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Effects of Dietary Conjugated Linoleic Acid and n-3 Fatty Acids on the Performance, Carcass Traits and Small Intestinal Morphology of Broiler Chickens

Research Article

M. Nosrati[1*], B. Navidshad[2] and M. Malecky[3]

[1] Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht, Iran
[2] Department of Animal Science, University of Mohaghegh Ardabili, Ardabil, Iran
[3] Department of Animal Science, Faculty of Agriculture, Bu Ali Sina University, Hamedan, Iran

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*Correspondence E-mail: nosrati@iaurasht.ac.ir

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ABSTRACT

An experiment was conducted on broiler chickens to study the effects of conjugated linoleic acid (CLA), fish oil, both at 7% of the diet, or their mixtures at 3.5% of the diet, on the performance, carcass traits and intestinal morphology of broiler chickens. The chicks fed with 7% fish oil or 7% CLA diets, were found to have an inferior weight gain in grower and finisher phases, respectively. A significant reduction in feed intake was observed with the diets containing 7% fish oil. However, adding CLA to the diets, did not affect birds feed intake. The dietary fish oil and CLA supplementation adversely affected the feed conversion ratio as well as carcass yield. CLA at the 7% level increased liver weight. There were no differences in the carcass, thigh or abdominal fat pad percentages between the experimental treatments; however the birds fed the diet containing 7% fish oil had the lowest breast and the highest liver percentages. Histological examination of small intestine revealed that, the diet containing 3.5% fish oil + 3.5% CLA resulted in higher villus height than the diets with 7% fish oil or CLA. Such an increase was observed in the crypt depth of the duodenum and jejunum, but no difference was detected in the crypt depth of the ileum. The small intestinal wall thickness and goblet cell numbers in the chickens fed the diet containing the mixture of fish oil and CLA were lower than those of the other treatments. The results of this study showed that a high dose of fish oil or CLA can reduce broiler chickens performance, but their combination can moderate this adverse effect.

KEY WORDS broiler chickens, carcass traits, CLA, fish oil, intestine morphology, performance.

INTRODUCTION

The lumen of the small intestine is equipped with villi, and their structure and height can be changed in response to different dietary agents. It has been reported that some chemicals, especially fats can influence the villus growth and consequently the absorptive capacity of the small intestine (Kruger *et al*. 1971; Antheony *et al.* 1991). Conjugated linoleic acid (CLA) is one of the unique fatty acids that could be supplemented into the chicken's ration. This compound is a specific isomer of linoleic acid, which in nature is produced as a by-product of the fatty acid biohydrogenation in the rumen. One of the interesting aspects of CLA is its fat-reducing effects in animal and human subjects (Park and Pariza, 2006). Fish oil is a good source of n-3 polyunsaturated fatty acids (PUFAs) (Farhomand and Checaniazar, 2009). The health promoting effects of n-3 PUFAs, especially EPA (C20:5 n-3) and DHA (C22:6 n-3), are well known (Knapp, 1991; Kinsella *et al.* 1990). Previous reports have demonstrated that dietary inclusion of fish oil

did not adversely affect mortality rate, weight gain or feed conversion ratio of broiler chickens as compared to the plant oils (Nash *et al.* 1995), However, some authors reported unfavorable taste in the meat of broiler chickens fed up to 2% dietary fish oil (Hardin *et al.* 1964). The body fat reducing effects of PUFAs have been shown in previous reports (Chashnidel *et al.* 2010). There are few reports on the effects of CLA or fish oil on the intestinal structure in broiler chickens. In the majority of the research that has been reported, less than 5% CLA or fish oil was used in formulating experimental diets. Therefore, the objective of the present study was to assess the effects of high dietary levels of CLA, fish oil and their mixture, on performance, various carcass characteristics and small intestine morphology of broiler chickens.

MATERIALS AND METHODS

A total of 240 Ross 308 mixed-sex broiler chickens were used in this study. Chicks that were ten days old were placed randomly into each of 12 litter pens (1.5×1.5 m^2). A lighting program of 23L:1D was used for the entire 42-d rearing period. The birds were housed in an environmentally-controlled room, and they had free access to feed (mash) and water. Experimental diets were formulated according to the Ross 308 manual. All chicks were fed a commercial starter diet from 0 to 10 days and the experimental diets from 11 to 28 days (grower phase) and 29 to 42 days (finisher phase). Three isocaloric and isonitrogenous diets were formulated to contain, 7% CLA (CLA), 7% fish oil (FO), or 3.5% CLA + 3.5% fish oil (CLA+FO) (Table 1). The CLA supplement used in this study was LUTA-CLA 60, prepared and supplied by BASF company (Ludwigshafen, Germany) and contained 30% isomer 9c, 11t and 30% isomer 10t, 12c of conjugated linoleic acid plus mostly oleic acid, so that dietary inclusion of 7 and 3.5% CLA supplied 4.2 and 2.1% CLA, respectively.

Group body weight (BW), average daily gain (ADG), daily feed intake (DFI) and feed conversion ratio (FCR) per pen were calculated for the grower (10-28 d) and finisher (29-42 d) phases. On d 42, two male birds per cage were weighed and slaughtered after an overnight feed withdrawal period. After scalding (63 ˚C) for 45 s, carcasses were mechanically defeathered and manually eviscerated. They were cut up after an internal carcass temperature of 4 ˚C was reached (approximately 4 to 6 h). Sex was verified at processing, and carcass, breast (pectoralis major+pectoralis minor) and thigh weights were recorded on 8 birds per treatment. Carcass yield was calculated as eviscerated carcass with neck, feet, and abdominal fat pad removed, as a percentage of live BW at the time of feed withdrawal. Data sets of completely randomized design with 3 treatments and 4 replicates were compared across the treatments using the one-way analysis of variance (ANOVA) procedure. The pen was considered as experimental unit for performance traits and each chicken was the experimental unit for carcass parameters and intestinal morphology. At 42 days of age, two chicks per replicate pen (1 male and 1 female) were randomly sampled for morphometric analysis. The intestinal tract was removed immediately and severed from the gizzard, and the pancreas was removed.

Three 1-centimeter tissue segments were taken from the proximal, middle and distal parts of the duodenum (from the gizzard to the pancreo-biliary ducts), jejunum (from the pancreo-biliary ducts to Meckel´s diverticulum) and ileum (from a Meckel´s diverticulum to the ileocaecal junction) sections.

All samples from each of those birds were taken from the same area of each section of the tract. Samples were stored in 10% buffered formalin for fixation, where they were gently shaken to remove any adhering intestinal contents.

Cross sections (5 µm thick) of each intestinal segment were processed in low-melt paraffin and stained with hematoxylin and eosin. This procedure provides a longitudinal section of villi. Using a Zeiss light microscope, 15 measurements per intestinal section were made for each parameter and averaged into one value per bird. Each histological data point was obtained from the mean of 45 records (3 sections and 15 villi per section). Significant means were then elucidated using the Duncan multiple range tests. All statistical tests were conducted at the 95% confidence level using the SAS program (SAS, 2002).

RESULTS AND DISCUSSION

Table 2 shows the effect of dietary fats on the performance of broiler chickens. During the growing phase, weight gain of the birds fed the diet containing 3.5% fish oil + 3.5% CLA was higher than other treatments ($P<0.05$). During the finisher phase, weight gain of the birds fed fed the 3.5% fish oil + 3.5% CLA or 7% fish oil were greater than those of the birds fed the 7% CLA diet ($P<0.05$). During the growing phase, the highest feed intake was observed in birds fed the CLA + fish oil mixture and then the 7% CLA ($P<0.05$). The lowest feed intake ($P<0.05$) was observed in the 7% fish oil treatment group during both the grower and finisher phases. The best feed conversion ratio for both the grower and finisher phases was related to the CLA + fish oil diet ($P<0.01$). The effects of the experimental diets on carcass parameters are shown in Table 3. There were no differences in carcass, thigh or abdominal fat pad among the experimental groups. However the birds fed the diet containing 7% fish oil had lower breast weight than the other treatments ($P<0.01$).

Table 1 Ingredients and nutrient content of the experimental diets

Ingredients	Starter	Grower (11-28 days of age)			Finisher (29-42 days of age)		
	(1-10 d)	FO[2]	CL	CLFO	FO	CL	CLFO
Corn (%)	60.23	53.99	55.8	54	57.98	59.5	58.92
Soy meal (%)	30.81	32.27	28.6	32.26	30.27	26.38	28.6
Fish meal (%)	5.37	3.00	5.00	3.01	1.00	2.99	1.51
Fish oil	-	7.00	-	3.50	7.00	-	3.50
Conjugated linoleic acid (CLA) (%)[1]	-	-	7.00	3.50	-	7.40	3.50
Oyster shell (%)	1.41	1.42	1.33	1.42	1.39	1.3	1.36
DCP (%)	0.51	0.66	0.52	0.66	0.84	0.71	0.82
Salt (%)	0.25	0.32	0.28	0.32	0.35	0.32	0.34
Vit-Min P (%)[3]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DL-Met (%)	0.26	0.25	0.23	0.25	0.18	0.16	0.18
L-Lys (%)	0.15	0.09	0.24	0.09	-	0.25	0.27
Calculated content							
Dry matter (DM) (%)	89.40	90.14	90.17	90.15	90.10	90.18	90.11
Metabolizable energy (ME) (kcal/kg)	2860	3211	3175	3175	3241	3225	3225
Crude protein (CP) (%)	22.5	21.00	21.00	21.00	19.00	19.00	19.00
Ether extracts (EE) (%)	2.86	9.52	9.65	9.52	9.55	10.07	9.60
Crude fibre (CF) (%)	3.53	3.48	3.27	3.47	3.41	3.18	3.12
Linoleic acid (%)	1.46	1.39	5.27	3.31	1.46	5.56	3.4
Ca (%)	0.95	0.90	0.90	0.90	0.85	0.85	0.85
Ava P (%)	0.48	0.45	0.45	0.45	0.43	0.43	0.43
Na (%)	0.15	0.16	0.16	0.16	0.16	0.16	0.16
Lys (%)	1.37	1.23	1.34	1.23	1.02	1.20	1.20
Met (%)	0.66	0.60	0.60	0.60	0.48	0.48	0.49
Met + Cys (%)	1.04	0.95	0.95	0.95	0.80	0.80	0.80

[1] CLA used in this experiment was CLA LUTA60 which contains 60% CLA, then 7% and 3.5% dietary inclusion of CLA will be equal to 4.2% and 2.1% respectively.
[2] FO: diet containing 7% fish oil; CL: diet containing 7% CLA; CLSO: diet containing 3.5% CLA + 3.5% soybean oil.
[3] Mineral premix provided per kg of ration with: Fe: 50 mg; Mn: 70 mg; Zn: 50 mg; Cu: 7 mg; Co: 0.4 mg; Se: 0.17 mg and I: 0.75 mg.
Vitamin premix provided per kg of ration with: vitamin A: 6000 IU: vitamin D_3: 2200 IU; vitamin E: 2000 IU; vitamin K_3: 20 µg; vitamin B_{12} 33 IU; B_2: 19 µg and Pantothenic: 60 mg.

Table 2 Effects of dietary, fish oil or conjugated linoleic acid (CLA)[1] and their mixtures on performance of broiler chickens[*]

Treatments	Grower phase (10-28 d)			Finisher phase (29-42 d)			Total experiment (10-42 d)		
	DWG (g/b/d)	DFI (g/b/d)	FCR	DWG (g/b/d)	DFI (g/b/d)	FCR	DWG (g/b/d)	DFI (g/b/d)	FCR
FO[2]	24.2^{c}	52.52^{c}	2.17^{a}	58.9^{a}	114.9^{b}	1.95^{c}	39.5^{b}	79.8^{b}	2.03^{b}
CL	35.9^{b}	72.42^{b}	2.02^{ab}	50.9^{b}	141.9^{a}	2.79^{a}	42.4^{ab}	102.4^{a}	2.42^{a}
CLFO	43.8^{a}	82.98^{a}	1.90^{b}	63.1^{a}	148.2^{a}	2.36^{b}	52.6^{a}	111.3^{a}	2.13^{b}
SEM	2.18	3.44	0.09	3.13	5.31	0.05	4.79	8.8	0.06

[*] Four replicates within each treatment, 20 birds per replicate.
[1] CLA used in this experiment was CLA LUTA60 which contains 60% CLA, then 7% and 3.5% dietary inclusion of CLA will be equal to 4.2% and 2.1% respectively.
[2] FO: diet containing 7% fish oil; CL: diet containing 7% CLA and CLFO: diet containing 3.5% CLA + 3.5% fish oil.
BW: body weight; DWG: daily weight gain; DFI: daily feed intake and FCR: feed conversion ratio.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Effects of fish oil or conjugated linoleic acid (CLA)[1] and their mixtures on carcass parameters of broiler chickens (as a percent of live weight)[*]

Treatments	Carcass	Breast	Whole leg	Liver	Fat pad
FO	54.5	18.1^{b}	18.1	2.6^{b}	2.0
CL	56.3	20.3^{a}	18.3	3.3^{a}	2.1
CLFO	56.3	21.2^{a}	18.4	2.6^{b}	2.1
SEM	1.31	0.35	0.23	0.04	0.08

[*] n= 8.
[1] CLA used in this experiment was CLA LUTA60 which contains 60% CLA, then 7% and 3.5% dietary inclusion of CLA will be equal to 4.2% and 2.1% respectively.
[2] FO: diet containing 7% fish oil; CL: diet containing 7% CLA and CLFO: diet containing 3.5% CLA + 3.5% fish oil.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The liver weight of the birds fed the 7% fish oil diet was comparable to that of the CLFO treatment which was significantly less than that of the CL treatment group (P<0.01).

Table 4 shows that the diet containing 3.5% fish oil + 3.5% CLA resulted in longer villi than the two other diets (P<0.01).

Table 4 Effects of dietary fish oil, conjugated linoleic acid (CLA)[1] and their mixtures on small intestine morphology of broiler chickens

Treatments	Duodenum					Jejunum				
	Villus height (µm)	Goblet cell numbers (in 100 µm^2)	Epithelum thicknes (µm)	Crypt depth (µm)	Crypt depth to villus height ratio	Villus height (µm)	Goblet cell numbers (in 100 µm^2)	Epithelum thicknes (µm)	Crypt depth (µm)	Crypt depth to villus height ratio
FO[2]	0.155	126^b	39^a	9.5^a	816^c	0.082	142^b	46^a	9.1^a	1716^c
CL	0.150	130^b	37^a	9.6^a	863^b	0.079	141^b	41^b	9.2^a	1787^b
CLFO	0.158	141^a	33^b	6.8^b	894^a	0.083	153^a	34^c	5.6^b	1850^a
SEM	0.007	5.4	1.7	0.91	14.68	0.003	5.2	4.2	0.9	19

Treatments	Ileum				
	Villus height (µm)	Goblet cell numbers (in 100 µm^2)	Epithelum thicknes (µm)	Crypt depth (µm)	Crypt depth to villus height ratio
FO	0.120	97.8	37^a	10.16^a	811^b
CL	0.119	98.1	32^b	9.66^{ab}	824^b
CLFO	0.116	102.5	26^c	8.00^b	880^a
SEM	0.006	5.16	2.36	0.96	25.3

[1] CLA used in this experiment was CLA LUTA60 which contains 60% CLA, then 7% and 3.5% dietary inclusion of CLA will be equal to 4.2% and 2.1% respectively.
[2] FO: diet containing 7% fish oil; CL: diet containing 7% CLA and CLFO: diet containing 3.5% CLA + 3.5% fish oil.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The significant effect was observed for crypt depth in the duodenum and jejunum (P<0.01). The epithelial thickness and goblet cell numbers in the small intestinal tissue of birds fed the diet containing fish oil + CLA mixture were lower than the other two groups (P<0.01). There were no differences in the ratio of the crypt depth to villus height in any segments of the small intestine among the experimental groups. In this study, the chickens fed the diets containing 7% fish oil or 7% CLA showed a reduced daily weight gain. The lower feed intake in the chickens fed the 7% dietary fish oil as compared to the other experimental diets could be attributed to the lower palatability of the higher dietary fish oil. The unfavorable effect of CLA on body weight gain of chickens has been reported by Szymczyk *et al*. (2001) and Suksombat *et al*. (2007). However, in the majority of the previous reports dietary CLA did not adversely affect feed intake of the birds. These findings were obtained using a variety of dietary CLA rates, such as 1% (Takahashi *et al*. 2002), 1.5% (Szymczyk *et al*. 2001), 1.8% (Simon *et al*. 2000), 2 to 3 % (Du *et al*. 2003) and 4% (Sirri *et al*. 2003). In most of the previous reports dietary CLA did not affect FCR (Simon *et al*. 2000; Du and Ahn, 2002), but in the present study the CLA-containing diets improved FCR in the grower phase. This trend was reversed in the finisher phase and for the whole experimental period, which may be a result of a prolonged feeding period with CLA. It seems that the growth rate of broiler chickens was more susceptible than other performance traits, and more than 1% dietary CLA decreases birds' growth. In the study of Szymczyk *et al.* (2001), the breast and thigh percent showed different trends in response to increased dietary CLA levels, so that the breast was not altered but the thigh percentage significantly increased.

In some other reports the dietary CLA at the rates of 1.5% (Suksombat *et al*. 2007) or 4% (Sirri *et al*. 2003) did not influence the carcass, breast or thigh yields. Suksombat *et al*. (2007) showed that a reduced abdominal fat pad in birds fed CLA was not accompanied with an increase in carcass, breast or thigh percentages. These controversial results could imply that the effects of CLA on the carcass parameters of broiler chickens possibly are not a simple result of changes in abdominal fat deposition. In the present study the type of dietary fat did not affect abdominal fat pad deposition; however, there are controversial reports in this respect. Du and Ahn (2002) found that consumption of 0.5% dietary CLA by broiler chickens for a three-week experimental period increased abdominal fat pad. Nevertheless, there are reports on reducing the effects of CLA on abdominal fat (Simon *et al*. 2000; Szymczyk *et al*. 2001). The higher liver weight in broiler chickens fed CLA has been reported by previous authors too (Du and Ahn, 2004).

The results of the present study showed that 7% fish oil or CLA resulted in an adverse effect on broiler chicken performance. It seems that the dietary CLA level and mixing it with n-3 fatty acids and also the birds' age could moderate the negative effects of CLA on broiler chickens' growth rate, so that during the finisher phase (but not during the growth phase) the chickens fed the lower level of dietary CLA level showed a better weight gain that those fed a higher level of dietary CLA. One of the aims of this study was to survey any possible interaction between CLA and PUFAs. Both categories of fatty acids can alter lipid metabolism in birds. It was proposed that the combination of CLA and PUFAs in the bird's diet may enhance their performance and reduce the body fat deposition (Zanini *et al*. 2006).

The origin of this idea was the lower n-6:n-3 ratio in the diets containing the mixture of CLA and n-3 fatty acids (Aydin *et al*. 2001). Based on former reports, CLA can reduce lipogenesis in the adipose tissue, but not in the hepatic tissue (Du and Ahn, 2002). This may explain the inefficiency of CLA to reduce abdominal fat deposition in broiler chickens, because the liver is the main site of lipogenesis in birds. The longer villi in the intestine of chickens fed the diet containing the mixture of fish oil and CLA agrees well with more weight gain and feed intake in this group as compared to those fed 7% CLA or 7% fish oil diets. The epithelial thickness in the jejunum influenced by the experimental diets and thinner epithelium was observed in the groups fed the CLA + fish oil mixture. The thinner epithelium in the small intestine can facilitate absorption, increase nutrient uptake and decrease the metabolic demands of the digestive tract (Visek, 1978). The lower epithelial thickness may prevent the formation of polyamines and volatile fatty acids and increase electrolyte turnover and intestinal cell activity, so the extra energy could serve for meat production (Bedford, 2000). The crypt depth in the duodenum and jejunum sections was increased in the chickens fed the 3.5% CLA + 3.5% fish oil diet; however, the crypt depth in the ileum was not changed by the experimental diets. Crypts act as the villi production factory and deeper crypt is a sign of more active cellular turnover and higher demand for newly formed tissues (Yason *et al*. 1987).

The crypt depth to villus height ratio was not influenced by experimental diets. This criterion represents the possible digestive capacity of the small intestine and a reduction in this ratio may result in a more efficient digestion and absorption process (Montagne *et al*. 2003). With respect to the positive relationship between the villus height and performance traits, it seems that at least in this study the villus height was more correlated to chicken performance than the crypt depth to villus height ratio.

CONCLUSION

The results of this study showed that the high dosage of fish oil or CLA can reduce broiler chicken performance, but their combination can moderate this adverse effect. In other word, the dietary inclusion of 7% CLA was more toxic than 3.5% CLA and 7% fish oil was more toxic than 3.5% fish oil. If the CLA and fish oil toxicities act through different ways, then they were not additive and the 3.5% CLA + 3.5% fish oil would not be as toxic as either one at 7%.

REFERENCES

Antheony C., Nguyen J. and Griffian A. (1991). *In vitro* and *in vivo* evaluation of effects of sodium caprate on enteral peptide absorbtion and on mucosal morphology. *Int. J. Pharm*. **191,** 15-24.

Aydin R., Pariza M.W. and Cook M.E. (2001). Olive oil prevents the adverse effects of dietary conjugated linoleic acid on chick hatchability and egg quality. *J. Nutr*. **131,** 800-806.

Bedford M. (2000). Removal of antibiotic growth promoters from poultry diets: implications and strategies to minimize subsequent problems. *World's Poult. Sci. J*. **56,** 347-365.

Chashnidel Y., Moravej H., Towhidi A., Asadi F. and Zeinodini S. (2010). Influence of different levels of n-3 supplemented (fish oil) diet on performance, carcass quality and fat status in broilers. *African J. Biotechnol*. **9,** 687-691.

Du M. and Ahn D.U. (2002). Effect of dietary conjugated linoleic acid on the growth rate of live birds and on the abdominal fat content and quality of broiler meat. *Poult. Sci*. **81,** 428-433.

Du M. and Ahn D.U. (2003). Dietary CLA affects lipid metabolism in broiler chicks. *Lipids*. **38,** 505-511.

Du M. and Ahn D.U. (2004). Dietary Conjugated Linoleic Acid (CLA) Effects Lipid Metabolism in Broiler Chicks. R1934 Iowa state University. Animal Industry Report.

Farhomand P. and Checaniazar S. (2009). Effect of graded levels of dietary fish oil on the yield and fatty acid composition of breast meat in broiler chickens. *J. Appl. Poult. Res*. **18,** 508-513.

Hardin J.O., Milligan J.L. and Sidwell V.D. (1964). The influence of solvent extracted fish meal and stabilized fish oil in broiler rations on performance and on the flavor of broiler meat. *Poult. Sci*. **43,** 858-860.

Kinsella J.E., Lokesh B. and Stone R.A. (1990). Dietary n-3 polyunsaturated fatty acid and amelioration of cardiovascular disease: possible mechanisms. *J. Food Sci. Technol*. **52,** 1-28.

Knapp H.R. (1991). Effects of dietary fatty acids on blood pressure: epidemiology and biochemistry. Pp. 94-106 in Health Effects of Dietary Fatty Acids. G.J. Nelson, Ed. Am. Oil Chem. Soc., Champaign, IL.

Kruger W.F., Bradley J.W. and Pitterson V. (1971). The interaction of gentian violet and lactobacillus organisms in the diet of leghorn hens. *Poult. Sci*. **56,** 480-486.

Montagne L., Pluske J.R. and Hampson D.J. (2003). A review of interactions between dietary fiber and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. *Anim. Feed Sci. Technol*. **108,** 95-117.

Nash D.M., Hamilton R.M.G. and Hulan H.W. (1995). The effect of dietary herring meal on the omega-3 fatty acid content of plasma and egg yolk lipids of laying hens. *Canadian J. Anim. Sci*. **75,** 247-253.

Park Y. and Pariza M.W. (2006). Mechanisms of body fat modulation by conjugated linoleic acid. *Food Res. Int*. **40,** 311-323.

SAS Institute. (2002). SAS STAT User's Guide. Version 9.1. SAS Institute, Inc., Cary, NC. USA.

Simon O., Manner K., Schafer K., Sagredos A. and Eder K. (2000). Effects of conjugated linoleic acid on protein to fat proportions, fatty acids and plasma lipids in broilers. *European J. Lipid Sci. Technol*. **102,** 402-410.

Sirri F., Tallarico N., Meluzzi A. and Franchini A. (2003). Fatty acid composition and productive traits of broiler fed diets containing conjugated linoleic acid. *Poult. Sci*. **82,** 1356-1361.

Suksombat W., Boonmee T. and Lounglawan P. (2007). Effects of various levels of conjugated linoleic acid supplementation on fatty acid content and carcass composition of broilers. *Poult. Sci*. **86,** 318-324.

Szymczyk B., Pisulewski P.M., Szczurek W. and Hancazakowski P. (2001). Effects of conjugated linoleic acid on growth performance, feed conversion efficiency and subsequent carcass quality in broiler chickens. *Br. J. Nutr*. **85,** 465-473.

Takahashi K., Kawamata K., Akiba Y., Iwata T. and Kasai M. (2002). Influence of dietary conjugated linoleic acid isomers on early inflammatory responses in male broiler chickens. *Br. Poult. Sci*. **43,** 47-53.

Visek W.J. (1978). The mode of growth promotion by antibiotics. *J. Anim. Sci*. **46,** 1447-1469.

Yason C.V., Summers B.A. and Schat K.A. (1987). Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: pathology. *Am. J. Vet. Res*. **48,** 927-938.

Zanini S.F., Colnago G.L., Pessotti B.M.S., Bastos M.R., Casagrande F.P. and Lima V.R. (2006). Body fat of broiler chickens fed diets with two fat sources and conjugated linoleic acid. *Int. J. Poult. Sci*. **5,** 241-246.