EDUCATION AND PRODUCTION

Growth Performance, Intestinal Microbial Populations, and Serum Cholesterol of Broilers Fed Diets Containing *Lactobacillus* Cultures

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ABSTRACT A study was conducted to determine the effects of adherent *Lactobacillus* culture on growth performance, intestinal microbial population, and serum cholesterol level of broilers. Four dietary treatments, consisting of the basal diet (control), basal diet + 0.05, 0.10, or 0.15% *Lactobacillus* culture (LC), were fed to 2,000 Arbor Acres broiler chicks from 1 to 42 d of age (DOA). The chicks were randomly assigned to 40 cages (50 chicks per cage, 10 cages per diet). The experimental period was 42 d. Body weights and feed to gain ratio were measured at 21 and 42 DOA. The intestinal microbial populations and serum cholesterol levels were determined at 10, 20, 30, and 40 DOA.

The results showed that body weights and feed to gain ratios were improved significantly ($P < 0.05$) when compared to control broilers for broilers fed diets containing 0.05 or 0.10% LC, but not 0.15% LC, at 21 and 42 DOA. Coliform counts in the cecum of birds receiving 0.05% LC at 10, 20, and 30 DOA, and 0.10% at 10 and 20 DOA were significantly lower ($P < 0.05$) than those of the control birds. The total aerobes, total anaerobes, lactobacilli, and streptococci in the small intestines and ceca of the control birds were not significantly different from those of the treated groups. Serum cholesterol levels were significantly lower ($P < 0.05$) in broilers fed the three diets containing LC at 30 DOA, and in the birds fed 0.05 or 0.10% LC at 20 DOA.

(*Key words*: *Lactobacillus*, broiler, serum cholesterol, probiotics, intestinal microbial population)

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INTRODUCTION

There is a worldwide attempt to reduce antibiotic use in animal production because increased microbial resistance to antibiotics and residues in animal products can be harmful to consumers. Over the last two decades, probiotics (direct-fed microbials), which include *Lactobacillus* cultures, have been used as an alternative to antibiotics in animal production. However, the results of feeding *Lactobacillus* cultures to chickens were not conclusive. Tortuero (1973) pioneered the use of *Lactobacillus* cultures in broilers and found that growth rate increased in chicks given a *Lactobacillus acidophilus* culture. Similar improvements on the growth performance have been reported by several researchers (Dilworth and Day, 1978; Watkins *et al*., 1982; Jin *et al*., 1996a; Mohan *et al*., 1996; Yeo and Kim, 1997). Consistent improvements in body weight gain of chicken fed *Lactobacillus sporegenes* culture have also been reported by Han *et al*. (1984), Mohan-Kumar and Christopher (1988), and Kalbande *et al*. (1992). However, several other workers (Watkins and Kratzer, 1983; 1984; Maiolino *et al*., 1992) reported that there were no significant differences in weight gain of chicken given diets with or without *Lactobacillus* cultures. Inconsistent results of using probiotics in animal production have been a constraint to the promotion of their uses. Variations in the efficacy of probiotics may be due to differences in microbial species or strains of microorganisms used or the methods of preparing the supplement.

It is speculated that the benefit derived from probiotics is a result of the organisms growing and contributing some beneficial function in the intestinal tract. Therefore, one of the most important considerations in achieving the desired effect from using lactobacilli as growth promotants is to ensure that the organisms survive passage through the stomach and proliferate in the intestinal tract. To establish successfully in the intestinal tract, bacterial strains must be able to adhere physically and multiply on the intestinal surfaces. We have previously isolated some *Lactobacillus* spp. from the chicken digestive tract. These isolates have

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Abbreviation Key: DOA = days of age; LC = *Lactobacillus* culture.

TABLE 1. Composition of the basal diet fed to broilers

Ingredient and composition	0 to 21 d	22 to 42 d
	(%)	
Ground yellow corn (8.9% CP)	56.00	61.60
Soybean meal (44.6% CP)	29.00	26.00
Corn gluten meal (42% CP)	3.50	0.00
Meat-born meal (50% CP)	3.50	0.00
Fish meal (50% CP)	1.50	7.00
Palm oil	3.50	2.60
Limestone	0.50	0.60
Dicalcium phosphate	0.85	0.85
Salt (NaCl)	0.30	0.30
Vitamin premix[1]	0.50	0.50
Mineral premix[2]	0.50	0.50
DL-methionine	0.35	0.05
Calculated composition		
CP (N × 6.25)	21.9	20.6
ME, kcal/kg	3,158	3,194
Lysine	1.18	0.99
Methionine and cystine	0.96	1.75
Calcium	0.90	0.89
Phosphorus	0.44	0.42

[1]The vitamin premix supplied the following per kilogram of complete feed: vitamin A, 4,500 IU (retinyl acetate); cholecalciferol, 1,000 IU; vitamin E, 25 IU (dl-α-tocopheryl acetate); vitamin B_{12}, 0.02 mg; menadione, 1.5 mg; riboflavin, 3 mg; thiamine, 1.5 mg; pantothenic acid, 5 mg; niacin, 20 mg; choline, 150 mg; folic acid, 0.5 mg; biotin, 0.5 mg; pyridoxine, 2.5 mg.

[2]The mineral premix supplied the following per kilogram of complete feed: manganese ($MnSO_4 \cdot H_2O$), 60 g; zinc (ZnO), 40 mg; iron ($FeSO_4 \cdot 7H_2O$), 80 mg; copper ($CuSO_4 \cdot 5H_2O$), 8 mg; selenium (Na_2SeO_3), 0.2 mg; iodine (Iodized NaCl), 0.8 mg; cobalt ($CoCl_2$), 0.4 mg.

shown a strong ability to attach to the intestinal epithelium of chicken (Jin *et al*., 1996d) and to survive in acidic and bile conditions *in vitro*. The objective of the present study was to determine the effects of the adherent *Lactobacillus* cultures on the growth performance, intestinal microbial populations, and serum cholesterol of broilers under tropical conditions.

MATERIALS AND METHODS

Animals and Diets

Two thousand 1-d-old Arbor Acres broiler chicks were randomly divided into 40 groups of 50 chicks each. Each group was assigned to a cage (3 m × 3 m) that had raised wire floors and contained a self-feeder and waterer to provide *ad libitum* access to feed and water. Chicks were brooded with a 100 W bulb for 10 d. The chicks were divided into four treatment groups (10 cages per treatment), and fed one of the four dietary treatments. The diets were 1) basal diet (control), 2) basal diet + 0.05% *Lactobacillus* culture (LC), 3) basal diet + 0.10% LC, and 4) basal diet + 0.15% LC. The basal diet used in the study was a typical corn-soybean diet and formulated to meet nutrient requirements (NRC, 1984) for starter (0 to 21 d) and grower (22 to 42 d) periods (Table 1). Chickens were weighed at 1, 21, and 42 d on cage basis to determine weight gain. Feed consumed was recorded daily, the uneaten discarded, and feed efficiencies were calculated (total feed:total gain). Mortality was recorded as it occurred and percentage mortality was determined at the end of the study. The experiment was carried out for 42 d under typical tropical conditions (hot and humid). All animal management and sampling procedures were in accordance with the guidelines of the Consortium Guide (1988).

Lactobacillus *Culture Preparation*

Twelve strains of *Lactobacillus*, which belong to four species (*L. acidophilus*, *L. fermentum*, *L. crispatus*, and *L. brevis*), isolated from chicken intestine, were used (Jin *et al*., 1996d). They were inoculated into Man, Rogosa, and Sharpe (MRS) broth[3] and incubated at 39 C for 24 h, after which the bacterial cells were harvested by centrifugation at 2,000 × *g* for 10 min at 4 C.

The bacterial pellets were lyophilized and stored at –20 C. To obtain a concentration of 1×10^9 cells per gram, LC was diluted with appropriate amounts of cornstarch and skimmed milk powder, based on their original colony-forming units per gram determined on MRS agar. The LC was stored at 4 C and mixed into the feed each day to ensure viable bacterial cells in the feed throughout the experimental period. The viability of the bacterial cells was checked biweekly to ensure that the concentration of the viable bacterial cells remained at 1×10^9 cells per gram.

Sample Procedures

Blood and intestinal samples were collected on 10, 20, 30, and 40 d of age (DOA) to determine serum cholesterol and the populations of total aerobes, total anaerobes, coliforms, lactobacilli, and streptococci in the small intestine and cecum. Ten birds (one per cage) were randomly selected from each treatment on each of these days and euthanatized by severing the jugular vein. The blood was collected in a test tube to obtain serum. The carcasses were immediately opened and the entire intestine removed aseptically. The cut sections of the small intestine (entire section after the duodenal loop to the cecal junction) and the ceca were ligated with a nylon suture.

Bacteriological Examinations

Approximately 1 g of the small intestinal and cecal contents was mixed with 9 mL of prereduced sterile dilution blank solution (Bryant and Burkey, 1953), and homogenized for 3 min. From the initial 10^{-1} dilution, 10-fold serial dilutions were subsequently made in sterile prereduced dilution blank solution for anaerobic bacteria and in 0.1% peptone for aerobic bacteria. The samples from the small intestine and cecum were diluted to 10^{-5}, 10^{-7}, and 10^{-9}. For each dilution, 0.1 mL was inoculated in

[3]Oxoid Ltd., Basingstoke, Hampshire RG24 8PW, UK.

TABLE 2. Body weights and feed to gain ratios of broilers fed diets without (CS) or with either 0.05% *Lactobacillus* culture (LC) (CS + 0.05% LC), 0.10% *Lactobacillus* culture (CS + 0.10% LC), or 0.15% *Lactobacillus* culture (CS + 0.15% LC) from 1 to 42 d of age

	Body weight			Feed to gain ratio			Mortality
Diet	1 d	21 d	42 d	1 to 21 d	22 to 42 d	1 to 42 d	1 to 42 d
	(g)			(g:g)			(%)
CS	41.8[a]	645.9[c]	1,914.5[c]	1.63[a]	2.18[a]	2.00[a]	8.2
CS + 0.05% LC	41.4[a]	671.8[ab]	1,983.2[b]	1.54[b]	2.05[b]	1.88[b]	5.6
CS + 0.10% LC	41.4[a]	681.0[a]	2,077.9[a]	1.52[b]	1.85[c]	1.74[c]	3.2
CS + 0.15% LC	41.5[a]	647.9[bc]	1,925.3[bc]	1.64[a]	2.11[a]	1.95[ab]	7.0
SEM	1.51	20.9	52.5	0.051	0.094	0.053	ND
Source of variation	Probabilities						
Diet	NS	0.018	0.0001	0.0007	0.0001	0.0001	ND

[a–c]Means within columns with no common superscript differ significantly ($P < 0.05$); ND, no data.

an agar roll tube for anaerobes and on agar plate for aerobes. The medium (6 mL) in a roll tube used for culturing and counting total anaerobes was FM 98-5 medium (Jin *et al.*, 1996a). The plate media used were MRS agar for lactobacilli, Brain Heart Infusion agar (BHIA)[3] for total aerobic bacterial count, MacConkay agar[3] for coliforms, and KF Streptococcus agar[4] for streptococci. All the inoculated roll tubes and plates were incubated at 39 C. Total numbers of bacterial colonies were counted at the end of each incubation period. The roll tubes were incubated for 6 d, MRS agar plates were incubated anaerobically for 2 d in a Gas-Pak container,[3], BHIA and MacConkay agar plates were incubated aerobically for 1 d, and KF *Streptococcus* agar plates for 2 d.

Analysis of Serum Cholesterol

Blood samples (5 mL) were taken from the jugular vein. Serum was isolated by centrifugation at 2,000 × *g* for 10 min and stored at –70 C until used for analysis. Serum cholesterol was measured by using cholesterol diagnostic kits (Sigma Diagnostics, Catalog No. 352).[5]

Statistical Analysis

Data were analyzed in a completely randomized design using the General Linear Models procedures of SAS® (SAS Institute, 1988). The microbial population, volatile fatty acids (VFA), and non-VFA, and serum cholesterol levels were analyzed on individual broilers whereas growth performance was based on cage weights. Feed to gain ratio was calculated and analyzed on a cage basis.

RESULTS AND DISCUSSION

The body weight, cumulative feed to gain ratio, and mortality of broilers from 1 to 42 DOA fed diets without or with 0.05, 0.10, or 0.15% LC are summarized in Table 2. The treatment with 0.10% LC of feed produced a significantly greater BW ($P < 0.05$) and feed to gain ratio than the control or the treatments with 0.05 or 0.15% LC. A significant ($P < 0.05$) increase in BW was observed at 21 and 42 DOA in broilers fed the diets containing 0.05 or 0.10% LC, but not 0.15% LC. Similar improvements in BW have been reported in poultry fed with *Lactobacillus*-supplemented diets (Tortuero, 1973; Crawford, 1979; Kim, *et al.*, 1988; Jin *et al.*, 1996a; Mohan, *et al.*, 1996; Yeo and Kim, 1997). In the present experiment, the improvement of BW was consistent in both the growing period (0 to 3 wk) and the finishing period (4 to 6 wk). This result is contrary to the findings of Mohan *et al.* (1996), who observed that the beneficial effect of probiotic on chicken occurred only after the 4th wk of growth, and that of Yeo and Kim (1997), who reported that average daily weight gain of chickens fed probiotics was significantly increased during the first 3 wk of growth but not during the 4th to 6th wk of growth.

Feed to gain ratios were improved significantly ($P < 0.01$) for the broilers fed diets with 0.05 or 0.10% LC from 1 to 21, 22 to 42, or 1 to 42 DOA. The feed to gain ratios were decreased by 0.12 ($P < 0.05$) and 0.26 ($P < 0.01$) unit for the birds fed diets with 0.05 and 0.10% LC, respectively. However, there was no significant improvement in the feed to gain ratio of birds fed with the diet containing 0.15% LC. There was only a slight decrease in feed to gain ratio of 0.07 and 0.05 from 22 to 42, and 1 to 42 DOA, respectively. The present results agree with Mohan *et al.* (1996), who found that feed to gain ratio of broilers fed on diets supplemented with 75 or 100 mg/kg *Lactobacillus* was better than those of the control and those fed on 125 mg/kg *Lactobacillus.* Similar improvements in feed efficiency have been reported for poultry receiving probiotics (Tortueto, 1973; Crawford, 1979; Jin *et al.*, 1996a; Mohan *et al.*, 1996). Mortality was lower in broilers fed the three diets containing LC, but statistical analysis on mortality was not conducted, as it was only recorded on a whole treatment basis.

The improvements in BW and feed to gain ratio of broilers fed *Lactobacillus* supplement were probably due to the *Lactobacillus* spp. used in the supplement. It has

[4]Difco Laboratories, Detroit, MI 48232-7058.
[5]Sigma Chemical Co., St. Louis, MO 63178-9916.

TABLE 3. Coliform populations in broilers fed diets without (CS) or with either 0.05% *Lactobacillus* culture (LC) (CS + 0.05% LC), 0.10% *Lactobacillus* culture (CS + 0.10% LC), or 0.15% *Lactobacillus* culture (CS + 0.15% LC) from 10 to 40 d of age

	Counts of coliform			
Organ	10 d	20 d	30 d	40 d
	(log cfu/g)			
Small intestine				
CS	7.11	6.40	6.00	6.71
CS + 0.05% LC	6.30	6.04	6.15	6.13
CS + 0.10% LC	7.02	5.87	6.09	6.88
CS + 0.15% LC	6.80	5.86	6.31	6.44
SEM	0.83	0.90	0.56	0.62
Source of variation	Probabilities			
Diet	NS	NS	NS	NS
Cecum				
CS	8.28[a]	7.26[a]	7.35[a]	7.50[a]
CS + 0.05% LC	7.74[b]	6.48[b]	6.68[b]	7.55[a]
CS + 0.10% LC	7.83[b]	6.59[b]	7.69[a]	7.63[a]
CS + 0.15% LC	7.87[b]	6.89[ab]	7.37[a]	7.55[a]
SEM	0.18	0.38	0.61	0.19
Source of variation	Probabilities			
Diet	0.049	0.035	0.03	NS

[a,b]Means within columns with no common superscript differ significantly ($P < 0.05$).

been suggested that to obtain the best effects from *Lactobacillus* as a growth promotant, the bacteria used must be able to survive and later colonize the gastrointestinal tract so that their beneficial functions could be performed. The *Lactobacillus* spp. used in the present study have a strong ability to attach to the intestinal epithelium of chicken (Jin *et al.*, 1996d), are resistant to the bile and acidic conditions and are able to antagonize and competitively exclude some pathogenic bacteria *in vitro* (Jin *et al.*, 1996b,c).

The results on the intestinal microbial population are presented in Tables 3 to 5. There was no significant difference in coliform populations in the small intestine of broilers fed on diets with or without *Lactobacillus* supplementation during the whole experimental period (Table 3). However, the number of coliforms was reduced significantly ($P < 0.05$) in the ceca of broilers fed diets containing all the three concentrations of LC at 10 DOA (Table 3). A significant reduction in coliforms was also observed in the birds receiving 0.05% LC at 20 and

TABLE 4. Lactobacilli populations in broilers fed diets without (CS) or with either 0.05% *Lactobacillus* culture (LC) (CS + 0.05% LC), 0.10% *Lactobacillus* culture (CS + 0.10% LC), or 0.15% *Lactobacillus* culture (CS + 0.15% LC) from 10 to 40 d of age

	Counts of lactobacilli			
Organ	10 d	20 d	30 d	40 d
	(log cfu/g)			
Small intestine				
CS	7.67[b]	7.43	7.22	6.94
CS + 0.05% LC	8.21[ab]	6.67	7.73	7.19
CS + 0.10% LC	8.12[ab]	7.32	7.33	6.94
CS + 0.15% LC	8.38[a]	6.85	7.23	7.01
SEM	0.53	0.86	0.38	0.32
Source of variation	Probabilities			
Diet	0.05	NS	NS	NS
Cecum				
CS	8.80[b]	8.88	8.32	8.01
CS + 0.05% LC	9.38[a]	8.72	8.38	7.93
CS + 0.10% LC	9.29[a]	8.53	8.75	7.89
CS + 0.15% LC	8.84[b]	8.68	8.46	8.19
SEM	0.26	0.23	0.32	0.17
Source of variation	Probabilities			
Diet	0.047	NS	NS	NS

[a,b]Means within columns with no common superscript differ significantly ($P < 0.05$).

TABLE 5. Total aerobe, total anaerobe, and streptococci populations in broilers fed diets without (CS) or with either 0.05% *Lactobacillus* culture (LC) (CS + 0.05% LC), 0.10% *Lactobacillus* culture (CS + 0.10% LC), or 0.15% *Lactobacillus* culture (CS + 0.15% LC) from 10 to 40 d of age

Population	Organ	10 d	20 d	30 d	40 d
		(log cfu/g)			
Counts of total aerobe	Small intestine				
	CS	7.71	7.09	6.90	7.18
	CS + 0.05% LC	7.61	6.68	7.24	6.80
	CS + 0.10% LC	7.25	6.79	6.69	7.30
	CS + 0.15% LC	7.61	6.41	7.00	6.83
Counts of total anaerobe					
	CS	7.68	6.94	6.81	6.91
	CS + 0.05% LC	7.69	7.04	6.92	7.08
	CS + 0.10% LC	7.77	7.03	6.89	6.88
	CS + 0.15% LC	7.96	6.93	6.85	6.63
Counts of streptococci					
	CS	7.87	7.32	6.59	6.39
	CS + 0.05% LC	7.81	6.60	7.00	6.43
	CS + 0.10% LC	8.08	6.71	6.34	6.92
	CS + 0.15% LC	8.05	6.70	6.86	6.52
Counts of total aerobe	Cecum				
	CS	8.36	8.48	7.76	7.86
	CS + 0.05% LC	7.99	8.11	7.82	7.73
	CS + 0.10% LC	8.24	8.38	8.05	7.72
	CS + 0.15% LC	8.17	8.31	7.89	7.75
Counts of total anaerobe	CS	10.45	10.23	9.71	9.69
	CS + 0.05% LC	10.05	9.87	9.83	9.82
	CS + 0.10% LC	10.33	9.71	9.81	9.72
	CS + 0.15% LC	10.39	9.75	9.71	9.87
Counts of streptococci	CS	8.34	8.50	7.98	7.00
	CS + 0.05% LC	8.39	8.45	7.88	6.88
	CS + 0.10% LC	8.57	7.90	7.76	7.16
	CS + 0.15% LC	8.29	7.89	8.11	7.21

30 DOA, and 0.10% LC at 20 DOA. No differences in coliform population were found in the ceca of the broilers fed the four diets at 40 DOA. These results concur with that of Watkins and Kratzer (1983), who reported that chicks dosed with *Lactobacillus* strains had lower numbers of coliforms in cecal macerates than the control. Francis *et al.* (1978) also reported that the addition of *Lactobacillus* product at 75 mg/kg of feed significantly decreased the coliform counts in the ceca and small intestine of turkeys. Using gnotobiotic chicks, Fuller (1977) found that host-specific *Lactobacillus* strains were able to decrease *Escherichia coli* in the crop and small intestine. Watkins *et al.* (1982) similarly observed that competitive exclusion of pathogenic *E. coli* occurred in the gastrointestinal tract of gnotobiotic chicks dosed with *L. acidophilus.* The *Lactobacillus* strains used in the present experiment had been found to be able to inhibit the growth of three serotypes of *Escherichia coli,* O1:K1, O2:K1, and O78:K80 *in vitro* (Jin *et al.,* 1996b).

There were no significant differences in the numbers of lactobacilli in the small intestine and cecum of broilers fed with or without LC at 20, 30, and 40 DOA. However,

TABLE 6. Serum cholesterol levels in broilers fed diets without (CS) or with either 0.05% *Lactobacillus* culture (LC) (CS + 0.05% LC), 0.10% *Lactobacillus* culture (CS + 0.10% LC), or 0.15% *Lactobacillus* culture (CS + 0.15% LC) from 10 to 40 d of age

	Serum cholesterol			
Diet	10 d	20 d	30 d	40 d
	(mg/dL)			
CS	148	111[a]	158[a]	128[a]
CS + 0.05% LC	151	89[b]	130[b]	126[a]
CS + 0.10% LC	151	82[b]	123[b]	106[b]
CS + 0.15% LC	148	98[ab]	133[b]	117[ab]
SEM	25	16	17	19
Source of variation	Probabilities			
Diet	NS	0.004	0.0005	0.05

[a,b]Means within columns with no common superscript differ significantly ($P < 0.05$).

at 10 DOA, the *Lactobacillus* populations in the cecum of birds receiving 0.05 or 0.10% LC and in the small intestine of birds receiving 0.15% LC were significantly ($P < 0.05$) higher than those of the control birds (Table 4). It is not known, from this study, why the addition of the adherent *Lactobacillus* culture failed to increase significantly the number of lactobacilli in the small intestine and cecum of chicken, although the *Lactobacillus* used has demonstrated a strong ability to attach to the ileal epithelial cells of chicken *in vitro* (Jin *et al*., 1996d) and densely colonized lactobacilli on the surface of the chicken ceca have been observed using electron microscopy.

There were no significant differences in the total aerobes, total anaerobes, and streptococci in the small intestine and cecum of broilers fed with or without LC (Table 5). Generally, total microbial numbers, irrespective of LC additions, were higher in the cecum than in the small intestine and the numbers decreased slightly with increasing age of the broiler. Salanitro *et al.* (1978) and Jin *et al.* (1997) have also reported that the cecum contains higher numbers of microorganisms than the small intestine.

Serum cholesterol levels were significantly lower ($P < 0.01$) in broilers fed the three diets containing LC at 30 DOA (Table 6). At 20 DOA, the serum cholesterol levels of broilers receiving 0.05 or 0.10% LC were also significantly lower ($P < 0.01$) than those of the control birds, and at 40 DOA only the serum cholesterol level of broilers receiving 0.10% LC was significantly lower. At 10 DOA there was no significant difference in the serum cholesterol levels of birds fed with or without LC. A similar reduction of serum cholesterol levels has been found in broilers (Mohan *et al*., 1996), layers (Tortuero *et al*., 1975; Abdulrahim *et al*., 1996), germ-free pigs (Mott *et al*., 1973), rats (Grunewald, 1982), and humans (Harrison and Peat, 1975) fed diets supplemented with *Lactobacillus*. The decrease in cholesterol level could be due to cholesterol assimilation (or uptake) by the *Lactobacillus* cells (Gilliland *et al*., 1985; Buck and Gilliland, 1994), or to the coprecipitation of cholesterol with deconjugated bile salts (Klaver and Van der Meer, 1993).

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