near the luminal surface of the mucosa. Necrotic tissue was usually intensely eosinophilic with dark basophilic elongated and distorted nuclei. Small numbers of heterophils were sometimes noted in adjacent viable tissue. The *Clostridia* were rod-shaped, moderately large, and often in chains. Coccidia were widely scattered and tended to be seen as solitary clusters with the organisms in various stages of development. Most appeared to be developing within absorptive epithelial cells consistent with *E. acervulina*. There was no evidence of villus atrophy associated with the coccidia.

DISCUSSION

Various degrees of intestinal necrosis were observed; however, typical field-type lesions (diphtheritic membrane) of necrotic enteritis were not observed in this study. The reason for absence of field-type lesions is unknown; however, Al-Sheikhly and Truscott (1977) observed similar lesion results 20 h subsequent to intraduodenal infusion of *C. perfringens* cells harvested from broth cultures and resuspended in PBS or fresh sterile TBG. Al-Sheikhly and Truscott (1977) stated that *C. perfringens* in sufficient numbers and slight intestinal damage were apparently required for disease production when a broth culture was utilized. However, in the present study, infusion of *E. acervulina* 5 d prior to inoculation of dietary treatments yielded no different intestinal lesions. Thus, these results indicate that whereas the aforementioned may be major factors, other factors, including the physical nature of the diet and the interaction of other intestinal microflora, may contribute to pathogenesis of the disease.

In the present study, intestinal lesion scores of the two groups of chickens provided the corn diets emphasizes the importance of unknown factors in the pathogenesis of necrotic enteritis. In both groups crop-infused with *E. acervulina*, lesion scores were the same despite one group being fed the corn diet mixed with *C. perfringens*. Similarly, influence of cereal grain source of the diet is obvious, as evidenced by comparison of the lesion scores of the corn + *C. perfringens* and the wheat + *C. perfringens* treatments.

The physiological action of guar gum has been reported to depend on the capacity of guar gum to hydrate rapidly and thus increase the viscosity of digesta in the gizzard and small intestine (Blackburn *et al.*, 1984; Edwards and Read, 1990; and Roberts *et al.* 1990 a,b), whereas pectin, a nontoxic carbohydrate, is a gelling agent thought to be responsible for the sticky feces observed when chickens are fed diets containing high concentrations of rye (Wagner and Thomas, 1977). Rough litter has been suggested to result in minor intestinal damage, which, together with *C. perfringens*, may cause necrotic enteritis (Al-Sheikhly and Truscott, 1977). Lesion scores of the wheat diets with added pectin, guar gum, or new, ground, pine shavings suggest that the diets were conducive to the development of necrotic enteritis. However, the diets with pine

shavings, pectin, or guar gum failed to yield lesion scores as high as that of the wheat-based diet itself.

During the course of the two trials, it was noted that addition of *C. perfringens* culture to the wheat-based diet resulted in a slurry that appeared to adhere to the stirring paddle. Addition of the guar gum, pectin, or ground pine shavings may have resulted in diets with enhanced intestinal digesta movement as compared with the all-wheat diet. Further study is necessary to determine the contribution of this factor to necrotic enteritis lesions.

No deaths occurred in either trial that could be attributed to necrotic enteritis. One chicken in the wheat + pectin + *C. perfringens* treatment in Trial 2 was found dead on the 2nd d the various diets were provided; however, upon necropsy, no wheat diet was found in the gastrointestinal tract of that chicken. The corn diet that the bird had consumed prior to placement on the wheat diet was found in the gizzard but no pathology was found to explain its death.

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