

Effect of Dietary Conjugated Linoleic Acid on the Growth Rate of Live Birds and on the Abdominal Fat Content and Quality of Broiler Meat¹

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ABSTRACT Two experiments were conducted to investigate the influence of dietary conjugated linoleic acid (CLA) on the growth, body composition, abdominal fat accumulation, and meat quality in broilers. In Experiment 1, 50 broilers that were 3 wk old (total of 200 birds) were assigned to one of the four diets containing 0, 0.25, 0.5, or 1% CLA and were fed for 3 wk. In Experiment 2, 40 broilers that were 3 wk old (total of 120 birds) were assigned to one of the three diets containing 0, 2, or 3% CLA and fed for 5 wk. At the end of the 3-wk feeding trial, the average body weight of broilers for Experiment 1 was about 2.20 kg per bird for all treatments. For Experiment 2, after 5-wk feeding trial, the average body weights of birds were 4.04, 3.99, and 3.93 kg for the control, 2% CLA, and 3% CLA groups, respectively, with a non sig-

nificant reduction in body weight as the levels of dietary CLA increased. There was no difference in abdominal fat weight, the total body fat, and protein content in broilers among the CLA treatments for Experiment 1. However, when the dietary CLA was increased to 2 or 3%, the total body fat content was reduced. The whole body fat content decreased from 14.2% in the control to 11.9 and 12.2% for 2 and 3% CLA, respectively. Dietary CLA at 2 and 3% levels influenced meat quality. After cooking, the breast meat from 2 or 3% dietary CLA treatment was harder and drier, and the color was a little darker than that of the control. These changes could be caused by the decreased unsaturated fatty acid content in meat after CLA feeding, which increased the melting point of the fat.

(*Key words:* conjugated linoleic acid, broiler growth, abdominal fat, meat color, sensory characteristics)

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INTRODUCTION

The major effect of conjugated linoleic acid (CLA) on animal performance is reducing fat accumulation and promoting muscle growth. Park et al. (1997) reported that CLA repartitioned body fat to lean and improved feed efficiency in rats. In a study with mice, DeLany et al. (1999) found that CLA feeding induced a rapid and marked decrease in fat accumulation and an increase in protein deposition. Park et al. (1999) observed that a 0.5% CLA diet significantly reduced body fat, increased whole body protein, water, and ash in rats. Dugan et al. (1997) reported that pigs fed CLA reduced feed intake by 5.2%, improved feed conversion efficiencies by 5.9%, decreased subcutaneous fat accumulation by 6.8%, and gained 2.3% more lean than pigs fed sunflower oil. However, Scimeca (1998) reported that feeding a diet supplemented with 1.5% CLA for 36-wk had no effect on feed consumption and body weight of rats. Stangl (2000) reported that feeding a diet containing up to 5% CLA mixture does not significantly

influence growth and body partitioning in rats. With 70-kg live-weight pigs, Mueller et al. (2000) showed that supplementation of CLA in iso-energy diets with a strongly positive energy balance has no marked effect on total lipid metabolism, but a slightly enhanced deposition of protein is evident. CLA supplementation at 3.0-3.4 g/d had no significant effect on body composition, body weight, and body mass index (BMI) in humans (Berven et al., 2000; Zambell et al., 2000).

Several studies showed that CLA influences meat quality. CLA improved pork composition by increasing marbling scores and intramuscular fat deposition, while having no detrimental effect on the sensory characteristics of pork (Dugan et al., 1999). Feeding CLA to pigs tended to increase the firmness of belly and increased lean meat content, and improved other aspects of meat quality in growing-finishing pigs (O'Quinn et al., 2000). Du et al. (2001) found that dietary CLA improved the oxidative stability of cooked chicken meat patties during aerobic storage.

The objective of this study was to assess the effect of dietary CLA on the growth, abdominal fat accumulation, and meat quality in broilers. Because breast meat is the

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Abbreviation Key: CLA = conjugated linoleic acid; GC = gas chromatography; MSD = Mass spectrometry detector.

TABLE 1. Percentage composition of diets fed to broilers

Ingredient	Diet (1 to 3 wk)	Diet (4 to 6 wk)	Diet (7 to 8 wk)
Corn	51.15	50.34	51.94
Soy meal	38.38	28.57	22.32
Wheat middlings	22.85	10.26	15.35
Meat and bone meal	3.00	3.00	3.00
Limestone	1.05	0.96	0.87
Dicalcium phosphate	0.85	0.85	0.59
Mineral premix ¹	0.30	0.30	0.30
Vitamin premix ²	0.30	0.30	0.30
DL-methionine	0.25	0.15	0.08
Sodium chloride (iodized)	0.09	0.25	0.24
Bacitracin methylene disalicylate	0.025	0.025	0.025
Soybean oil	4.61	5.0 to 0 ³	5.0 to 0 ³
Conjugated linoleic acid (CLA) source	0	0 to 5.0 ³	0 to 5.0 ³
Calculated analysis			
Metabolizable energy, kcal/kg	3,100	3,100	3,100
Protein	23.00	20.00	18.00
Total sulfur amino acids	0.95	0.77	0.65
Methionine	0.59	0.45	0.35
Lysine	1.33	1.11	0.96
Arginine	1.56	1.34	1.20
Glycine + serine	2.31	2.02	1.82
Histidine	0.60	0.52	0.46
Isoleucine	0.96	0.81	0.72
Leucine	1.91	1.66	1.50
Phenylalanine + tyrosine	1.97	1.67	1.47
Threonine	0.87	0.74	0.66
Tryptophan	0.31	0.26	0.23
Valine	1.06	0.92	0.83
Calcium	1.00	0.95	0.85
Available phosphate	0.45	0.45	0.40
Total phosphate	0.69	0.72	0.68
Sodium	0.18	0.25	0.25

¹Mineral premix provided per kilogram of diet: Mn, 80 mg; Zn, 90 mg; Fe, 60 mg; Cu, 12 mg; Se, 0.147 mg; sodium chloride, 2,247 g.

²Vitamin premix supplies per kilogram of diet: retinyl acetate, 8,065 IU; cholecalciferol, 1,580 IU; 25-hydroxycholecalciferol, 31.5 µg, dl- α -tocopheryl acetate, 15 IU; vitamin B₁₂, 16 µg; menadrene, 4 mg; riboflavin, 7.8 mg; pantothenic acid, 12.8 mg; niacin, 75 mg; Choline chloride, 509 mg; folic acid, 1.62 mg; biotin, 0.27 mg.

³Experiment 1: 0% CLA diet, soybean oil, 5.00%; CLA, 0%. In the 0.25% CLA diet, soybean oil, 4.57%; CLA, 0.43%. In 0.50% CLA diet, soybean oil, 4.14%; CLA, 0.86%. In 1.00% CLA diet, soybean oil, 3.33%; CLA, 1.67%. Experiment 2: 0% CLA diet, soybean oil, 5.00%; CLA, 0%. In 2.0% CLA diet, soybean oil, 2.67%; CLA, 3.33%. In 3.0% CLA diet, soybean oil, 0%; CLA, 5%.

most valuable part in chicken carcass, the quality of breast meat after CLA feeding was analyzed.

MATERIALS AND METHODS

Dietary Treatments

Experiment 1. A total of 200, 3-wk-old broiler chickens were assigned to four dietary treatments containing 0, 0.25, 0.5, or 1.0% CLA. Diets were prepared by adding 0, 0.43, 0.85, or 1.67% a commercial CLA³ that contained 60% CLA. Broilers were allotted to eight pens and randomly assigned to each dietary CLA treatment and fed for 3 wk. A corn-soybean meal basal diet was used, and energy was adjusted using soybean oil (Table 1). Feed consumption and body weights were recorded at the beginning and end of the feeding trial. At the end of the 3-wk feeding period, broilers were slaughtered using the USDA guidelines.

Eight birds from each dietary treatment were used for carcass composition analysis: whole broiler carcasses were ground three times each through 2-cm, 9-mm, and 3-mm plates using a heavy-duty grinder. Protein, fat, water, and ash content of carcass were analyzed with the Association of Official Analytical Chemists methods (AOAC, 1999). Whole liver was collected during slaughter and weighed.

Abdominal fat and carcass weight were also measured. Breast meat was separated from birds 24 h after slaughter for sensory characteristic analysis. The pH of raw breast meat and the texture and color of cooked breast meat were measured.

Experiment 2. One hundred twenty, 3-wk-old broiler chickens were assigned to three dietary treatments containing 0, 2.0, or 3.0% CLA. Broilers were allotted to six pens; two pens were randomly assigned to each dietary CLA treatment. A corn-soybean meal basal diet was used, and energy was adjusted using soybean oil (Table 1). Broilers were weighed at the beginning, 3 wk, and end of the 5-wk feeding trial. Other measurements and analyses were the same as in Experiment 1.

³Conlinco, Inc., Detroit Lakes, MN 56502.

Lipid Extraction

Approximately 3-g samples were accurately weighed into a 50-mL test tube. After adding 30 mL of Folch 1 (chloroform:methanol = 2:1, w/v, Folch et al., 1957), the samples were homogenized with a Brinkman polytron⁴ (Type PT 10/35) for 10 s at high speed. Twenty-five micrograms of butylated hydroxyanisole (BHA, 10%) dissolved in 98% ethanol was added to each sample prior to homogenization. The homogenate was filtered through a Whatman #1 filter paper into a 100-mL graduated cylinder and 1/4 volume (on the basis of filtrate) of 0.88% NaCl solution was added. After the cylinder was capped with a glass stopper, the filtrate was mixed well. The inside of the cylinder was washed twice with 2 mL of Folch 2 (3:47:48/CHCl₃:CH₃OH:H₂O), and the contents were stored until the aqueous and organic layers clearly separated. The upper layer was siphoned off, and the lower layer was transferred to a glass scintillation vial and dried at 50 C under a nitrogen flow.

Analysis of Fatty Acid Composition

One milliliter of methylating reagent (anhydrous methanolic-HCl-3N)⁵ was added to a test tube containing 50 μ L of total lipid and was incubated in a water bath at 60 C for 40 min. After cooling to room temperature, 2 mL of hexane and 5 mL of water were added, mixed thoroughly, and left at room temperature overnight for phase separation. The top (hexane) layer, containing methylated fatty acids, was used for gas chromatographic analysis. Analysis of fatty acid composition was performed by a gas chromatograph⁶ (HP 6890) equipped with an autosample injector⁶ and flame ionization detector. A capillary column⁶ (HP-5; 0.25 mm i.d., 30 m, 0.25- μ m film thickness) was used. A splitless inlet was used to inject samples (1 μ L) into the capillary column. Ramped oven temperature conditions (180 C for 2.5 min, increased to 230 C at 2.5 C/min, then held at 230 C for 7.5 min) were used. Temperatures of the inlet and detector were 280 C. Helium was used as a carrier gas, and a constant column flow of 1.1 mL/min was used. Flame ionization detector air, hydrogen (H₂), and make-up gas (helium) flows were 350, 35, and 43 mL/min, respectively. Fatty acids were identified using a mass spectrometer (MS) detector⁶ (Model 5973). Gas chromatography-MS was performed with the same column and oven temperature conditions as described previously. The ionization potential of the MS was 70 eV, and the scan range was 45 to 450 m/z.

Identification of fatty acids was achieved by comparing mass spectral data with those of the Wiley library⁶ and

fatty acid standards. CLA isomers in meat lipids were identified by comparing with CLA standards purchased.^{7,8} The compositions of CLA isomers and fatty acids were reported as percentage composition of total lipids, and total peak area (pA*sec) was used to calculate fatty acid composition.

Sensory Evaluation

For the sensory characteristics, the breast meat kept at 4 C for 2 d was cooked to an internal temperature of 74 C in a 250 C electric oven. The cooked breast meat was vacuum packaged within an hour after cooking and stored at 4 C overnight. Immediately before sensory analysis, breast meat was transversely sliced into thin pieces and presented to sensory panelists. Sixteen trained sensory panelists characterized attributes of breast rolls. Panelists were selected based on interest, availability, and performance in screening tests conducted with samples similar to those being tested. Three training sessions were conducted to let panelists familiarize themselves with the odor, color and texture of cooked chicken breast meat, and the scale to be used. Linear horizontal scales of 15 cm, anchored with descriptors at opposite ends, were used to rate the stimuli of color (white to dark), aroma (weak to strong), hardness (soft to hard), and juiciness (dry to juicy) of chicken breast meat. The responses from the panelists were expressed in numerical values ranging from 0 to 15. All samples presented to panelists were labeled with random three-digit numbers.

Instrument Measurement

After storage at 4 C for 2 d, breast meat from Experiment 2 was used for instrumental analyses. The pH value of raw breast meat was measured after homogenizing 2 g of meat in 10 mL of distilled water. Cooked breast meat stored at 4 C overnight was tempered under room temperature for 2 h before texture measurement. Resistance was analyzed using an Instron⁹ equipped with a star probe. For color measurement, the cooked breast meat was transversely cut into two halves, and L*, a*, and b* values of the newly cut surface were measured with a Hunter color meter.¹⁰

Statistical Analysis

The effect of dietary CLA on the growth and feed consumption of live broilers, and fatty acid composition, chemical composition, color and sensory attributes of breast meat were analyzed with general linear models procedure (GLM) of SAS software (SAS Institute, 1989). Student-Newman-Keul's multiple-range test was used to compare differences among mean values ($P < 0.05$). Mean values and standard errors of the mean were reported.

The significance of overall treatment effect for quality was not analyzed because CLA level was the only treatment. The error (residue) equaled the overall variation of measurements minus the variation due to CLA treatment.

⁴Brinkman Instruments, Inc., Westbury, NY 11590-0207.

⁵Sigma-Aldrich, St. Louis, MO 63178.

⁶Hewlett Packard Co., Wilmington, DE 16808-1610.

⁷Matreya, Inc., Pleasant Gap, PA 16823.

⁸Nuchek, Elysian, MN 56028.

⁹Instron Corporation, Canton, MA 02021.

¹⁰Hunter Associated Labs. Inc., Reston, VA 22090.

TABLE 2. Growth of broilers as affected by dietary conjugated linoleic acid (CLA) treatment¹

Treatment	Weighing period (wk)				Daily gain (avg.)
	3	4	5	6	
Experiment 1					
0% CLA	0.70	1.18	1.76	2.18	0.10
0.25% CLA	0.70	1.18	1.77	2.21	0.11
0.5% CLA	0.70	1.16	1.73	2.17	0.10
1.0% CLA	0.71	1.19	1.74	2.19	0.10
SEM	0.009	0.020	0.029	0.037	0.002
	3	6	8		
Experiment 2					
0% CLA	0.66	2.70	4.04		0.12
2% CLA	0.67	2.67	3.99		0.11
3% CLA	0.67	2.64	3.93		0.11
SEM	0.008	0.057	0.074		0.002

¹For Experiment 1, n = 50; for Experiment 2, n = 40.

RESULTS AND DISCUSSION

There was no difference in the live weight of chicken after feeding up to 1% CLA for 3 wk (Table 2, Experiment 1). When the dietary CLA level increased to 2 and 3%, and fed for 5 wk (Experiment 2); however, the body weight of broilers and daily gain showed a nonsignificant decline as the dietary CLA level increased. This result was in agreement with other reports. DeLany et al. (1999) showed that when the dietary CLA level in rats was low, dietary CLA did not influence the growth rate of rats. Twibell et al. (2000) also reported that the growth rate of fish decreased as the dietary CLA level increased. Stangl (2000), however, found that rats feeding a diet with up to 5% CLA mixture did not significantly influence growth.

Up to 1.0% dietary CLA had no effect on feed consumption, and 2.0 and 3.0% dietary CLA reduced feed consumption of broilers only slightly. Feed conversion rates were constant and were not influenced by dietary CLA (Table 3). However, Twibell et al. (2000) found that the feed efficiency in fish was improved after feeding CLA. Dugan et al. (1997) also found improved feed conversion rates in pigs after feeding CLA diets. The difference in the effect of dietary CLA on animal performance could be related to the differences in animal species, dietary CLA levels, feeding duration, and nutritional status of animals.

No differences in carcass weight among dietary CLA treatments were found in either experiment. In Experiment 1, 0.5% dietary CLA treatment significantly increased the abdominal fat content in the broilers. However, there was no difference in abdominal fat content in Experiment 2. Fat content in whole carcasses was not significantly influenced by dietary CLA level of up to 1%, but was decreased after feeding 2 or 3% dietary CLA. This result differs from the result of DeLany et al. (1999) who showed that CLA feeding at a low level produced a rapid, marked decrease in fat accumulation and an increase in protein deposition in mice. It appears that birds are not as sensitive to dietary CLA treatments as mice. Moisture contents of whole carcasses were also not influenced by dietary CLA (Table 4). Protein content in carcass showed an increasing trend as the dietary CLA levels increased, but this was not statistically significant. Several reports indicated increased protein content in animals after feeding CLA (Park et al., 1999; DeLany et al., 1999), but the reason for the increased protein content after feeding CLA is not clear. The dietary CLA levels up to 3% did not change the ash content of whole carcass.

Up to 1% dietary CLA did not produce an effect on the hardness, color, pH, and sensory characteristics of broiler meat (data not shown). When the dietary levels of CLA were increased to 2 or 3%, however, dietary CLA had

TABLE 3. Feed consumption and efficiency influenced by dietary conjugated linoleic acid (CLA) levels

	Dietary CLA (%)						SEM
	0%	0.25%	0.5%	1.0%	2%	3%	
Feed consumption per bird (kg)							
Experiment 1 ¹	2.96	2.96	2.94	2.95			0.03
Experiment 2 ²	3.29				3.26	3.26	0.06
Experiment 2 ³	2.92				2.88	2.86	0.03
Feed transversion rate							
Experiment 1	0.50	0.51	0.50				0.001
Experiment 2	0.54				0.54	0.53	0.001

¹Average feed consumption starting from the beginning of third week to the end of sixth week.

²Average feed consumption starting from the beginning of third week to the end of sixth week.

³Average feed consumption starting from the beginning of seventh week to the end of eighth week. The feed consumption of each pen as one replication, n = 2.

TABLE 4. Carcass weights and abdominal fat content of broilers fed different dietary conjugated linoleic acid (CLA) levels¹

	0%	0.25%	0.5%	1.0%	2%	3%	SEM
Carcasses weight (g)							
Experiment 1	1,642	1,684	1,638	1,635			37
Experiment 2	2,919				2,924	2,919	61
Abdominal fat (g)							
Experiment 1	30 ^b	37 ^b	41 ^a	37 ^{ab}			2
Experiment 2	60				60	57	4
Carcass fat content (%)							
Experiment 1	14.5	14.6	14.9	15.2			0.7
Experiment 2	14.2 ^a				11.9 ^b	12.1 ^b	0.4
Carcass moisture content (%)							
Experiment 1	66.0	65.8	65.4	65.1			0.6
Experiment 2	65.3				66.7	66.5	0.5
Carcasses protein content (%)							
Experiment 1	16.50	16.47	16.59	16.42			0.2
Experiment 2	17.59				18.18	18.19	0.3
Carcasses ash content (%)							
Experiment 1	2.81	2.89	2.86	2.88			0.2
Experiment 2	2.78				2.96	2.97	0.3

^{a,b}Means within a row with no common superscript differ significantly ($P < 0.05$).

¹For Experiment 1, $n = 30$; for Experiment 2, $n = 40$.

significant effect on the quality of breast meat (Table 5). The resistance of breast meat to the penetration of the star probe increased as the dietary CLA level increased, indicating that dietary CLA increased the hardness of breast meat. In pork, Dugan et al. (1999) showed that dietary CLA did not affect the quality of meat.

There was no difference in the pH of breast meats from broilers fed different levels of CLA. Dietary CLA significantly influenced the color of breast meat. The L^* , a^* , and b^* values decreased as dietary CLA level increased (Table 5), as a result the darkness of breast meat increased after feeding CLA. This result was in agreement with the observation of our sensory panelists (Table 5).

Sensory panelists did not recognize a significant increasing trend in the darkness of breast meat as dietary CLA level increased (Table 5). There was no significant difference in chicken aroma, although the intensity of aroma increased as the dietary CLA increased. The reason for the increased aroma of meat from birds with high dietary CLA treatment could be due to specific flavor compounds that

might be increased in samples with high dietary CLA treatments. Several sensory panelists mentioned that they noticed a special flavor in CLA-treated breast meats, but could not describe it accurately. The hardness of breast meat increased as the dietary CLA level increased, whereas the juiciness decreased slightly. The increased hardness and decreased juiciness could be due to the changes in fatty acid composition of muscle lipids. Dietary CLA increased the proportion of saturated fatty acids (Table 6), and thus, increased the melting point of that fat, which would make the meat drier and harder. Another reason could be due to the increased protein content (Table 4), which increased the hardness of breast meat after cooking. There were no significant differences in sensory measurements due to relatively large variations. However, instrumental analyses (Instron measurement and color) showed significant differences. Based on the instrumental results and also the numerical difference in sensory evaluation, we suggest that there was a difference in meat quality.

TABLE 5. Effect of conjugated linoleic acid (CLA) on the texture and sensory characteristics of cooked breast fillets in Experiment 2¹

Texture	0% CLA	2% CLA	3% CLA	SEM
Instrument measurement				
Resistance (Instron)	3.45 ^b	3.62 ^{ab}	3.81 ^a	0.11
pH ²	5.82	5.81	5.81	0.09
Color L^* value	80.70 ^a	80.21 ^a	79.08 ^b	0.25
Color a^* value	8.44 ^a	8.28 ^a	7.80 ^b	0.14
Color b^* value	22.11 ^a	21.11 ^b	19.22 ^c	0.20
Sensory measurement				
Color (darkness)	5.94	6.00	6.11	0.67
Aroma	6.60	7.03	7.00	0.65
Hardness	6.27	6.30	6.43	0.60
Juiciness	5.22	5.12	5.06	0.61

^{a-c}Means within a column with no common superscript differ significantly ($P < 0.05$).

¹The d.f. for CLA treatment = 2. For resistance and pH measurement, $n = 30$ and the d.f. for error = 87. For sensory evaluation, $n = 16$ and the d.f. for error = 45.

²Measured before cooking.

TABLE 6. Lipid content and fatty acid composition of breast fillets as affected by dietary conjugated linoleic acid (CLA)

Fatty acid	0% CLA	2.0% CLA	3.0% CLA	SEM
Tetradecanoic acid	0.38 ^b	0.57 ^a	0.64 ^a	0.04
Hexadecenoic acid	2.01 ^a	1.19 ^b	0.94 ^b	0.14
Hexadecanoic acid	20.47 ^b	24.35 ^a	23.16 ^a	0.75
Octadecadienoic acid	26.34 ^a	18.10 ^b	14.33 ^c	0.64
Octadecenoic acid	30.61 ^a	22.13 ^b	22.50 ^b	0.62
Octadecatrienoic acid	2.78 ^a	1.88 ^b	1.45 ^c	0.05
Octadecanoic acid	9.12 ^b	13.73 ^a	13.27 ^a	0.43
<i>Cis</i> -9, <i>trans</i> -11 CLA	0 ^c	3.28 ^b	5.37 ^a	0.07
<i>Trans</i> -10, <i>cis</i> -12 CLA	0 ^c	4.10 ^b	7.11 ^a	0.14
<i>Trans</i> -9, <i>trans</i> -11 CLA	0 ^c	0.85 ^b	2.23 ^a	0.08
Other CLA isomers	0 ^c	2.28 ^b	3.04 ^a	0.08
Eicosatetrenoic acid	4.49 ^a	4.05 ^a	2.87 ^b	0.36
Docosahexenoic acid	0.90 ^a	0.78 ^a	0.47 ^b	0.05
Unconfirmed PUFA ¹	2.93	2.67	2.11	0.32
Total Saturated fatty acid	29.97	38.65	37.07	
Total monounsaturated fatty acid	32.62	23.32	23.44	
Total polyunsaturated fatty acid-CLA	37.44	27.48	21.23	
Total CLA	0	10.51	17.75	

^{a-c}Means within a row with no common superscript differ significantly ($P < 0.05$); $n = 4$.

¹Polyunsaturated fatty acids.

Table 6 shows the fatty acid composition of breast fillets after different dietary CLA treatments. Total saturated fatty acids increased greatly as dietary CLA level increased, whereas the total monounsaturated fatty acids and polyunsaturated fatty acids decreased, which confirmed many previous reports (Lee et al., 1998; Bretillon et al., 1999; Ahn et al., 1999; Du et al., 1999). CLA has a high melting point, similar to saturated fat. The changes in fatty acid composition greatly increase the melting point of fats and, thus, can make meat feel tougher.

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