

Effects of Dietary Conjugated Linoleic Acid on the Growth Performance of Fish: A Review

¹Ling Gao and ²Huajia Shan

¹College of Binzhou Polytechnic, 256600 Binzhou, Shandong Province, China

²Department of Grassland Science, College of Animal Science and Technology, China Agricultural University, 100193 Beijing, China

Abstract: Conjugated Linoleic Acid (CLA) is a mixture of structure and geometric isomers of C_{18:2} fatty acids. Currently, it has been widespread concerned for the special physiological functions. This review elucidated the significance of the studies in the effects of dietary CLA on the growth performance, body composition, lipid contents and fatty acid composition of fish. Sorting earlier literatures, researchers found that dietary CLA can affect the growth performance and body composition of fish while the differences between different fish species are significant. Earlier studies in a number of species show that dietary CLA may have effects on the growth rate of fish but the results are diverging. This difference is mainly because of the mixed amount and the isomer composition ratio of CLA. There are evidences demonstrated that the impacts of CLA on fish's growth were closely related to the isomers of CLA. Dietary CLA (cis-9, trans-11 and trans-10, cis-12 isomers) inclusion resulted in significant increases of the contents of Saturated Fatty Acids (SFA) and Polyunsaturated Fatty Acids (PUFA) (p<0.05) and decreased Monounsaturated Fatty Acid (MUFA) content (p<0.05) in both muscle and liver tissues.

Key words: Conjugated linoleic acid, growth performance, fish, SFA, PUFA

INTRODUCTION

Aquaculture is the fastest growing animal-based agricultural food production sector, expanding at >9% per year and currently contributes over one third of all the fish in the human food basket (Tacon, 2003; Kennedy *et al.*, 2007). Nutrition is one of the most important and the most easily controlled factors which affect the growth and immunity of fish. The current trend in aquatic products is to increase the lipid content in diets to spare proteins, improve Feed Conversion Ratio (FCR) and decrease the amount of waste produced by fish (Valente *et al.*, 2007). Fatty acid as one of the most important and essential nutrients in the growth and reproduction processing of fish have momentous biological and physiological regulating functions. It is an important theory and approach to achieve the aim of healthy breeding, green aquatic products production and aquaculture sustainable development by improving the immunity and disease resistance with nutrition method (Zhanyu *et al.*, 2009).

Conjugated Linoleic Acid (CLA), the collective term for positional and geometric isomers of C_{18:2} fatty acids is one of the natural unsaturated fatty acids with many

physiological functions. It has been testified by experiments on human beings and animals that CLA are characterized for having many beneficial effects to humans including protection against cancer, obesity, heart disease and immune dysfunction (Subbaiah *et al.*, 2010; Pariza, 2004; Belury, 2002). Some researches indicated that CLA is responsible for antiatherosclerotic, antioxidative, antibacterial and immunomodulative properties in humans (Gerhard *et al.*, 2000; Roche *et al.*, 2001).

STRUCTURE AND FUNCTIONS OF CLA

Structure and geometric isomers: CLA refers to a group of Polyunsaturated Fatty Acids (PUFA) that exist as positional and stereoisomers of octadecadienoic acid. There is no methylene group separating the double bonds of CLA as there is in Linoleic Acid (LA). Instead, conjugated double bonds (i.e., the two double bonds are separated by one single bond) in either cis (c) or trans (t) configuration are present predominantly in positions 8 and 10, 9 and 11, 10 and 12 or 11 and 13 (Azain *et al.*, 2000; Brown *et al.*, 2001b; Chen *et al.*, 2004) (Fig. 1).

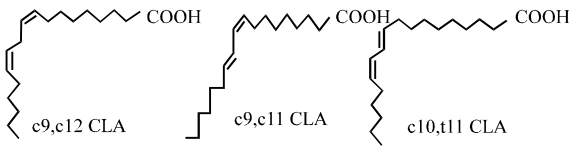


Fig. 1: Structures of c9, c12 CLA, c9, t11 CLA and c10, t12 CLA

Although, there are four above structural isomers and >28 geometric isomers in theory, in accordance with the present experimental test results, there are only minority isomers have better physiological activity in all natural and synthetic CLA isomers such as c9, t11 CLA, t10, c12 CLA, t9, t11 CLA, t10, t12 CLA and t10 c12 CLA (Belury, 2002; Larson *et al.*, 2003; Terpstra, 2004; Brown and McIntosh, 2003).

Physiological functions: Ever since been found, numerous reports pointed out that CLA have several beneficial effects in health-related disorders using animal models and cell cultures derived from humans and animals. Studies on CLA got increasingly concern in recent years for the reason that CLA have the specific physiological functions. The research scope and depth on the physiological functions and the related mechanism of CLA become more and more expanding and deepening.

Animal experiments have showed that CLA can affect the tumor cells by regulating and inhibiting the activities of cytochrome P450 and some enzymes involved in the carcinogenic process such as ornithine ammonialyase, decarboxylase and protein kinase C as well as inhibiting the protein and nucleic acid synthesis of cancer cells. Bhattacharya *et al.* (2006) and Kelley *et al.* (2007) showed that CLA can reduce the risk of various types of cancer including skin cancer, stomach cancer, colon cancer, breast cancer and liver cancer. It has been suggested that CLA not only reduce initiation, promotion and progression steps of cancer development but also reduce metastasis of cancer (Lee *et al.*, 2005; Bhattacharya *et al.*, 2006; Kelley *et al.*, 2007). The latest studies showed that CLA as a Peroxisome Proliferator Activated Receptor γ (PPAR γ) high-affinity ligand can inhibit the accumulation of mRNA in mouse tumor cells. Especially c9, t11 CLA is one of the best types of fatty acid PPAR ligands which can express the anticancer effect by activating PPAR (Harvatine and Bauman, 2011; Dilzer and Park, 2012). They pointed out that CLA may be involved in restraining the synthesis of eicosanoids production, interfering with cell signaling pathways, regulating and controlling RNA synthesis, promoting apoptosis as well as inhibiting and

reducing the matrix metalloproteinase and vascular endothelial growth factors (Lee *et al.*, 2005; Bhattacharya *et al.*, 2006; Kelley *et al.*, 2007; Yeonhwa, 2009).

Cook *et al.* (1993) firstly reported that CLA has the properties in promoting cell-division, prevent muscle degeneration, improving the immune capacity, reducing inflammatory concurrent and so on. Bhattacharya *et al.* (2006) reported that CLA can also prevent cardiovascular diseases by reducing various types of cholesterol, lowering blood pressure and participating in the activation of peroxidase receptors. CLA has been shown to improve immune related responses by modulating Tumor Necrosis Factor- α (TNF- α), cytokines (i.e., interleukin-1, 4, 6 or 8), prostaglandins or nitric oxides while reducing allergic type immune responses (Yeonhwa, 2009). Kritchevsky have proven that CLA was able to reduce the atherosclerotic lesions of rabbit and hamster by animal experiments. Yamasaki *et al.* (2000) pointed out that adding CLA in the pig lymphocyte culture medium can promote through mitotic inducer lymphocytes induced embryo cell transformation, lymphocytes virulence and macrophage lethality. Some studies demonstrated that 1% of CLA in the diet can make the young mouse spleen cell proliferation accelerated and <0.5% of CLA can also enhance the *in vivo* splenic lymphocytes produce immune factors. It has been proven that add a certain dose of CLA into the dietary feed can promote the increase of the spleen and serum immunoglobulin IgG, IgM and IgA and at the same time prevent the weight loss by reducing the animal growth inhibition caused by immune stimulation (Yamasaki *et al.*, 2000; Bhattacharya *et al.*, 2006).

Numerous *in vitro* and *in vivo* experiments have demonstrated that CLA can affect bone metabolism (Platt *et al.*, 2007; Kelly *et al.*, 2003; Kelly and Cashman, 2004; Brownbill *et al.*, 2005). Berge *et al.* (2004) and Burr *et al.* (2006) demonstrated CLA has a positive effect on bone health for that CLA can promote the division and regeneration of bone tissue as well as the synthesis of cartilage tissue and mineral deposition in bone. Banu *et al.* (2006) pointed out that CLA can affect many aspects and improve the bone health including the quality of bone ash, bone density, bone dry weight, length and calcium, magnesium, phosphorus and other mineral content. This is mainly due to the interaction of the calcium and CLA in the bones (Park *et al.*, 2008). The PPAR γ 2 not only militate the differentiation and proliferation of fat cells but also participate in the differentiation and proliferation process of bone marrow hematopoietic stem cells, bone marrow mesenchymal stem cells and osteoblasts (Maurin *et al.*, 2005). The effects

and mechanism of CLA on bone metabolism in recent years increasingly reported for the reason that it is one of the most important PPAR γ 2 natural activated ligand. A study on the bones of rodents showed that the supplement of CLA can increase bone mineral content and promote bone formation (Kelly *et al.*, 2003). Watkins *et al.* (2003) reported that CLA can change the runx2 level of rodents calvaria bone cells and promote the osteoblast differentiation. It has been reported that high concentrations of c9, t11 CLA and t10, c12 CLA can significantly improve the Atherogenic Lipoprotein Propile (ALP) activity of MG63 cells and SaOS2 cell of bone cell lines, increase the number of mineralized nodules in cell maturation and enhance its role in promoting the process of osteogenesis (Platt *et al.*, 2007; Cusack *et al.*, 2005). Platt and El-Soheby (2009) proved that c9, t11 CLA can promote the differentiation of *in vitro* cultured human Mesenchymal Stem Cells (MSCs) to fat cells without affecting the differentiation into osteoblasts.

Park *et al.* (1995) firstly reported that CLA had the function of reducing the fat content of animal body and increasing the proportion of lean meat. Ostrowska reported that CLA in daily ration can increase feed efficiency, increase lean meat and reduce carcass fat deposition. With the progress of the study confirmed that CLA is closely related to fat metabolism and fat cell differentiation. Many animal experiments and human studies showed that intake of CLA can reduce the body fat and inhibit fat increase (Brown *et al.*, 2001a, b; Kang *et al.*, 2003. Park and Pariza (2007) showed that it is the results of variety of physiological mechanisms for the physiological functions of CLA can reduce the animal fat, such as increasing energy consumption, reducing the accumulation of adipose tissue, inhibiting fat cell differentiation and promoting fat cell death, regulating cytokines, increasing muscle fatty acid content. Park *et al.* (1999) confirmed in subsequent studies that the special function is due to the isomer of t10, c12 CLA. The t10, c12 CLA can reduce the content of triglycerides and PPAR γ 2 (Evans *et al.*, 2001). Researchers reported that the effects of CLA on PPAR γ 2 changed over time, PPAR γ 2 increased in the first three days and followed by a decrease. Evans *et al.* (2000) reported that t10, c12 CLA can inhibit the gene expression of 3T3-L1 preadipocytes stearoyl-coenzyme A dehydrogenase SCD1. It has been demonstrated that c9, t11 CLA do not change the gene expression of fat cells and it may increase the content of triglycerides. According to experiments on the fat cells, the reduction of fat is not because of the cells decreasing but because that the fat droplets getting smaller and smaller (Choi *et al.*, 2000; Evans *et al.*, 2001). Different isomers of CLA played different physiological roles on

fat metabolism, t10, c12 CLA plays an important role in reducing PPAR γ 2 expression and inhibiting 3T3-L1 preadipocytes differentiation (Kang *et al.*, 2003).

EFFECTS ON GROWTH PERFORMANCE OF FISH

Effects on growth rate: Growth performance is one of the most important evaluation indexes of the aquaculture industry and also is a primary basis of measuring the effectiveness of feed. The primary aim of the present study was to determine if dietary CLA has any important effects on growth parameters and lipid metabolism. Nevertheless, current studies have apparent disputes regard to the influence of dietary CLA on the growth performance of fish. Valente *et al.* (2007) determined the daily gain rate of body weight, feed intake, feed efficiency and other growth indicators of European sea bass (*Dicentrarchus labrax*) fed with the feeds which respectively added 0, 0.5, 0.75, 1.0 and 2.0% of CLA. The results show that different groups had no significant difference in the indicators of growth parameters and FCR. Berge *et al.* (2004) fed the Atlantic salmon (*Salmo salar*) with different levels of dietary CLA (0, 0.5, 1 and 2%) and after 12 weeks of feeding trial they found that CLA had no obvious facilitation on the growth of Atlantic salmon (*Salmo salar*). Similarly, Leaver reported that 4% CLA supplement had no apparent effect on the growth and FCR of Atlantic salmon (*Salmo salar*). Twibell and Wilson (2003) added 0, 0.5 and 1% CLA, respectively to the feed of channel catfish (*Ictalurus punctatus*) and after 8 weeks of feeding trial they determined the Rate of body Weight Gain (WGR), Specific Growth Rate (SGR), feed efficiency, feed intake and other growth indexes. They found by the experiments that the growth performance of channel catfish (*Ictalurus punctatus*) had no significant difference for adding CLA. Studies in the influence of CLA on the growth of large yellow croaker (*Pseudosciaena crocea*) had also obtained a similar conclusion (Zhang, 2008; Zhao, 2008; Zhanyu *et al.*, 2009). Effects of different CLA levels on the growth performance of large yellow croaker (*Pseudosciaena crocea*) are presented in Table 1. The growth indexes of yellow large croaker have declined

Table 1: Effects of different dietary CLA levels on the growth of large yellow croaker (*Pseudosciaena crocea*)

Feed composition	WGR (%)	SGR (%)	LGR (%)	CF (%)
Control	66.32±21.00 ^a	0.72±0.18 ^a	15.91±5.74 _a	1.47±0.19 ^a
5% fish oil	51.26±40.12 ^b	0.54±0.39 ^c	8.58±9.16 ^b	1.77±0.47 ^b
4% fish oil+1%CLA	58.43±20.20 ^b	0.65±0.17 ^b	10.00±4.16 ^b	2.25±0.29 ^c
3% fish oil+2%CLA	47.60±17.34 ^b	0.55±0.16 ^c	5.14±3.15 ^b	1.69±0.20 ^b
1% fish oil+4%CLA	53.48±22.06 ^b	0.60±0.19 ^b	8.96±4.43 ^b	1.81±0.26 ^b

The adjacent superscript letters in the same row mean significant at p<0.05 level, the isolated superscript letters mean significant difference at p<0.01 level. WGR: Rate of body Weight Gain; SGR: Specific Growth Rate; LGR: Length Growth Rate; CF: Condition Factor (Zhang, 2008)

when add a certain level of CLA. Nevertheless, there is no significant difference. Another trial indicated that growth of the cod was unaffected by CLA with neither having any significant effect on final weight, SGR, nor Thermal Growth Coefficient (TGC) (Kennedy *et al.*, 2007).

However, some trials have demonstrated the opposite results. Choi *et al.* (1999) fed crap (*Ctenopharyngodon idellus*) and tilapia nilotica (*Oreochromis niloticus*) with different dietary CLA supplement levels. The experimental results demonstrated that 1.0% CLA supplement could promote the growth performance of carps significantly. Whereas, large doses (>2%) of CLA for other fish growth has showed different degrees of inhibition. Chen *et al.* (2010) reported that added 0.5% of CLA into the feed can slightly promote the WGR of grass carp (*Ctenopharyngodon idellus*). But the WGR and SGR were decreasing with the CLA amount continues to increase and were significantly lower than control ($p < 0.05$) when the additive amount of CLA reached to 3%. In a present trial, CLA at 0.5% inclusion resulted in a higher Hepato-Somatic Index (HSI) in the cod and increased HSI in response to feeding CLA and been earlier reported in hybrid striped bass (*Morone saxatilis* x *M. chrysops*) (Twibell *et al.*, 2000), yellow perch (*Perca flavescens*) and tilapia (*Oreochromis niloticus*) (Kennedy *et al.*, 2007). Another study also proved the same conclusion, CLA at 1% inclusion has no significant influence on the growth performance and FCR of golden perch and channel catfish (*Ictalurus punctatus*) while obviously reduced the feed intake and WGR of stripe hybrid bass (Twibell *et al.*, 2001; Twibell and Wilson, 2003).

Effects on body composition: Table 2 shows the effects of dietary CLA on the proximate composition (%) and lipid content (%) of large yellow croaker (*Pseudosciaena crocea*). Moisture, crude protein and ash contents of fish were aggrandized gradually along with the increasing CLA concentration in daily ration though the difference was not significant ($p > 0.05$). Zhao (2008) hold the view that adding the CLA content of daily ration not only can reduce the fat content of the fish

whole-bodies but also reduce the fat content in the liver and muscles. Michael and Douglas (2006) reported that 4% supplement of CLA can significantly reduce the fat content and improve the protein proportion of Atlantic salmon (*Salmo salar*). Chen (2009) conducted an in-depth study through a feeding experiment and he pointed out that the muscle crude fat of black carp (*Mylopharyngodon piceus*) fed with diets containing 0.6, 1.2, 1.8, 2.4 and 3% CLA, respectively were lower than control. Especially a supplement of 3.0% CLA can significantly decrease the fat content of black carp ($p < 0.05$). But some studies show a different conclusion. Valente *et al.* (2007) reported that a 0-2% supplement of CLA in the experimental diets of large-size rainbow trout (*Oncorhynchus mykiss*) had no significant influence on the whole-body composition between dietary treatments ($p > 0.1$). Figueiredo-Silva *et al.* (2005) fed rainbow trout (*Oncorhynchus mykiss*) with the experimental diets which added 0, 0.5, 0.75, 1 and 2% of CLA, respectively during 12 weeks. The result of the whole-body composition and retention of rainbow trout (*Oncorhynchus mykiss*) fed the different diets has no significant difference on the protein, lipid and energy retention values. Twibell and Wilson (2003) fed channel catfish (*Ictalurus punctatus*) with the diets added 0, 0.5 and 1% CLA, respectively. The result of 8 weeks feeding trail showed there were no significant difference in the whole-body composition of different dietary treatments. This suggests that whether dietary CLA had the special physiological functions on promoting the protein deposition, improving the mineral metabolism and reducing the fat accumulation of fish need further research.

Effects on fatty acid composition: Table 3 is the fatty acid composition in the muscle of large yellow croaker (*Pseudosciaena crocea*) reflected the influence of dietary CLA. Zhao (2008) reported that dietary CLA had significantly effects on SFA, MUFA and PUFA in both muscle and liver of large yellow croaker (*Pseudosciaena crocea*). MUFA decreased significantly whereas SFA and PUFA showed a significant increase.

Table 2: Effects of dietary CLA on the proximate composition (percentage) and lipid content (percentage of wet tissue weight) of large yellow croaker (*Pseudosciaena crocea*)

CLA content (%)	Body composition				Lipid content	
	Moisture	Crude fat	CP	Ash	Liver	Flesh
0.00	12.14±0.11	4.42±0.09	68.74±0.68	5.77±0.10	3.09±0.04	5.43±0.07
1.25	12.16±0.13	4.39±0.07	69.01±0.88	5.78±0.10	3.04±0.02	5.34±0.06
2.50	12.16±0.18	4.36±0.07	69.74±0.83	5.81±0.13	2.95±0.02	5.29±0.07
5.00	12.17±0.15	4.34±0.06	69.94±1.00	5.81±0.10	2.91±0.03	5.24±0.08

The values are mean±SD (n = 6) (Zhao, 2008)

Table 3: Main fatty acids and CLA isomers (percentage of total fatty acids) in the muscle of large yellow croaker (*Pseudosciaena crocea*) with different dietary CLA levels

Fatty acid	CLA content (%)			
	0	1	2	4
C14:0	3.36±0.30	3.44±0.02	3.53±0.01	3.63±0.03
C16:0	29.15±0.18 ^a	29.25±0.03 ^a	29.41±0.06 ^{ab}	29.60±0.06 ^b
C18:0	4.45±0.11 ^a	4.58±0.03 ^{ab}	4.64±0.02 ^b	4.81±0.02 ^c
Other saturated	2.08±0.12	2.13±0.06	2.16±0.04	2.21±0.09
Σsaturated ¹	39.04±0.14 ^a	39.40±0.04 ^b	39.74±0.04 ^c	40.25±0.08 ^d
C16:1 n-7	11.53±0.15 ^a	10.56±0.08 ^b	9.19±0.02 ^c	7.49±0.02 ^d
C18:1 n-9	28.05±0.33 ^a	27.21±0.14 ^b	26.29±0.07 ^c	25.10±0.17 ^d
C22:1 n-9	0.43±0.01 ^a	0.39±0.02 ^a	0.33±0.02 ^b	0.30±0.02 ^b
Other	3.42±0.06 ^a	3.32±0.08 ^a	3.03±0.13 ^b	2.90±0.09 ^b
monounsaturated				
Σmonounsaturated ²	43.42±0.14 ^a	41.49±0.07 ^b	38.84±0.22 ^c	35.79±0.15 ^d
C18:2 n-6	0.29±0.04 ^a	0.33±0.03 ^{ab}	0.37±0.02 ^{bc}	0.41±0.02 ^c
C18:2 cis-9, trans-11	0.04±0.01 ^a	0.56±0.02 ^b	0.99±0.02 ^c	1.89±0.02 ^c
C18:2 trans-10, cis-12	0.02±0.01 ^a	0.51±0.02 ^b	0.97±0.02 ^c	1.87±0.02 ^d
C20:4 n-6	0.73±0.06 ^a	0.79±0.08 ^a	0.90±0.07 ^{ab}	1.08±0.02 ^b
C20:5 n-3	5.00±0.02 ^a	5.09±0.03 ^a	5.22±0.15 ^{ab}	5.45±0.08 ^b
C22:3 n-6	0.26±0.04	0.28±0.02	0.30±0.01	0.31±0.02
C22:5 n-3	1.24±0.07 ^a	1.30±0.04 ^{ab}	1.38±0.05 ^b	1.43±0.01 ^b
C22:6 n-3	6.11±0.13 ^a	6.28±0.08 ^a	7.21±0.06 ^b	7.34±0.05 ^b
Other	3.86±0.11	3.97±0.09	4.08±0.09	4.18±0.19
polyunsaturated				
Σpolyunsaturated ³	17.54±0.19 ^a	19.11±0.03 ^b	21.42±0.23 ^c	23.96±0.23 ^d
Total CLA ⁴	0.06±0.01 ^a	1.08±0.01 ^b	1.96±0.02 ^c	3.77±0.03 ^d

The values are mean±SD (n = 3). Different superscript letters in the same row denote significant differences (p<0.05) between diets as determined by One-way Analysis of Variance (ANOVA) with Tukey's post-test; ¹Saturated: C14:0, C15:0, C16:0, C17:0, C18:0, C20:0; ²Monounsaturated: C15:1 n-7, C16:1 n-7, C17:1 n-9, C18:1 n-9, C20:1 n-9, C22:1 n-9, C24:1 n-9; ³Polyunsaturated: C16:2 n-4, C20:2 n-6, C16:2 n-6, C18:1 n-6, C18:2 n-6, C18:2 cis-9, trans-11, C18:2 trans-10, cis-12, C20:4 n-6, C22:3 n-6, C16:3 n-3, C18:3 n-3, C18:4 n-3, C20:5 n-3, C22:5 n-3, C22:6 n-3; ⁴Sum of CLA isomers cis-9, trans-11 and trans-10, cis-12

CLA supplementation also resulted in a significant increase of C16:0 and C18:0 whereas a significant decrease of C16:1 n-7 and C18:1 n-9 was observed in fish fed with 4% CLA. Concerning the PUFA fraction, a gradual increasing of C18:2 n-6, C20:5 n-3 and C22:6 n-3 was observed in both muscle and the liver of fish fed with high CLA. Similarly, Berge *et al.* (2004) reported that the deposition ratio of n-3 fatty acid was higher in fish fed diets with 1 or 2% CLA than it was in the control diet and in the 0.5% CLA diet, even though the amounts of n-3 fatty acid observed in the fish at the end of the experiment were similar in all deposition ratio of n-3 fatty acid with increasing CLA inclusion in the diet (Table 4). The deposition ratio of C22:6 n-3 was in the range 1.35-1.46 indicating a net deposition of this fatty acid in all dietary groups. The linear increase in deposition ratio of C22:6 n-3 was highly significant (p<0.0001) with increasing levels of CLA.

Earlier studies indicate that the effects of dietary CLA on the growth performance for different varieties of fish were not the same. Numerous studies have demonstrated that effects of CLA on the growth performance of different fish species had significant difference. More scholars suggested that the influence of dietary CLA on growth performance of fish mainly due to the mixed amount and the isomers composition ratio of CLA. Especially in recent years more and more evidence demonstrated that the impacts of CLA on animal's growth were closely related to the isomers of CLA.

Table 4: Fatty acid contents given as percentage of total fatty acids, in juvenile Atlantic salmon (*Salmo salar*) sampled at the start of the experiment and after feeding the experimental diets for 12 weeks (means±SEM n = 3)

Fatty acid	Initial samples	Diet 1 (control)	Diet 2 (0.5% CLA)	Diet 3 (1.0% CLA)	Diet 4 (2.0% CLA)	ANOVA (p-value)
14:0	3.9±0.10	6.0±0.070 ^a	5.8±0.070 ^b	5.6±0.060 ^b	5.3±0.010 ^c	0.0002
15:1	-	0.4±0.004	0.4±0.060	0.4±0.010	0.4±0.030	0.3300
16:0	13.5±0.04	14.1±0.20	14.0±0.080	13.8±0.120	13.9±0.070	0.3300
16:1 n-7	5.2±0.08	5.3±0.090 ^a	4.8±0.030 ^b	4.6±0.030 ^b	4.3±0.030 ^c	0.0001
17:1	-	0.4±0.010 ^a	0.4±0.020 ^a	0.3±0.002 ^b	0.3±0.010 ^b	0.0200
18:0	2.5±0.04	2.4±0.080 ^a	2.4±0.070 ^b	3.6±0.060 ^b	4.0±0.120 ^a	0.0001
18:1 n-7	2.6±0.09	2.4±0.030 ^a	2.3±0.004 ^b	2.3±0.020 ^b	2.3±0.030 ^b	0.0200
18:1 n-9	16.3±0.10	15.2±0.21 ^a	14.5±0.030 ^b	14.4±0.060 ^b	14.7±0.030 ^b	0.0040
18:2 n-6	5.5±0.03	3.2±0.400 ^a	3.1±0.003 ^a	3.1±0.020 ^a	3.0±0.040 ^b	0.0100
18:3 n-3	1.7±0.04	1.0±0.030 ^a	0.9±0.070 ^a	0.9±0.020 ^a	0.8±0.010	0.0200
18:4 n-3	2.2±0.03	1.8±0.030 ^a	1.8±0.003 ^a	1.7±0.020 ^b	1.6±0.010 ^c	0.0004
20:0	-	0.3±0.040	0.2±0.080	0.3±0.000	-	0.4700
20:1 n-9	5.1±0.26	11.7±0.37 ^a	11.7±0.090 ^a	10.6±0.270 ^b	10.0±0.350 ^b	0.0070
20:2 n-6	0.7±0.18	0.4±0.090	0.4±0.020	0.4±0.003	0.3±0.004	0.1500
20:4 n-3	1.3±0.07	0.8±0.030 ^a	0.8±0.020 ^a	0.7±0.020 ^a	0.7±0.010	0.0200
20:4 n-6	0.6±0.03	0.3±0.002	0.3±0.010	0.3±0.010	0.3±0.010	0.4900
20:5 n-3	5.2±0.12	3.4±0.050 ^a	3.4±0.090 ^a	3.4±0.030 ^a	3.2±0.030 ^b	0.0300
22:1 n-11	6.1±0.07	14.2±0.07 ^a	13.7±0.200 ^b	13.3±0.090 ^c	12.0±0.030 ^d	0.0001
22:5 n-3	2.2±0.03	1.4±0.050 ^a	1.3±0.060 ^a	1.3±0.020 ^a	1.2±0.040 ^b	0.0400
22:6 n-3	17.9±0.40	10.2±0.06 ^a	10.0±0.160 ^a	10.0±0.030 ^a	9.4±0.040 ^b	0.0020
CLA*	0	0 ^a	1.9±0.040 ^c	3.8±0.090 ^b	7.3±0.060 ^a	0.0001
SumSFA	22.7±0.06	23.8±0.47	24.4±0.080	24.4±0.340	24.4±0.160	0.4700
SumMUFA	35.4±0.27	49.7±0.57 ^a	47.7±0.130 ^b	45.9±0.260 ^c	44.1±0.310 ^d	0.0001
Sum n-3	30.9±0.06	19.0±0.48	18.7±0.630	19.4±0.240	17.8±0.390	0.1800
Sum n-6	6.9±0.16	3.9±0.040 ^a	3.8±0.030 ^a	3.8±0.010 ^a	3.6±0.050 ^b	0.0100
Identified	97.7±0.57	97.1±0.61	97.2±0.780	97.9±0.590	97.8±0.180	0.6400

Values with different superscript letters within a row are significantly different at p<0.05 level; *Sum of isomers t10, c12 CLA and c9, t11 CLA. (Berge *et al.*, 2004)

DISCUSSION

Dietary CLA has some unobvious effects on the growth performance of fish while the difference is not significant. Earlier studies in a number of species show that CLA may have effects on the growth rate of fish but the results are diverging. More importantly, the impacts of dietary CLA on the growth rate for different fish species showing absolutely different results. There are some evidences suggest that this may due to the mixed amount and the isomers composition ratio of dietary CLA. Dietary CLA (cis-9, trans-11 and trans-10, cis-12 isomers) inclusion resulted in significant increases of the contents of Saturated Fatty Acids (SFA) and Polyunsaturated Fatty Acids (PUFA) ($p < 0.05$) and decreased Monounsaturated Fatty Acid (MUFA) content ($p < 0.05$) in both muscle and liver tissues.

Results from studies in other animal species are varied but several studies support the findings of most fish studies. The reason why dietary CLA can affect the growth performance of fish is that CLA involved in metabolism and the synthesis and degradation of lipid. CLA, a new type of nutrient redistribution agent, relates to fat deposition and nitrogen distribution. It influences the feed utilization of animal by participating in the body metabolism. Therefore, dietary CLA can reduce the weight of animal and improve the efficiency of feed utilization at the same time. Brodie *et al.* (1999) and Evans *et al.* (2000, 2001) found by laboratory processing the vegetative cells of rodents that CLA can decrease the differentiation of preadipocytes in a dose-dependent manner. CLA can significantly increase the lipid burning activity and promote fatty acid oxidation by enhancing the activity of carnitine palmitic acid shift enzyme which responsible for transporter of fatty acid into mitochondria for oxidation. What more, it could inhibit the activity of Lipoprotein Lipase (LPL) and then reduce the accumulation of triglyceride in the body tissues. All these features are conducive to the protein synthesis and the decomposition of body fat.

It also suggested that CLA changes the body fat content of animals by influencing the pathway of fat synthesis and metabolic. In the process of glucose synthesis of fatty acid, Glucose Transporter Protein (GLUT) is one of the regulatory factors during the facilitated diffusion procedure of glucose to the cell membrane. There are a variety of GLUT (GLUT1, GLUT5, GLUT7) in animals body which encoded by different homologous genes.

CONCLUSION

The amount of glucose entered into cell membrane is mainly depends on the expression level of

genes encoding these protein which were influenced by CLA. Adding CLA to the diets plays a negative regulatory role in the adipose tissue *GLUT4* gene expression of animals (Kahn, 1994). Takahashi and Kushi (2002) reported that CLA supplement could significantly reduce the weight and the *GLUT4* mRNA level of mice white adipose tissue and brown adipose tissue. Rahman *et al.* (2001) found that could reduce the glycerophosphate dehydrogenase activity of mice. Similarly, Tsuboyama-Kasaoka *et al.* (2000) reported that CLA could inhibit the activities of Fatty Acid Synthase (FAS) and Acetyl-Coenzyme a Carboxylase (ACCase) as well as the expression of LPL. Aforementioned enzymes have close relationship with the fat synthesis. Therefore, dietary CLA in all probability reduce the fat content by decreasing the amounts and inhibiting the activities of those enzymes.

ACKNOWLEDGEMENTS

Researchers gratefully acknowledge the financial supports from the 12th Five Year Plan National Science and Technology Support Projects (2011BAD17B02) and the National Natural Science Foundation of China (30800800).

REFERENCES

- Azain, M.J., D.B. Hausman, M.B. Sisk, W.P. Flatt and D.E. Jewell, 2000. Dietary CLA reduces rat adipose tissue cell size rather than cell number. *J. Nut.*, 130: 1548-1554.
- Banu, J., A. Bhattacharya, M. Rahman, M. O'Shea and G. Fernandes, 2006. Effects of conjugated linoleic acid and exercise on bone mass in young male Balb/C mice. *Lipids Health Dis. Elect. Res.*, Vol. 5, 10.1186/1476-511X-5-7.
- Belury, M.A., 2002. Dietary conjugated linoleic acid in health: Physiological effects and mechanisms of action. *Annu. Rev. Nut.*, 22: 505-531.
- Berge, G.M., B. Ruyter and T. Asgard, 2004. Conjugated linoleic acid in diets for juvenile Atlantic salmon (*Salmo salar*); effects on fish performance, proximate composition, fatty acid and mineral content. *Aquaculture*, 237: 365-380.
- Bhattacharya, A., J. Banu, M. Rahman, J. Causey and G. Fernandes, 2006. Biological effects of conjugated linoleic acids in health and disease. *J. Nutr. Biochem.*, 17: 789-810.
- Brodie, A.E., V.A. Manning and K.R. Ferguson, 1999. CLA inhibits differentiation of pre-and post-confluent 3T3-L1 preadipocytes but inhibits cell proliferation only in p refluenc cells. *J. Nut.*, 129: 381-387.

- Brown, J.M. and M.K. McIntosh, 2003. CLA in humans: Regulation of adiposity and insulin sensitivity. *J. Nut.*, 133: 3041-3046.
- Brown, J.M., Y.D. Halvorsen and Y.R. Lea-Currie, 2001a. Trans-10, cis-12, but not cis-9, trans-11 conjugated linoleic acid attenuates lipogenesis in primary cultures of stromal vascular cells from human adipose tissue. *Nutr*, 131: 2316-2321.
- Brown, J.M., Y.D. Halvorsen, Y.R. Lea-Currie, C. Geigerman and M. McIntosh, 2001b. t10, c12 but not c9,t11. CLA attenuates lipogenesis in primary cultures of stromal vascular cells from human adipose tissue. *J. Nut.*, 131: 2316-2321.
- Brownbill, R.A., M. Petrosian and J.Z. Ilich, 2005. Association between dietary conjugated linoleic acid and bone mineral density in postmenopausal women. *J. Am. Coll. Nutr.*, 24: 177-181.
- Burr, L.L., C.G. Taylor and H.A. Weiler, 2006. Dietary conjugated linoleic acid does not adversely affect bone mass in obese fa/fa or lean Zucker rats. *Exp. Biol. Med. (Maywood, N.J.)*, 231: 1602-1609.
- Chen, B.Q., Y.M. Yang and Q. Wang, 2004. Effects of t9, t11-CLA on adhesion of human gastric carcinoma cellline SGC-7901. *World J. Gastroenterol.*, 10: 1392-1396.
- Chen, C., 2009. Effects of supplement with conjugated linoleic acids on the growth performance, muscle composition and hepatobiliary function in black carp (*Mylopharyngodon piceus*). Ph.D. Thesis, University of Michigan.
- Chen, C., F. Huang, Q.Y. Shu, L. Zhang and Y.P. Zhou, 2010. Effects of dietary conjugated linoleic acids on growth performance, muscle composition, AST and ALT activities in serum in juveniles Grass carp (*Ctenopharyngodon idellus*). *Acta Hydrobiol. Sinica*, 3: 647-654.
- Choi, B.D., S.J. Kang, Y.L. Ha and R.C. Ackman, 1999. Accumulation of Conjugated Linoleic Acid (CLA) in Tissues of Fish Fed Diets Containing Various Levels of CLA. In: *Quality Attributes of Muscle Foods*. Xiong, Y.L., C.T. Ho and E. Shahidi (Eds.). Kluwer Academic Publishing, Plenum, pp: 61-71.
- Choi, Y., Y.C. Kim and Y.B. Han, 2000. The trans-10, cis-12 isomer of conjugated linoleic acid down-regulates stearoyl-CoA desaturase 1 gene expression in 3T3-L1 adipocytes. *J. Nut.*, 130: 1920-1924.
- Cook, M.E., C.C. Miller, Y. Park and M. Pariza, 1993. Immune modulation by altered nutrient metabolism: Nutritional control of immune-induced growth depression. *Poult. Sci.*, 72: 1301-1305.
- Cusack, S., C. Jewell and K.D. Cashman, 2005. The effect of conjugated linoleic acid on the viability and metabolism of human osteoblast-like cells. *Prostaglandins Leukot Essent Fatty Acids*, 72: 29-39.
- Dilzer, A. and Y. Park, 2012. Implication of Conjugated Linoleic Acid (CLA) in human health. *Crit. Rev. Food Sci. Nut.*, 6: 488-513.
- Evans, M., C. Geigerman and J. Cook, 2000. Conjugated linoleic acid suppresses triglyceride accumulation and induces apoptosis in 3T3-L1 preadipocytes. *Lipids*, 35: 899-910.
- Evans, M., Y. Park and M. Pariza, 2001. Trans-10, cis-12 conjugated linoleic acid reduces triglyceride content while differentially affecting peroxisome proliferator activated receptor gamma2 and alpha2 expression in 3T3-L1 preadipocytes. *J. Lipids*, 36: 1223-1232.
- Figueiredo-Silva, A.C., E. Rema, N.M. Bandarrac, M.L. Nunes and L.M.P. Valente, 2005. Effects of dietary conjugated linoleic acid on growth, nutrient utilization, body composition and hepatic lipogenesis in rainbow trout juveniles. *Aquaculture*, 248: 163-172.
- Gerhard, J., K.T.F. Jana, S. Friedrich and L. von Christian, 2000. Conjugated linoleic acids: Physiological effects in animal and man with special regard to body composition. *Eur. J. Lipid Sci. Technol.*, 102: 695-703.
- Harvatine, K.J. and D.E. Bauman, 2011. Characterization of the acute lactational response to trans-10, cis-12 conjugated linoleic acid. *J. Dairy Sci.*, 12: 2256-2257.
- Kahn, B.B., 1994. Dietary regulation of glucose tRNA's porter gene expression: Tissue specific effects in adipose cells and muscle. *J. Nutr.*, 124: 1289-1294.
- Kang, K., W. Liu, K.J. Albright, Y. Park and M.W. Pariza, 2003. 2003 Trans-10, cis-12 CLA inhibits differentiation of 3T3-L1 adipocytes and decreases PPAR γ expression. *Biochem. Biophys. Res. Commun.*, 303: 795-799.
- Kelley, N.S., N.E. Hubbard and K.L. Erickson, 2007. Conjugated linoleic acid isomers and cancer. *J. Nutr.*, 137: 2599-2607.
- Kelly, O. and K.D. Cashman, 2004. The effect of conjugated linoleic acid on calcium absorption and bone metabolism and composition in adult ovariectomised rats. *Prostaglandins Leukot Essent Fatty Acids*, 71: 295-301.
- Kelly, O., S. Cusack, C. Jewell and K.D. Cashman, 2003. The effect of polyunsaturated fatty acids, including conjugated linoleic acid, on calcium absorption and bone metabolism and composition in young growing rats. *Br. J. Nutr.*, 90: 743-750.
- Kennedy, S.R., R. Bickerdike, R.K. Berge, A.R. Porter and D.R. Tocher, 2007. Influence of dietary conjugated linoleic acid (CLA) and tetradecylthioacetic acid (TTA) on growth, lipid composition and key enzymes of fatty acid oxidation in liver and muscle of Atlantic cod (*Dadus morhua* L.). *Aquaculture*, 264: 372-382.

- Larson, T.M., S. Toubros and A. Astrup, 2003. Efficacy and safety of dietary supplements containing conjugated acid for the treatment of obesity-evidence from animal and human studies. *J. Lipid Res.*, 8: 1-23.
- Lee, K.W., H.J. Lee, H.Y. Cho and Y.J. Kim, 2005. Role of the conjugated linoleic acid in the prevention of cancer. *Crit. Rev. Food Sci. Nut.*, 45: 135-144.
- Maurin, A.C., P.M. Chavassieux and P.J. Meunier, 2005. Expression of PPARgamma and beta/delta in human primary osteoblastic cells: Influence of polyunsaturated fatty acids. *Calcif Tissue Int.*, 76: 385-392.
- Michael, J.L. and R. Douglas, 2006. Effect of dietary Conjugated Linoleic Acid (CLA) on lipid composition metabolism and gene expression in Atlantic salmon (*Salmo salar*) tissues. *Comp. Physiol.*, 145: 258-267.
- Pariza, M.W., 2004. Perspective on safety and effectiveness of conjugated linoleic acid. *Am. J. Clin. Nut.*, 79: 1132S-1136S.
- Park, Y. and M.W. Pariza, 2007. Mechanisms of body fat modulation by Conjugated Linoleic Acid (CLA). *Food Res. Int.*, 40: 311-323.
- Park, Y., J.M. Storkson, K.J. Albright, W. Liu and M.W. Pariza, 1999. Evidence that the trans-10,cis-12 isomer of conjugated linoleic acid induces body composition changes in mice. *Lipids*, 34: 235-241.
- Park, Y., K.J. Albright, W. Liu, M.E. Cook and M.W. Pariza, 1995. Dietary Conjugated Linoleic Acid (CLA) reduces body fat content and isomers of CLA are incorporated into phospholipid fraction. Institute of Food Technologists Annual Meeting: Book of Abstracts, January 1995, Pages: 183.
- Park, Y., M.W. Pariza and Y. Park, 2008. Co-supplementation of dietary calcium and Conjugated Linoleic Acid (CLA) improves bone mass in mice. *J. Food Sci.*, 73: C556-C560.
- Platt, I., L.G. Rao and A. El-Sohemy, 2007. Isomer-specific effects of conjugated linoleic acid on mineralized bone nodule formation from human osteoblast-like cells. *Exp. Biol. Med. (Maywood)*, 232: 246-252.
- Platt, I.D. and A. El-Sohemy, 2009. Regulation of osteoblast and adipocyte differentiation from human mesenchymal stem cells by conjugated linoleic acid. *J. Nutr. Biochem.*, 20: 956-964.
- Rahman, S.M., Y.M. Wang, S.Y. Han, J.Y. Chaa, N. Fukudab, H. Yotsumotoa and T. Yanagita, 2001. Effects of short-term administration of conjugated linoleic acid on lipid metabolism in white and brown adipose tissues of starved/refed Otsuka Long-Evans Tokushima fatty rats. *Food Res. Int.*, 34: 515-520.
- Roche, H.M., E. Noone and A.N. Gibney, 2001. Conjugated linoleic acid: A novel therapeutic nutrient. *Nutr. Res. Rev.*, 14: 173-188.
- Subbaiah, P.V., D. Sircar, B. Aizezi and E. Mintzer, 2010. Differential effects of conjugated linoleic acid isomers on the biophysical and biochemical properties of model membranes. *Biochimica Biophysica Acta (BBA)- Biomembranes*, 1798: 506-514.
- Tacon, A.G.J., 2003. Global Trends in Aquaculture and Compound Aquafeed Production. In: *Aquafeed Directory and Buyer's Guide 2003*, Tacon, A.G.J. (Ed.). International Turret RAI, Uxbridge, pp: 8-23.
- Takahashi, Y. and M. Kushiro, 2002. Dietary conjugated linoleic acid reduces body fat mass and affects gene expression of proteins regulating energy metabolism in mice. *Comp. Biochem. Physiol. Part B.*, 133: 395-404.
- Terpstra, A.H.M., 2004. Effect of CLA on body composition and plasma lipids in humans: An overview of the literature. *Am. J. Clin. Nut.*, 79: 352-361.
- Tsuboyama-Kasaoka, N., M. Takahashi, K. Tanemura, H.J. Kim and T. Tange *et al.*, 2000. Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice. *Diabetes*, 49: 1534-1542.
- Twibell, R.G. and R.P. Wilson, 2003. Effects of conjugated linoleic acids and total dietary lipid concentrations on growth responses of juvenile channel catfish, *Ictalurus punctatus*. *Aquaculture*, 221: 621-628.
- Twibell, R.G., B.A. Watkins and P.B. Brown, 2001. Dietary conjugated linoleic acid and lipid source alter fatty acid composition of juvenile yellow perch, *Perca flavescens*. *J. Nutr.*, 131: 2322-2328.
- Twibell, R.G., B.A. Watkins, L. Rogers and P.B. Brown, 2000. Effects of dietary conjugated linoleic acids on hepatic and muscle lipids in hybrid striped bass. *Lipids*, 35: 155-161.
- Valente, L.M.P., N.M. Bandarra, A.C. Figueiredo-Silva, P. Rema and P. Vaz-Pires *et al.*, 2007. Conjugated linoleic acid in diets for large-size rainbow trout (*Oncorhynchus mykiss*): Effects on growth, chemical composition and sensory attributes. *Br. J. Nut.*, 97: 289-297.
- Watkins, B.A., Y. Li, H.E. Lippman and S. Feng, 2003. Modulatory effect of omega-3 polyunsaturated fatty acids on osteoblast functions and bone metabolism. *Prostaglandins Leukot. Essent. Fatty Acids*, 68: 387-398.

- Yamasaki, M., K. Mansho, Y. Ogino, M. Kasai, H. Tachibana and K. Yamada, 2000. Acute reduction of serum leptin level by dietary conjugated linoleic acid in Sprague-Dawley rats. *J. Nut. Biochem.*, 11: 467-471.
- Yeonhwa, P., 2009. Conjugated Linoleic Acid (CLA): Good or bad trans fat. *J. Food Comput. Anal.*, 22: 4-12.
- Zhang, J.Z., 2008. Effects of conjugated linoleic acid on the growth, body composition, lipometabolism and immunity of large yellow croaker (*Pseudosciaena crocea*). Ph.D. Thesis, University of Washington.
- Zhanyu, Z., W. Tianxing and T. Honggang, 2009. Effects of conjugated linoleic acid on growth and immune indices of large yellow croaker (*Pseudosciaena crocea*, R). *Chinese J. Anim. Nut.*, 21: 205-211.
- Zhao, Z.Y., 2008. Effects of Conjugated Linoleic Acid (CLA) on the lipometabolism, immunity, flesh quality and *PPAR* gene expression of large yellow croaker (*Pseudosciaena crocea*). Ph.D. Thesis, Computational Mechanics Institute.