

Metabolizable Energy Value of Conjugated Linoleic Acid for Broiler Chicks and Laying Hens¹

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ABSTRACT Two experiments with broiler chicks and one experiment with laying hens were conducted to determine the ME_n value of conjugated linoleic acid (CLA). In Experiment 1, for 8 d, 16-d-old chicks were fed diets in which 4, 8, or 12% of CLA Source A or 4, 8, or 12% of soybean oil (SO) was substituted for glucose. Dietary ME_n increased linearly ($P \leq 0.001$) with increments of CLA Source A or SO. Regression analysis relating increases in dietary ME_n and increments of the dietary fat sources showed that the ME_n values of CLA Source A and SO, when evaluated separately, were 7,419 and 8,429 kcal/kg, respectively. In Experiment 2, feed was withheld from laying hens for 38 h and then the hens were force-fed diets containing 15% glucose, 15% CLA Source A, or 15% SO (two feedings of 30 g each). Excreta samples were

collected for 36 h after the last feeding. The ME_n values obtained for CLA Source A and SO were 8,517 and 8,437 kcal/kg, respectively. The ME_n of CLA Source B (higher in unsaturated fatty acids than CLA Source A) was determined in Experiment 3 by feeding diets containing 4, 8, or 12% CLA Source B to 14-d-old chicks. Increases in dietary ME_n with increments of CLA Source B were curvilinear, with resulting ME_n of 9,375 to 9,588 kcal/kg of fat when CLA Source B was fed at 4 or 8% of the diet and 7,917 kcal/kg when fed at 12% of the diet. Results of this research show that CLA sources can contribute substantial energy to diets, but the ME_n value of CLA sources for young chicks varies with fatty acid composition and dietary concentration.

(Key words: conjugated linoleic acid, nitrogen-corrected metabolizable energy, chicks, hens)

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INTRODUCTION

Considerable attention has been focused on dietary conjugated linoleic acid (CLA) since Pariza and Hargraves (1985) and Ha et al. (1990) reported that CLA seemed to have anticarcinogenic properties in several animal systems. In 1993, Cook et al. observed that 0.5% dietary CLA modified some consequences of an immune response in chicks. They found that chicks injected with *Escherichia coli* lipopolysaccharide and not fed CLA lost weight, whereas chicks injected with lipopolysaccharide and fed 0.5% CLA continued to gain weight, albeit slowly. Cook et al. (1996) also reported that dietary CLA impaired hatchability of chicken eggs and thus may provide an effective mechanism for controlling populations of wild birds. Working with laying hens, Chamruspollert and Sell (1999) found that the CLA concentration in egg yolk lipids increased linearly as dietary CLA concentration increased from 0.5 to 5% of the diet, indicating that eggs could be made to supply appreciable CLA to diets of humans. In the latter instance, the ME_n value of the CLA source used

to formulate laying hen diets was assumed to be approximately the same as that of soybean oil (SO). To the authors' knowledge no published data exist that document the ME_n value of CLA sources for poultry. Assuming that CLA sources may be used in poultry feeding programs in the future, knowledge of the ME_n value of these sources will be important. Research reported here was conducted to determine the ME_n value of CLA sources, obtained commercially, for young broiler chickens and for laying hens.

MATERIALS AND METHODS

Procedures used in the experiments reported herein were approved by the Laboratory Animal Resources Committee of Iowa State University.

Animals and Experimental Design

Experiment 1. One hundred-twenty 16-d-old, male broiler chicks (Ross × Ross) were used. The chicks were fed a 23% CP, 3,100 kcal of ME_n/kg starter diet from 1 to 16 d of age. Five chicks were assigned randomly to

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Abbreviation Key: CLA = conjugated linoleic acid; SO = soybean oil.

TABLE 1. Composition of glucose basal diets used for broiler chicks in Experiments 1 and 3 and for White Leghorn hens in Experiment 2

Item	Broiler chicks		Laying hens, Experiment 2
	Experiment 1	Experiment 3	
	(%)		
Corn	43.03	43.81	46.12
Soybean meal	35.10	34.16	24.06
Glucose monohydrate	12.00	12.00	15.00
Soybean oil	3.36	3.45	1.17
Meat and bone meal	3.00	3.00	3.00
Limestone	0.94	0.97	8.80
Dicalcium phosphate	1.02	1.03	0.32
Mineral premix ¹	0.30	0.30	0.30
Vitamin premix ²	0.30	0.30	0.30
DL-methionine	0.15	0.18	0.13
Sodium chloride	0.10	0.10	0.10
Celite™, ³	0.70	0.70	0.70
Calculated analysis			
ME _n , kcal/kg	3,100.00	3,050.00	2,850.00
CP	20.69	21.10	15.62
TSAA	0.82	0.82	0.65
Met	0.48	0.49	0.39
Lys	1.23	1.18	0.90
Ca	1.00	1.00	3.80
Nonphytate phosphorus	0.45	0.45	0.30

¹Supplied per kilogram of diet: manganese, 70 mg; zinc, 40 mg; iron, 37 mg; copper, 6 mg; selenium, 0.15 mg; sodium chloride, 2.60 g.

²Supplied per kilogram of diet: vitamin A (retinyl acetate), 8,090 IU; cholecalciferol, 1,575 IU; dl- α -tocopheryl acetate, 12 IU; vitamin B12, 16 μ g; vitamin K (menadione sodium bisulfite), 2.0 mg; riboflavin, 4.0 mg; pantothenic acid, 12.8 mg; niacin, 75 mg; choline, 509 mg; folic acid, 1.62 mg; biotin, 75 μ g; ethoxyquin, 15 mg.

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each of 24 pens located in a Petersime³ brooder battery. Chicks were supplied with light 24 h/d and an ambient temperature of 27 C. Seven dietary treatments were used. These included a glucose basal diet, which contained 12% glucose monohydrate, and diets in which 4, 8, or 12% SO or 4, 8, or 12% CLA Source A were substituted for glucose on an equal weight basis. Composition of the glucose basal diet is shown in Table 1. The CLA Source A was supplied by Conlinco, Inc.⁴ All diets were formulated to be isonitrogenous and to meet or exceed National Research Council (NRC, 1994) recommended nutrient concentrations. Metabolizable energy contents of the experimental diets were not equalized. Celite™,⁵ was included at 0.7% of all diets to serve as an indigestible marker. Fatty acid analysis of CLA Source A and SO was done at a commercial laboratory⁶ while the experiment was in progress. Results presented in Table 2 show that CLA Source A contained about 49.5% total CLA.

The glucose basal diet and diets containing SO were assigned randomly to three pens of chicks, whereas diets containing CLA Source A were assigned to four pens of chicks. Diets and water were provided ad libitum, starting

when the chicks were 16 d old. Body weight data were recorded when chicks were 16 and 24 d old, and feed consumption data for the 8-d experiment also were recorded. Excreta samples were collected from each pen on Days 7 and 8 of the experiment. Excreta and diet samples were freeze-dried in preparation for analysis. Samples of excreta and diets were analyzed for gross energy by bomb calorimetry⁷ and for nitrogen by the Kjeldahl procedure (Association of Official Analytical Chemists, 1980; Method 14.068) and for acid-insoluble ash (Scott and Bodaji, 1997). These data were used to calculate ME_n of the diets, and the ME_n values of the diets were used to estimate the ME_n of CLA Source A and SO by the procedure described by Hill and Anderson (1958). In this procedure, the ME_n value assigned to glucose was 3,640 kcal/kg (Hill and Anderson, 1958).

Experiment 2. Fifteen 70-wk-old White Leghorn laying hens were used. The hens were kept in individual metabolism cages that were equipped for collection of excreta. Until the start of the experiment, hens were supplied with a laying hen diet (16% CP, 2,850 kcal of ME_n/kg) and water for consumption ad libitum. Hens were provided with 14 h of light and 10 h of darkness per day. A force-feeding method was used to determine the ME_n value of the experimental diets. This method involved depriving all hens of feed for 38 h. At the end of 38 h, five hens were assigned to each of three experimental diets, and 30 g of the appropriate diet was force-fed to each hen, as described by Sibbald (1986). Another 30 g of each diet also was force-fed to the appropriate hens 24 h later. Excrement was collected from each hen for 36 h after the last feeding. Water was supplied ad libitum during the experiment.

The three experimental diets were a glucose basal diet that contained 15% glucose and diets in which CLA Source A (same source as in Experiment 1) or SO was substituted, on a weight basis, for glucose of the glucose basal diet. Composition of the glucose basal laying hen diet is presented in Table 1. All diets were formulated to meet or exceed nutrient concentrations recommended by the NRC (1994). The ME_n of diets containing the test fats were allowed to increase with the inclusion of CLA Source A or SO. As in Experiment 1, 0.7% Celite™ was included

TABLE 2. Fatty acid composition of soybean oil and conjugated linoleic acid (CLA) Source A and CLA Source B

Fatty acid	Soybean oil	CLA Source A	CLA Source B
	(%) of methyl esters		
Palmitic	10.65	13.12	5.40
Stearic	4.40	14.24	6.10
Oleic	23.72	19.15	19.97
Linoleic	52.99	3.27	6.65
Linolenic	7.62	ND ¹	ND
CLA (<i>cis</i> 9, <i>trans</i> 11)	ND	8.66	17.94
CLA (<i>trans</i> 10, <i>cis</i> 12)	ND	10.39	20.27
CLA (<i>cis</i> 9, <i>trans</i> 11)	ND	2.92	4.41
CLA (<i>cis</i> 11, <i>trans</i> 13)	ND	18.65	15.34
Other CLA isomers	ND	8.85	3.57
Other	0.62	0.75	0.35

¹ND = none detected.

³Petersime, Gettysburg, OH 45328.

⁴Conlinco, Inc., Detroit Lakes, MN 56502.

⁵Sigma Chemical, St. Louis, MO 63178.

⁶CN Laboratories, Courtland, MN 56021.

⁷Parr Instruments Co., Moline, IL 61265.

in the diets as an indigestible marker. Excrement samples collected from each hen were freeze-dried in preparation for analyses. These samples were analyzed for gross energy, nitrogen, and acid-insoluble ash by procedures described for Experiment 1. Calculations of ME_n values of test diets and estimations of ME_n values of CLA Source A and SO also were made as described for Experiment 1.

Experiment 3. This experiment was conducted to determine the ME_n value of another source of CLA (CLA Source B). Laboratory analysis of CLA Source A during Experiments 1 and 2 showed that the fatty acid composition of this CLA source was notably different from that of CLA sources used by Ahn et al. (1999), Chamruspollert and Sell (1999), and Du et al. (1999). CLA Source B contained lower concentrations of the saturated fatty acids, palmitic and stearic, than did CLA Source A and also contained a greater concentration of total CLA than CLA Source A (Table 2). The proportional distribution of isomers of CLA also was different for CLA Sources A and B. Because of these differences and because the fatty acid composition of CLA Source B was similar to CLA sources used by Ahn et al. (1999), Chamruspollert and Sell (1999), and Du et al. (1999), Experiment 3 was conducted to determine the ME_n of CLA Source B with broiler chicks.

Eighty 14-d-old male broiler chicks (Ross × Ross) were used. As in Experiment 1, the chicks were fed a 23% CP, 3100 kcal of ME_n/kg starter diet from 1 to 14 d of age. Five chicks were assigned to each of 16 pens located in a Petersime brooder battery. Average chick weight at the start of the experiment was 430 g. Four pens of chicks were assigned randomly to each of four test diets. The test diets were a glucose basal diet (Table 1) and diets in which 4, 8, or 12% CLA Source B were substituted for glucose of the basal diet on a weight:weight basis. Celite™ was included in all diets as an indigestible marker. Diets and water were supplied for consumption ad libitum, starting when the chicks were 14 d old. Body weight gain and feed consumption data were recorded when chicks were 19 d old. Excreta samples were collected on Days 4 and 5 of the 5-d experiment. Excreta and diet samples

were processed and analyzed as described for Experiment 1. Analytical values of excreta samples and diets were used to calculate dietary ME_n values as described for Experiment 1, again using an ME_n value for glucose of 3,640 kcal/kg.

Statistical Analysis

All data were analyzed statistically by the general linear models procedure of SAS® (SAS Institute, 1985) to determine the effects of dietary SO and CLA sources. Regression analysis was used to assess the relationship between dietary concentrations of SO or CLA source on BW gain, feed consumption, dietary ME_n values, and changes in dietary ME_n data of Experiments 1 and 3, whereas multiple regression analysis (Robbins, 1986) was used to determine comparative effects of SO and CLA Source A on changes in dietary ME_n in Experiment 1. Analysis of variance was used to determine the effects of SO or CLA Source A on dietary ME_n for laying hens in Experiment 2.

RESULTS

Experiment 1

Weight gain and feed consumption data for the 7-d experiment are shown in Table 3. Regression analysis showed that weight gain was not affected by dietary concentrations of SO or CLA Source A. Feed consumption and feed-to-gain ratio, however, were decreased linearly ($P \leq 0.01$ and $P \leq 0.02$, respectively) as dietary SO or CLA Source A concentrations increased. Regression coefficients for changes in feed consumption related to SO and CLA Source A did not differ ($P = 0.19$). Data presented in Table 4 show that dietary ME_n increased linearly ($P \leq 0.01$) with increments of SO or CLA Source A. The same was true for the change in dietary ME_n related to increases in SO or CLA Source A after adjusting for the deletion of glucose from the diets. Multiple regression analysis of

TABLE 3. Weight gain and feed consumption of broiler chicks fed diets containing soybean oil (SO) or conjugated linoleic acid source A (CLA Source A) from 16 to 24 d of age, Experiment 1

Dietary treatment	Weight gain ^{1,2}	Feed consumed ³	Feed-to-gain ratio ³
	(g/chick)		
Glucose basal	437	625	1.43
4% SO	477	634	1.33
8% SO	453	576	1.27
12% SO	439	535	1.22
4% CLA Source A	438	618	1.41
8% CLA Source A	451	603	1.34
12% CLA Source A	445	576	1.29
SEM	10	12	0.02

¹Means of three pens of chicks per dietary treatment for the glucose basal and SO diets and four pens per dietary treatment for the CLA Source A diets. Chicks averaged 432 ± 11 g each at the start of the experiment.

²Regression analysis showed that weight gain was not affected ($P = 0.15$) by dietary concentration of SO or CLA Source A.

³Regression analysis showed that feed consumed and feed-to-gain ratio were decreased ($P \leq 0.01$ and 0.02 , respectively) with increments of SO or CLA Source A. Regression coefficients for changes related to SO and CLA Source A did not differ.

TABLE 4. Dietary ME_n values and increases in dietary ME_n associated with increments of dietary soybean oil (SO) and the conjugated linoleic acid (CLA) Source A, Experiment 1

Dietary treatment	Determined dietary ME _n ¹	Increases in dietary ME _n related to increases in supplemental fat source ^{1,2,3,4}
		(kcal/kg)
Glucose basal	3,243	...
4% SO	3,523	426
8% SO	3,679	728
12% SO	3,905	1,100
4% CLA Source A	3,404	307
8% CLA Source A	3,518	567
12% CLA Source A	3,706	901
SEM	24	26

¹Effects of SO and CLA were linear ($P \leq 0.001$).

²Increases in dietary ME_n after adjustments for the deletion of glucose from the diets as fat sources were included in the diets.

³Multiple regression analysis with common intercept resulted in the equation: $Y = 31.95 + (70.57X) + (89.12Z)$; $R^2 = 0.97$. Where Y = increase in dietary ME_n with increase in dietary SO or CLA Source A, X = percentage CLA Source A in the diet, and Z = percentage SO in the diet. The coefficients for CLA Source A and SO differed significantly ($P \leq 0.001$).

⁴Separate regression analysis for CLA Source A and SO data resulted in the following equations: ME_n of CLA Source A kcal/kg = $-1.83 + (74.19X)$, $r^2 = 0.97$; and ME_n of SO, kcal/kg = $77.0 + (84.29Z)$, $r^2 = 0.97$.

these increases in dietary ME_n as related to concentrations of SO or CLA Source A resulted in the following equation:

$$Y = 31.95 + (70.57 \times \% \text{ CLA}) + (89.12 \times \% \text{ SO})$$

where $R^2 = 0.97$, $P \leq 0.001$, and Y = increase in dietary ME_n.

The regression coefficients for CLA source and SO differed ($P \leq 0.001$). These coefficients were used to estimate the ME_n values of the fat sources, and the results were 7,057 and 8,912 kcal/kg for CLA Source A and SO, respectively. The ME_n values calculated using the coefficients obtained when changes in dietary ME_n were regressed separately for each fat source showed that the ME_n of CLA Source A and SO were 7,419 and 8,429 kcal/kg, respectively.

Experiment 2

The determined ME_n values of the diets and the increases in dietary ME_n values related to the substitution

of the fat sources for glucose are shown in Table 5. The latter values were obtained by assuming that the ME_n value of glucose was 3,640 kcal/kg (Hill and Anderson, 1958). Additional calculation resulted in estimated ME_n values of 8,517 and 8,437 kcal/kg for the CLA Source A and SO, respectively. These ME_n values were not different ($P > 0.05$).

Experiment 3

Gain in BW was not affected ($P > 0.05$) by dietary CLA Source B during the 5-d experiment (Table 6). There was a significant nonlinear effect ($P = 0.002$) of CLA Source B on feed consumption, whereby feed intake by chicks fed 4% CLA Source B was greater than that of chicks fed diets containing 8 or 12% CLA Source B. Feed-to-gain ratio decreased linearly ($P = 0.02$) with increments of CLA Source B. Data presented in Table 7 show that dietary ME_n, as determined experimentally, increased in a curvilinear manner as dietary concentration of CLA Source B increased. Increases in dietary ME_n also were curvilinear, after adjustments were made for the deletion of glucose from the diets as CLA Source B was included in the diets. In the latter instance, dietary ME_n increased 94 to 95 kcal/kg for each 1% inclusion of CLA Source B up to an inclusion rate of 8%. When CLA Source B, however, was included at 12% of the diet, dietary ME_n increased by only 79 kcal/kg for each 1% inclusion, resulting in the observed curvilinear response. Thus, the ME_n values of CLA Source B were 9,375, 9,588, and 7,917 kcal/kg when this fat source was used at 4, 8, and 12% of the diet, respectively.

DISCUSSION

To the authors' knowledge, there are no data in the literature describing the ME_n value of CLA for chickens. Results of the current studies show that the ME_n of CLA for young broiler chicks varies according to fatty acid composition. One source of CLA, CLA Source A, tested in Experiment 1, contained relatively high concentrations of saturated fatty acids and low unsaturated fatty acid concentrations. The ME_n value of CLA Source A was determined to be 7,419 kcal/kg (Table 8), a value comparable to that obtained with beef tallow for chicks (Renner

TABLE 5. Dietary ME_n values and increases in dietary ME_n associated with substitution of 15% soybean oil (SO) or conjugated linoleic acid (CLA) Source A for 15% glucose in diets of laying hens, Experiment 2

Dietary treatment	Dietary ME _n	Increases in dietary ME _n related to substitution of 15% fat source for 15% glucose	Estimated ME _n of fat sources
	(kcal/kg)	(kcal)	
Glucose basal	3,040 ^b
15% SO	3,760 ^a	1,266	8,440
15% CLA Source A	3,772 ^a	1,278	8,520
SEM	59	51	...

^{a,b}Means within column not followed by a common superscript letter differ significantly ($P \leq 0.05$).

TABLE 6. Weight gain and feed consumption of broiler chicks fed diets containing conjugated linoleic acid (CLA) Source B from 14 to 19 d of age, Experiment 3

Dietary treatment	Weight gain ¹	Feed consumed	Feed-to-gain ratio ²
	(g/chick)		
Glucose basal	284	406	1.43
4% CLA Source B	301	415	1.38
8% CLA Source B	280	380	1.36
12% CLA Source B	291	390	1.34
SEM	11	3	0.017
	(P)		
Effect of diet	0.18	0.002	0.02

¹Means of four pens per dietary treatment, five chicks per pen. Chicks averaged 430 ± 12 g each at the start of the experiment.

²The decrease in feed-to-gain ratio was linear ($P \leq 0.02$) with increments of dietary CLA Source B.

and Hill, 1960). This ME_n value was observed irrespective of whether CLA Source A was included at 4, 8, or 12% of the diet. The results of Experiment 3 showed that the ME_n value of CLA Source B, which contained less saturated and more unsaturated fatty acids than CLA Source A, was greater for chicks than that observed for CLA Source A, especially when CLA Source B was fed at 4 or 8% of the diet (Table 8). The ME_n values for CLA Source B presented in Table 8 were calculated, in one instance, using the nonlinear regression equation shown in Table 7. These calculations indicated an ME_n of about 10,200 kcal/kg when CLA Source B constituted 4 or 8% of the diet and about 8,000 kcal/kg when CLA Source B was fed at 12% of the diet. A second set of ME_n was calculated by relating increases in dietary ME_n to each concentration of CLA Source B in the diets. Resulting values were 9,375, 9,588, and 7,917 kcal/kg for dietary concentrations of 4, 8, and 12%, respectively. Both sets of ME_n show clearly that CLA Source B was superior to CLA Source A as an

TABLE 7. Dietary ME_n values and increases in dietary ME_n values associated with increments of dietary conjugated linoleic acid (CLA) Source B, Experiment 3

Dietary treatment	Determined dietary ME _n	Increases in dietary ME _n related to increases in CLA Source B ¹
	(kcal/kg)	
Glucose basal	3,100 ²	...
4% CLA Source B	3,329	375 ²
8% CLA Source B	3,572	767
12% CLA Source B	3,612	950
SEM	22	22
	(P)	
Effect of diet	0.001	0.001

¹Increases in dietary ME_n after adjustments were made for the deletion of glucose from the diets as CLA Source B was included in the diets.

²Effects of CLA Source B were curvilinear. The relationship between increases in dietary ME_n, after adjusting for the deletion of glucose, and the inclusion of different concentrations of CLA Source B is described by the equation $Y = 11.36 + (117.16X) - (3.01X^2)$. Linear, $P < 0.001$, quadratic, $P = 0.0015$, $r^2 = 0.986$; where $Y =$ increase in dietary ME_n with increase in dietary CLA Source B, and $X =$ percentage of dietary CLA Source B.

TABLE 8. Estimated ME_n values of conjugated linoleic acid (CLA) Sources A and B for broiler chicks, Experiments 1 and 3

Source of CLA	Dietary concentration of CLA (%)	Estimated ME _n of CLA	
		Experiment 1	Experiment 3
		(kcal/kg)	
A	4 to 12	7,419 ¹	...
B	4	...	10,225 ²
B	8	...	10,291
B	12	...	8,009
			9,375 ³
			9,588
			7,917

¹Estimated on the basis of linear relationship between increases in dietary ME_n and increments of CLA Source A.

²Estimated for inclusion of 4, 8, or 12% CLA Source B in diets by using curvilinear regression equation.

³Estimated for inclusion of 4, 8, or 12% CLA Source B in diets by relating the increase in dietary ME_n to the specific concentration of CLA Source B in the diet.

energy source for broiler chicks. However, it also was evident that utilization of dietary energy was decreased when CLA Source B was fed at 12% of the diet, compared with including 4 or 8% CLA Source B in the diet.

Results of Experiment 2 showed that the ME_n value of CLA Source A for laying hens was equivalent to that of SO, when included in the diet at 15%. Thus the relatively high proportion of saturated fatty acids in CLA Source A did not seem to impair utilization of energy from the diet by laying hens as much as was observed with broiler chicks. This age-related difference would be expected on the basis of the report by Renner and Hill (1960) that the ME_n value of the relatively saturated fat, beef tallow, was greater for adult hens than for young chicks.

Results of the research reported here show that the ME_n values of CLA sources for chicks vary according to the fatty acid composition and dietary concentration of this fat source. Thus, it is important for CLA manufacturers to use adequate quality control during the manufacturing process to ensure a consistent product with respect to fatty acid composition. The CLA sources used in research reported by Ahn et al. (1999), Chamruspollert and Sell (1999), and Du et al. (1999) had a fatty acid profile similar to that of CLA Source B evaluated in the current study. Thus, the dietary energy supplied by all these sources should have been utilized relatively efficiently.

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