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Beneficial Effects of Dietary Anticarcinogenic Conjugated Linoleic Acid(CLA) on the Performances of Laying Hens and Broilers

Jai-Il Byon, Sook-Jahr Park, Kyung-Ahr Park, Jeung-Key Ha*, Jeong-Ok Kim** and Yeong-Lae Ha†

Dept. of Agricultural Chemistry and Gyeongsang Institute of Cancer Research, and
**Dept. of Animal Science, Gyeongsang National University, Chinju 660-701, Korea*
***Dept. of Chemistry, Pusan Women's University, Pusan 616-736, Korea*

Abstract

Effects of conjugated linoleic acid(CLA), known as an effective anticarcinogen in several aminal models, on the egg production and egg weight of laying hens, and the weight gains of broilers were investigated. CLA was synthesized from corn oil by the alkaline isomerization method and purified by the low-temperature precipitation method. Diets for laying hens and for broilers were synthesized to meet the specification of their NRC standard rationals. Two separated experiments(Experiment I and II) were conducted for laying hens. In experiment I, 45 hens(300 days of age) were divided into 15 hens per treatment group; each hen was housed in a wired cage located in a temperature and humidity-controlled house and adopted to the control diet. One week later, each group was subjected to one of the four treatment groups for 5 weeks : control, 1.0% CLA, 2.5% CLA and 5.0% CLA diets. Diet and water were *ad libitum.* The condition of experiment II was the same as that of experiment I except for the addition of 5% corn oil diet and the extension of feeding period to 7 weeks. Egg production, egg weight and feed intake were recorded every week. Forty-five broilers(10 days of age) were adopted to the control diet for a week and then switched to the treatment diets for 5 weeks: control, 1.0% CLA, 2.5% CLA, 5% CLA and 5% corn oil diets. Body weight and feed intake of broilers were measured every week. Diets supplemented with various amounts of CLA enhanced the egg production and increased the egg weight regardless laying hens' age(150 days or 300 days) as compared to control diet. The most effective diet for the egg production and egg weight of young hens(150 days of age) was found to be the 1.0% CLA diet, but relatively higher CLA diet(2.5% CLA) was required for old hens(300 days of age) to obtain similar results as seen in younger hens. All hens treated with CLA ate greater amount of feed than control hens. Broilers treated with various amount of CLA ate less feed as compared to control ones, but the body weight gain was greater than the control broilers. These results indicate that CLA enhanced the egg production and egg weight of laying hens, and increased the body weight gain of broilers with less diet consumption.

Key words: conjugated linoleic acid(CLA), egg production, egg weight, broilers

INTRODUCTION

CLA, the acronym for conjugated linoleic acid, was first isolated from grilled ground beef and established as a potent cancer inhibitor in several animal models (1-8). CLA was then shown to reduce the catabolic effects of immune stimulation in mice, rats, and chickens without adversely affecting immune function, and shown to enhance the growth and improve feed efficiency in some animal species(9,10). Recently CLA exhibited anti-athroscrerotic activity in rabbits(11), and reduced cholesterol content in eggs and enhanced the stability of chicken muscles against oxygen(12).

Now, CLA is of world-wide interest, including USA, Mexico, India, and Italy as well. Researches on CLA are mostly focused on its anticarcinogenic mechanism and application to food and feed uses(13). The principal sources of CLA are dairy products and other foods derived from ruminant animals(14-22). Small amounts of CLA is present in virtually all foods(22). Much effort was contributed to increase CLA contents in various foods like cheeses and soy sauce(14-19), but CLA content in foods was not substantially increased. Hence, at present, the only possible way to increase effectively CLA content in foods is to use the chemically-synthesized CLA as an food additive, but the CLA is not natural product which might be discriminated by consumers as a toxic substance.

†Corresponding author

Given these aspects of CLA implications, we are trying to use chemically-synthesized CLA as a supplement for chicken diets without adversely affecting the performances of chickens. Once the nature of chemically-synthesized CLA is neutralized by chickens, using as the supplementation of diet, the poultry products produced by such a way might be classified as non-toxic foods and not discriminated by consumers. This sort of research, regarding feed use of chemically-synthesized CLA for chickens, is the first trial in the world.

In the present study, we report the effect of chemically-synthesized CLA on the egg production and egg weight of laying hens, and the weight gain of broilers as well. We found that dietary CLA increased the egg production and egg weight of laying hens, and the weight of broilers

MATERIALS AND METHODS

Materials

Ethylene glycol, KOH, CaO, MgO and Mn_3O_4 were purchased from Chunji Chemical Co.(Japan). Hexane (Burdick, USA) was HPLC grade. All other ingredients for diets were purchased from local groceries or animal food markets. All other chemicals used were ACS grade.

Synthesis of CLA

CLA was chemically synthesized from corn oil by alkaline isomerization method and purified by the low-temperature precipitation method described by Ha et al.(2). The purified CLA was derivatized by 4% sulfuric acid in absolute methanol and analyzed by GC.

Preparation of synthetic diets

Diet for laying hens

Basal diet identical to the National Research Council (NRC) standard rationals' specification for the diet of laying hens was synthesized with the diet components shown in Table 1. Treatment diets(1, 2.5 and 5% CLA) were prepared by mixing an appropriate amount of the chemically-synthesized CLA with the basal diet by weight. A positive control diet(5% corn oil) was also prepared by mixing an appropriate amount of corn oil with the basal diet by weight.

Diet for broilers

Two basal diets identical to the NRC standard ra-

Table 1. Composition of the experimental basal diet for laying-hens[1)]

Ingredient	%
Yellow corn	45.0
Wheat	12.0
Rice bran	8.0
Wheat bran	10.0
Soybean oil meal	9 0
Fish meal	7.0
Brown seaweed	0.2
Fat[2)]	3.0
Bone meal[3)]	1.0
CaO	3.8
MgO	0.5
Salt	0.2
Vitamin premix	0.3

1)Diet contains amino acids and minerals identical to the NRC specifications for laying hen diet. Diet contains 2,912 ME kcal/kg. 14.8% protein and various amount of vitamins(A, 6893IU; D, 562ICU; E, 40IU; K, 0.75mg; riboflavin, 8.6mg; pantothenic acid, 19.5mg; niacin, 8.8mg; and B_{12}, 0.023mg per kg)

2)Fat was derived from the animals

3)Brown seaweed was roughly ground after washing and drying

Table 2. Composition of experimental basal diets for broiler chicks[1)]

Age(0~3 weeks)		Age(4~6 weeks)	
Ingredient	%	Ingredient	%
Yellow corn	58	Yellow corn	58
Soybean meal	20	Soybean meal	20
Fish meal	15	Fish meal	8
Fat[2)]	4	Wheat	7
Rice bran	1	Fat	4
Brown seaweed[3)]	0.2	Rice bran	1
Mn_3O_4	0.1	Brown seaweed	0.2
CaO	0.2	CaO	0.2
MgO	0 8	$MnSO_4$	0.1
Salt	0.1	MgO	0.8
Vitamin premix	0.2	Salt	0.1
Methionine	0.4	Methionine	0.4
		Vitamin premix	0.2

1)Diet contains amino acids and minerals identical to NRC specifications for the diet of broilers. Diet for 0~3 weeks of age contains 3,239ME kcal/kg, 23.2% protein and various amout of vitamins(A, 18,330IU; D, 4,680ICU; E, 52 IU; K, 2mg; riboflavin, 14mg; pantothenic acid, 16mg; niacin, 53mg; and B_{12}, 0.045mg per kg). Diet for 4~6 weeks of age contains 3,245ME kcal/kg, 20% protein and various amount of vitamins(A, 18,357IU; D, 4,680ICU; E, 54IU; K, 2mg; riboflavin, 10mg; pautothenic acid, 16mg; niacin, 53mg; and B_2; 0.02mg per kg)

2)Fat was derived from animals

3)Brown seaweed was roughly ground after washing and drying tionals' specification for broilers(0~3 weeks and 4~6 weeks of age) were prepared with the components shown in Table 2. The composition of the basal diets was slightly different with regard to age of broilers to be fed. Diets containing 1%, 2.5%, and 5% CLA were prepared by mixing an appropriate amount of chemically-synthesized CLA with the basal diets by weight.

All diets for laying hens and broilers were freshly prepared and stored at the cold room temperature(5°C). Peroxide value of the diets containing CLA or corn oil was not different from that of basal diet when determined before and after feeding.

Feeding experiments

Feeding conditions

Each chicken was housed in a wire cage(40×40×40cm) located in a temperature and humidity-controlled house(25°C and 70% RH) and subjected to an appropriate diet and water *ad libitum*. The light-dark cycle was a 12-12 hours for laying hens and a 8-14 hours for broilers.

Experiments for laying hens

Two separated experiments were performed. In experiment I, 45 laying hens(Highbrown, 300 days of age) purchased from Hyoge-ari-won(Hamyang, GN) and were divided into 4 groups(15 hens/group and one hen/cage), then adopted to the basal diet for a week (Table 1). Each group of hens was then subjected to one of either treatment diets for 5 weeks : control (basal diet), 1% CLA, 2.5% CLA, or 5% CLA diet. Feed intake, egg production and egg weight were measured before and every week after the initiation of treatments. In experiment II, supplemented CLA effect was investigated using young Highbrown(150 days of age). Each hen was housed and adopted to the basal diet until the average egg production reached approximately 55%. Each group was fed one of treatment diets for 7 weeks : control(basal diet), 1% CLA, 2.5% CLA, 5% CLA, or 5% corn oil diet. Other treatments were the same as experiment I.

Experiment for broilers

Forty-five broilers(Abike. 10 days of age) purchased from Hyoge-ari-won(Hamyang, GN) was adopted to the basal diet(Table 2) for one week, followed by subjecting to one of either treatment diets(control, 1.0% CLA, 2.5% CLA, 5% CLA, or 5% corn oil diet) for 5 weeks. Feed intake and body weight were measured every week post treatment.

Statistics

Tukey's w test was used to determine the significance of results obtained.

RESULTS

Synthesis of CLA for feeding experiment

Fig. 1 shows a GC chromatogram of the CLA synthe-

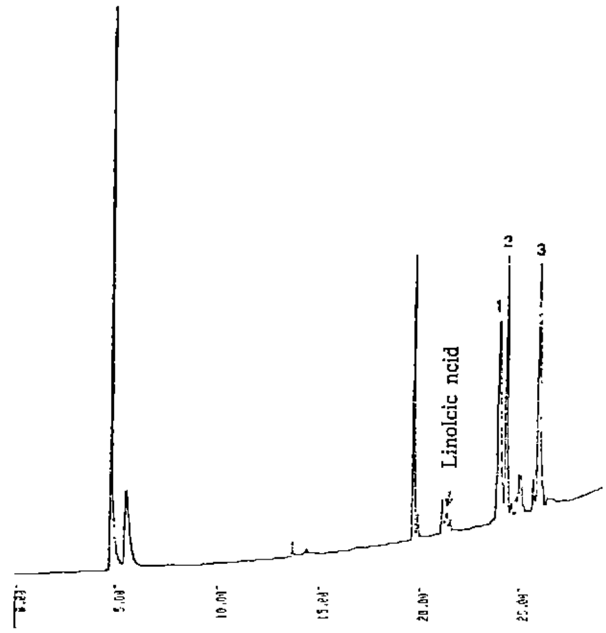

Fig. 1. A typical chromatogram of CLA synthesized from corn oil.
HP 5890 series II system equipped with FID was used. Supelcowax-10 capillary column(60m×0.25, i.d., 32μm film thickness) was used with oven temperature program(initial temperature, 5°C; 2°C/min; final temperature, 20°C). Peak identification: 1, cis, 9-trans,11-CLA; 2, trans,10-cis,12-CLA; 3, trans, 9-trans,11- and trans,10-trans,12-CLA.

Table 3. Low-temperature purification of CLA synthesized from corn oil[1)]

Treatment(°C)	cis,9-trans,11-CLA (%)[2)]	Total CLA(%)[3)]
5	45	40
0	45	70
-5	47	91

1)CLA was analyzed by capillary GC chromatography. Synthesized sample from corn oil was dissolved in hexane(30 g/70ml)
2)Percentage of cis,9-trans,11-CLA isomer for the total CLA
3)Percentage of total CLA(cis.9-trans,11-,trans,9-trans,11-, trans,10-cis,12-,trans,10-trans,12-CLA) for total weight of CLA purified

sized using freshly prepared corn oil and purified according to the precipitation method described in Ha et al. (12). Purity of the total CLA was found to be 91%, consisting of four major CLA isomers(cis,9-trans, 11-, trans,10-cis,12-,trans,9-trans,11- and trans,10-trans, 12-CLA). The purified CLA contained about 47% of the c9, t11-CLA isomer(Table 3). No linoleic acid, the precursor fatty acid of CLA, was found in the synthesized CLA.

Effect of dietary CLA on the performances of laying hens

Egg production

Table 4 shows the positive effect of dietary CLA on the egg production. In experiment I, the egg production by laying hens 300 days of age was ranging 41.5% to 70.6% over a period of experimental time of 5 weeks. The egg production of the control group was substantially lower than that of CLA treatment groups during entire experimental period. Within the CLA treatment groups, the egg production was not significantly different, but was high in 2.5% CLA treatment group. At week 5, the egg production of control, 1%, 2.5% and 5% CLA treatment groups were found to be 41.5%, 60.1%, 67.7%, 56.5%, respectively, indicating that CLA did not adversely affect the egg production, and thus most effective dietary CLA appeared to be the 2.5% CLA diet. Egg production tended not to decrease by feeding time, but not statistically significantly, except for the week 2 and 5, as compared to week 0.

In experiment II, hens(150 days of age) were fed for 7 weeks with the same diets used in experiment I, plus 5% corn oil diet. Beginning the experiment, the egg production was found to be about 55% on average after which it varied ranging from 55.1% to 88.4%, depending upon the treatments and feeding times. Very similar positive results in the egg production by CLA to those of experiment I were observed, indicating that the egg production was elevated by CLA as compared to control treatment group; however, in all treatment groups, including the control group, egg production was increased by the extended feeding time. No significant difference in the egg production was seen at an earlier stage(week 0~5) of the experimental period, but the significance was observed at a later stage(week 6~7). The 1.0% CLA treatment was most effective in the egg production. The egg production by CLA treatments was not different from that by 5% corn oil treatment. Thus, these results indicate that for younger age of laying hens, the 1% CLA diet effectively enhanced the egg production.

Egg weight

Table 5 shows egg weights by hens fed for 5 or 7 weeks various amount of dietary CLA. In experiment I, the egg weight was not significantly elevated by control group over a period of time, but it was elevated by CLA treatments. Within CLA treatment groups, the CLA treatment effect was clearly seen with regard to the amount, but not between 2.5% and 5.0% CLA diets. In experiment II, the egg weight was much smaller

Table 4. Effect of various amount of dietary CLA on the egg production(%)[1)]

Exp[2)]	Treatment[3)]	Weeks after treatment							
		0	1	2	3	4	5	6	7
Exp I	Control	60.8	60.0	51.0	53.0	54.4	41.5[4)]		
	CLA 1.0%	65.5	67.0	57.1	58.1	58.5	60.1		
	CLA 2.5%	68.3	62.4	71.7	58.5	67.6	67.7		
	CLA 5.0%	63.2	60.3	65.6	55.3	57.3	56.5		
Exp II	Control	55.1	69.1	80.5	84.5	82.5	78.6	75.9[4)]	71.2[4)]
	CLA 1.0%	56.2	77.2	79.9	89.6	82.5	82.4	86.5	88.3
	CLA 2.5%	56.2	86.2	88.4	88.3	84.4	78.9	80.9	80.1
	CLA 5.0%	52.3	66.5	71.4	80.7	82.5	81.2	80.3	83.4
	Corn oil 5%	57.0	84.5	79.2	81.0	81.3	80.2	80.4	79.2

1)Egg production was derived from which the number of eggs collected in a week was divided by the number of hens of the treatment group, and multiplied by 100

2)Experiment I and II performed using laying hens with 300 days and 150 days of ages, respectively

3)Each treatment contained 15 hens in both Experiments

4)Tukey's w at 0.05 : 8.3 for week 2 and 5.8 for week 5 of Experiment I; 4.3 for week 6 and 6.2 for week 7 of Experiment II

Table 5. Average egg weight(g) from hens treated with various amount of dietary CLA[1)]

Exp[2)]	Treatment[3)]	Weeks after treatment							
		0	1	2	3	4	5	6	7
Exp I	Control	66.3	67.9	69.0	67.1	69 4	64.5[4)]		
	CLA 1.0%	65.5	67.0	69.1	69.1	73.5	68.1		
	CLA 2.5%	66.3	67.4	76.7	73.5	77.6	70.7		
	CLA 5.0%	63.2	69.3	71.6	71.3	74.3	71.5		
Exp II	Control	50.1	52.2	53 2	54.3	53.7	54.7	55.5[4)]	56.7[4)]
	CLA 1.0%	49.9	53.9	54.9	56.1	56.4	57.5	57.8	59.1
	CLA 2.5%	51.2	53.3	53.6	54.1	55.5	56.5	57.4	57.2
	CLA 5.0%	45.9	54.7	54.5	53.8	55.2	58.3	58.9	58.1
	Corn oil 5%	48.5	52.7	53.4	54.3	54.6	54.1	54.5	55.1

1)Average egg weight in the same treatment group was derived from which the total egg weight collected in a week, then divided by the number of egg
2)Experiment I and II performed using laying hens with 300 days and 150 days of age, respectively
3)Each treatment contained 15 hens in both Experiments
4)Tukey's w at 0.05: 3.2 for week 5 of Experiment I; 2.2 for week 6 and 2.6 for week 7 of Experiment II

Table 6. Average feed intake(g/bird/day) by laying hens treated with various amount of dietary CLA[1)]

Exp[2)]	Treatment[3)]	Weeks after treatment					
		0	1	2	3	4	5
Exp I	Control	78 3	78.0	91.2	73.1	81.0	82.4[4)]
	CLA 1.0%	70 5	75.7	91.2	98.0	85.5	98 1
	CLA 2.5%	80.3	88.1	108.9	98 6	100.0	112.1
	CLA 5.0%	79.2	84.1	83.5	99.3	99.4	99.9
Exp II	Control	65 9	71.2	79.5	81.0	74.5	75.0[4)]
	CLA 1.0%	70.1	89.1	82.9	87.4	86.4	84 5
	CLA 2.5%	69.1	84.7	86.4	82.6	85.2	90.9
	CLA 5.0%	71.1	75.7	72.6	76.7	71.7	78.6
	Corn oil 5%	69.9	83.1	81.9	79.5	77.1	83.3

1)Average feed intake of each treatment group was calculated by subtracting the amount of left-over diet from amount of supplied diet. Feed intake of Experiment II was recorded only for 5 weeks
2)Experiment I and II performed using laying hens with 300 days and 150 days of age, respectively
3)Each treatment contained 15 hens in both Experiments
4)Tukey's w at 0.05: 13.9 for week 5 of Experiment I; 12.1 for week 5 of Experiment II

than that of experiment I, but the effect of CLA on the egg weight was similar to that observed in experiment I. CLA treatments elevated the weight of eggs as compared to control or 5.0% corn oil treatment. As results, 1.0% CLA concentration in the diet is believed to be the adequate amount for the egg weight gain of both ages of laying hens.

Feed intake

Table 6 shows the feed intake by laying-hens. In experiment I, feed intake was increased by extended feeding time. CLA treatment enhanced feed intake as compared to control treatment. Within CLA treatments, the most feed was consumed by the hens treated with the diet containing CLA 2.5%, but no significant difference was seen in between CLA 1% and CLA 5% treatments. At week 5, feed intake of control, CLA 1%, CLA 2.5%, and CLA 5% treatment groups was 82.4, 98.1, 112.1, 99.9g, respectively.

In experiment II, feed intake was recorded only for 5 weeks. The feed intake was similar to that of experiment I. Feed intake of 5% corn oil treatment was similar to that of all CLA treatments, but slightly higher than the control group.

Effect of dietary CLA on the performances of broilers

Table 7 shows the feed intake by broiler chickens treated with various diets. As compared to the control

Table 7. Average feed intake(g/bird/day) by broilers treated with various amount of dietary CLA[1)]

Treatment[2)]	Weeks after treatment					
	0	1	2	3	4	5
Control	99.0[3)]	132.9	152.4	183.2	183.3	260.0
CLA 1.0%	100.5	134.7	152.4	192.5	191.1	240.0
CLA 2.5%	110.1	134.3	147.9	178.6	175.6	232.9
CLA 5.0%	109.0	125.7	150 0	157.4	165.0	234.3
Corn oil 5%	100.1	131.4	147.4	180.7	182.8	264.4

1)Feed intake of each treatment group was calculated by the subtraction the amount of left-over diet from the amount supplied each week

2)Each treatment contained 15 broilers

3)Tukey's w at 0.05 : 11.5, 9.9, 6.0, 13.4, 10.5, 15.3 for week 0, 1, 2, 3, 4, and 5, respectively

Table 8. Effect of dietary CLA on the body weight (kg/bird) of broilers

Treatment	Weeks after treatment					
	0	1	2	3	4	5
Control	1.00[1)]	1.68	2.08	2.60	2.80	2.85
CLA 1.0%	1.03	1.96	2.15	2.80	3.05	3.10
CLA 2.5%	1.11	1.92	2.39	2.76	2.98	3.45
CLA 5.0%	1.11	1.85	2.22	2.90	3.30	3.45
Corn oil 5%	1.12	1.90	2.07	2.55	2.65	3.10

1)Value was an average body weight of 15 broilers

3)Tukey's w at 0.05 : 0.2, 0.4, ,0.3, 0.3, 0.3, 0.4 for week 0, 1, 2, 3, 4, and 5, respectively

group, 2.5% and 5.0% CLA treatments substantially suppressed the feed intake, but 1.0% CLA treatment slightly enhanced the feed intake. The feed intake by the 5% corn oil treatment group exhibited lower feed intake than control treatment, but not different from 2.5% and 5.0% CLA treatment groups.

Body weight of the broilers was somewhat inversely related to the feed intake(Tables 7 and 8). Among the treatments, 1.0% CLA treatment was the most effective for the body weight gain. The broilers treated with other CLA treatments(2.5% CLA and 5.0% CLA) did not gain the body weight relative to control diet, but they ate less feeds. After week 3 of treatment, the body weight gain by CLA treatments was significantly higher than that by control and 5.0% corn oil treatment, indicating higher feed efficiency of CLA at later stage.

DISCUSSION

Chemically-synthesized CLA, containing more than 90% of total CLA of which the concentration of the cis,9-trans,11 isomer is 47%(Table 3), enhanced the egg production and the egg weight by both young(150 days of age) and old(300 days of age) laying hens (Tables 4-5). The CLA effect was much more sensitive in younger laying hens than older ones. This effect might be related to the feed intake, because hens treated with CLA consumed more diet than the control hens or hens treated with 5% corn oil of which the calorie level is identical to that of 5% CLA diet(Table 6). In addition to calorie effect, some other biological actions of dietary CLA might affect the egg production and/or the egg weight, which are the subject for further researches.

Weight gain of the broilers was elevated by CLA supplementation, relative to control diet and 5% corn oil diets(Table 6), but the broilers treated with CLA ate less feed than the broilers treated with 5% corn oil or control diets(Table 7). These results indicate that CLA exhibited the high feed efficiency, the facts are in accordance with the results observed from rats treated with CLA(9). It is of interest to note that body fat treated with CLA diets was not significantly different from that with control diet(data not shown). Such an effect of CLA on rats has already been reported by Chin et al.(9). This data will be published elsewhere soon. In the point of the weight gain of the broilers, the most efficient dietary concentration of CLA was found to be 1% in the basal diet.

It is not well understood how the feed efficiency of CLA was improved in broilers, but not in laying hens. It might in part because CLA might be indirectly related to the growth of the broilers, but directly affect the egg production and egg weight of laying hens; however, exact mechanisms are not known and remained as further research topics. In this study, the effects of CLA on farm animals were achieved using a mixture of all possible CLA isomers present in the synthesized CLA sample, not individual isomers. It is also interesting research topics whether all four major CLA isomers are equally active for the performances of laying hens and broilers.

Beneficial effects of CLA on the performances of laying hens and broilers obtained here are based on the small scale of 15 animals per treatment. Thus, a farm scale experiment should be performed to ensure the beneficial action of CLA on farm animals.

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REFERENCES

1. Ha, Y. L., Grimm, N. K. and Pariza, M. W. : Anticarcinogens from fried ground beef : heat-altered derivatives of linoleic acid. *Carcinogenesis*, **8**, 1881(1987)
2. Ha, Y. L., Storkson, J. M. and Pariza, M. W. : Inhibition of benzo[a]pyrene-induced mouse forestomach neoplasia by conjugated dienoic derivatives of linoleic acid. *Cancer Res.*, **50**, 1097(1990)
3. Ip, C., Singh, M., Thompson, H. J. and Scimeca, J. A. : Conjugated linoleic acid suppresses mammary carcinogenesis and proliferative activity of the mammary gland in the rat. *Cancer Res.*, **54**, 1212(1994)
4. Scimeca, J. A., Thompson, H. J. and Ip, C. : Effect of conjugated linoleic acid on carcinogenesis. *Adv. Exp. Med. Biol.*, **364**, 59(1994)
5. Ip, C., Scimeca, J. A. and Thompson, H. J. : Conjugated linoleic acid. A powerful anticarcinogen from animal fat sources. *Cancer*, **54**(3 Suppl), 1050(1994)
6. Ip, C., Li, D. J. and Scimeca, J. A. : Potential of food modification in cancer prevention. *Cancer Res.*, **54**(7 Suppl), 1957s(1994)
7. Ip, C., Chin, S. F., Scimeca, J. A. and Pariza, M. W. : Mammary cancer prevention by conjugated linoleic acid. *Cancer Res.*, **51**, 6118(1991)
8. Shultz, T. D., Chew, B. P., Seaman, W. R. and Luedecke, L. O. : Inhibitory effect of conjugated dienoic derivatives of linoleic acid and β-carotene on the *in vitro* growth of human cancer cells. *Cancer Lett.*, **63**, 125(1992)
9. Chin, S. F., Storkson, J. M., Albright, K. J., Cook, M. E. and Pariza, M. W. : Conjugated linoleic acid is a growth factor for rats as shown by enhanced weight gain and improved feed efficiency. *J. Nut.*, **124**, 2344(1994)
10. Miller, C. C., Park, Y., Pariza, M. W. and Cook, M. E. : Feeding conjugated linoleic acid to animals partially overcomes catabolic responses due to endotoxin injection. *Biochem. Biophys. Res. Commun.*, **198**, 1107(1994)
11. Lee, K. N., Kritchevsky, D. and Pariza, M. W. : Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis*, **108**, 19(1994)
12. Ha, Y. L., Ha, J. K. and Kim, J. O. : Dietary anticarcinogenic conjugated dienoic derivatives of linoleic acid (CLA) on the reduction of egg cholesterol content and the improvement of chicken muscle stability against oxidation. KOSEF report, p.1(1994)
13. Hauman, B. F. : Conjugated linoleic acid offers research promise. *IFORM*, **7**, 152(1996)
14. Ha, Y. L., Grimm, N. K. and Pariza, M. W. : Newly recognized anticarcinogenic fatty acids : identification and quantification in natural and processed cheesed. *J. Agric. Fd. Chem.*, **37**, 75(1989)
15. Shantha, N. C., Ram, L. N., O'Leary, J., Hicks, C. L. and Decker, E. A. : Conjugated linoleic acid concentrations in dairy products as affected by processing and storage. *J. Food Sci.*, **60**, 695(1995)
16. Shantha, N. C., Decker, E. A. Q. and Ustunol, Z. : Conjugated linoleic acid concentration in processed cheese. *J. Am. Oil Chemists' Soc.*, **69**, 425(1992)
17. Frolich, W. : Conjugated linoleic acids in meat and milk fats from ruminants-natural defence mechanisms? *Meieriposten*, **83**, 351(1994)
18. Parodi, P. W. : Conjugated linoleic acid : an anticarcinogenic fatty acid present in milk fat. *Aust. J. Dairy Technol.*, **49**, 93(1994)
19. Shantha, N. C., Crum, A. D. and Decker, E. A. : Evaluation of conjugated linoleic acid concentrations in cooked beef. *J. Agri. Fd. Chem.*, **42**, 1757(1994)
20. Nadathur, S. R., Gould, S. J. and Bakalinsky, A. T. : Antimutagenicity of an acetone extract of yogurt. *Mut. Res.*, **334**, 213(1995)
21. Aneja, R. P. and Murthi, T. N. : Conjugated linoleic acid contents of Indian curds and ghee. *Ind. J. Dairy Sci.*, **43**, 231(1990)
22. Chin, S. F., Liu, W., Storkson, J. M., Ha, Y. L. and Pariza, M. W. : Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. *J. Food Comp. Anal.*, **5**, 185(1992)

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