

EXPRESSION OF ANTIOXIDANT ENZYME AND FATTY ACID TRANSPORT GENES UNDER CONDITIONS OF HIGH TUNA OIL AND CURCUMINOID DIETARY SUPPLEMENTATION IN SLOW-GROWING CHICKEN

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Abstract

This study investigated gene expressions of the antioxidant enzymes, superoxide dismutase, glutathione peroxidase, and catalase, and of the fatty acid transport proteins, fatty acid transport protein 4, lipoprotein lipase (LPL), and fatty acid translocase (FAT/CD36), and the relationships between the expressions of these genes in slow-growing chickens fed dietary curcuminoids combined with tuna oil. Four hundred eighty 1-day-old mixed-sex Korat chickens were randomly divided into 6 experimental groups with 4 replications per group and 20 chickens per replication. The control group was fed diets supplemented with 4% tuna oil only. The experimental groups were fed basal diets with 4% tuna oil and 200 ppm vitamin E or curcuminoids at 20, 40, 60, or 80 ppm. A completely randomized design with mixed sexes was used. At 84 days, 8 chickens were slaughtered per group. mRNAs were extracted from the breast meat and livers, and the thiobarbituric acid reactive substances (TBARS) were analyzed. Analysis of variance and correlational analysis were used to analyze significant effects and correlations, respectively. TBARS and mRNA did not significantly differ for any antioxidant enzyme genes. Conversely, the LPL and FAT/CD36 gene expressions in the livers and all transport gene expressions in the meat differed ($P < 0.00$). Moreover, correlations were found between both gene groups,

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particularly in meat. These results indicate that dietary curcuminoids may not affect antioxidant enzyme gene functions but do affect fatty acid transport genes under experimental conditions.

Keywords: Antioxidant enzyme gene, curcuminoid, fatty acid transport gene, slow-growing chicken, n-3 PUFA

Introduction

Slow-growing chickens is one solution to achieving the 1st (no poverty), 2nd (zero hunger), and 15th (life on land) sustainable development goals worldwide. Chicken meat functionality should be improved to meet the needs of consumers and to be more competitive in the broiler meat market. Knowledge of the interactions between diet and genetics can be applied to achieve this purpose.

Meat enriched with n-3 polyunsaturated fatty acids (PUFA) benefits consumer health (Bhalerao *et al.*, 2014). However, nonruminants cannot synthesize n-3 PUFA because they have no Δ -6-desaturase (Lee *et al.*, 2016). Therefore, n-3 PUFA in chicken meat can be increased via feed supplementation. From the previously study Tuna oil supplementation in chicken diet has no significant effect on Growth performance trait (Pryor; Rymer and Givens, 2006; Febel *et al.*, 2008; Farhoomand and Checaniazar, 2009; Hang *et al.*, 2018). However, enriching meat with n-3 PUFA can make it more susceptible to lipid oxidation (Cortinas *et al.*, 2005), which not only decreases n-3 PUFA content but impacts the meat quality, particularly the texture (Addis, 1986). Antioxidants can limit the oxidation rate in chickens and, subsequently, in poultry products (Leeson, 2012). Antioxidants from natural sources are usually “generally recognized as safe” (GRAS) and thus can be suitable alternatives to synthetic antioxidants (Hang *et al.*, 2018). The antioxidant properties of curcumin and its derivatives have been studied extensively (Masuda *et al.*, 1999; Barclay *et al.*, 2000). Our previous study (Hang *et al.*, 2018) found that curcuminoids from curcumin removed turmeric oleoresin and, when combined with tuna oil, exhibited antioxidant properties and enhanced fatty acid accumulation.

Theoretically, fatty acid accumulation in meat depends on antioxidant mechanisms and fatty acid transport (Kakkar *et al.*, 1995; Yadav *et al.*, 1997; Islam *et al.*, 2016; Longo *et al.*, 2016). Superoxide dismutase, glutathione peroxidase, and catalase are the main enzymes used for reducing reactive oxygen species (ROS) (Weydert and Cullen, 2010). Generally, Pro-oxidants those are chemicals can induce oxidative stress by generate ROS (Puglia and Powell, 1984). ROS can be produced in animals' bodies or can be stimulated by high-fat diets (Apel and Hirt, 2004) and can reduce n-3 PUFA by lipid oxidation (Barrera, 2012). However, antioxidants react to resist lipid oxidation and will reduce ROS to protect n-3 PUFA. These enzymes are encoded by the *SOD*, *GPX*, and *CAT* genes (Sies, 1993; Perez-Campo *et al.*, 1994; Gumulec *et al.*, 2013). The lipoprotein lipase (*LPL*), fatty acid translocase (*FAT/CD36*), and fatty acid transport protein 4 (*FATP4*) genes are involved in fatty acid transport. Huang *et al.* (2016) reported that *LPL* releases free fatty acids from lipoprotein and is correlated with intramuscular fatty acids in chicken breast and thigh. *FAT/CD36* is a membrane protein that facilitates long-chain fatty acid uptake in skeleton muscle (Guo *et al.*, 2013), and *FATP4* is important for long-chain fatty acid absorption (Gimeno, 2007).

Based on these genes' functions, we hypothesized that these genes should be impacted when chickens are fed the experimental curcuminoid diet. Thus, we investigated their expressions when chickens were fed the dietary curcuminoids combined with tuna oil in slow-growing chickens. The relationships between these genes were also elucidated. This study will provide information on the expressions of these genes and the relationships between them in slow-growing chickens. In addition,

this is the first step in identifying gene markers to aid in a selection program for improving antioxidant ability and fatty acid accumulation in slow-growing chickens. The current study continued the work of Hang *et al.* (2018).

Materials and Methods

Animal Ethics

This experiment was conducted at the Poultry Research Unit of the University Farm, Suranaree University of Technology (SUT). All procedures used in the present study were approved by the Ethics Committee on Animal Use of SUT, Nakhon Ratchasima, Thailand.

Experimental Design, Diet, and Management

Korat chickens (KR) that were crossbred between native Thai chickens, Lueng Hang Khoa (sires) and Suranaree University of Technology (SUT) chickens (dams) were used to represent slow-growing chickens in this study. Four hundred eighty 1-day-old mixed-sex KRs were raised in accordance with SUT farm guidelines. The KRs were fed a corn-soybean meal-based diet composed of 21% crude protein and 3100 kcal metabolizable energy per kilogram from 1 to 21 days of age. All diets were isonitrogenous and isocaloric diets under the temperature condition range max 32.4 and min 25.2 relative humidity 51%.

On day 21, the KRs were randomly allotted to 6 experimental diet groups in a completely randomized design. The 6 experimental diets were basal diets supplemented with 4% tuna oil [feed grade; T. C. Union Agrotech Co. Ltd., Bangkok, Thailand] and labeled as follows: 4% tuna oil only (control diet; CON), 200 ppm vitamin E [Zagro (Thailand) Co. Ltd., Pathum Thani, Thailand] (positive control; E-200), 20 ppm curcuminoids (CUR-20), 40 ppm curcuminoids (CUR-40), 60 ppm curcuminoids (CUR-60), and 80 ppm curcuminoids (CUR-80). Details of the experimental diets and nutrient compositions were presented in Hang *et al.* (2018). Each group had 4 replications with 20 chickens per replication.

The chickens were fed *ad libitum* throughout the experimental period. Automatic

water supply was used for this experiment. All experimental cage densities were 8 chickens/m². Chickens were reared on the floor in open-sided houses.

Sample Collection and RNA Extraction

Each week, the body weights (BW) and feed intake per pen were measured, and the average daily gain (ADG) and feed conversion ratio (FCR) were calculated. Any mortality was also recorded. These records were previously reported by Hang *et al.* (2018).

On day 84, eight chickens per group (4 males and 4 females) were slaughtered, and the breast meat and livers were collected, frozen in liquid nitrogen, and stored at -80°C for mRNA extraction. Total RNA from the breast and liver tissues was extracted using a High Pure RNA tissues kit (Roche, Mannheim, Germany). RNA quality was assessed using a spectrophotometer (Thermo Scientific, Nanodrop 2000, Wilmington, DE, USA). First-strand cDNA was synthesized using the transcriptor first-strand cDNA synthesis kit (Applied Biosystems, Foster City, CA, USA).

cDNA (2 µl) was mixed with 6 µL of deionized water, 10 µL of SYBR Green I Master Mix (Applied Biosystems), and 1 µL of each forward and reverse primer. The annealing temperatures of *SOD*, *CAT*, *GPX*, *FATP4*, *LPL*, and *FAT/CD36* followed the studies of Yarru *et al.* (2009), Guo *et al.* (2013), Wang *et al.* (2013), Huang *et al.* (2016) and are shown in Table 1. The mixes were used for real-time PCR (Roche480, Mannheim, Germany). Expression quantities of the genes were compared with the expression level of glyceraldehyde-3-phosphate dehydrogenase (housekeeping gene).

Thiobarbituric Acid Reactive Substance (TBARS) Measurements in Meat

Duplicate 5-g tissues samples were blended for 30 s in a blender (Waring Products, Torrington, CT, USA) with 1 mL of 0.2 mg/mL butylated hydroxytoluene and 45.5 mL of 10% trichloroacetic acid in 0.2 M phosphoric acid. The homogenate was filtered through Whatman No. 1 filter paper, and the filtrate was collected in a flask. Two 5-mL aliquots of filtrate were collected from each flask and placed in glass

Table 1. Forward and reverse primer information and annealing temperatures of SOD = superoxide dismutase; CAT = catalase; GPX = glutathione peroxidase; FATP4 = fatty acid transport protein 4; LPL = lipoprotein lipase; FAT/CD36 = fatty acid translocase

gene	Primer forward primer and reverse primer	Product length	Annealing temperatures (°C)	Reference
<i>FATP4</i>	F: 5'-CATCACCATTCTCCAACTCCAG-3' R: 5'-GACTCAGGGCTTCTCCT-3'	274	60	Yan Wang. (2012)
<i>LPL</i>	F: 5'-AGGAGAAGAGGCAGCAATA- 3' R: 5'-AGCCAGCAGCAGATAAG- 3'	127	59	Guo J. (2013)
<i>FAT/CD36</i>	F: 5'-TGCAAAGCAGGGAGGGTGAAA- 3' R: 5'-GGTGCAAAGGCCACAAAGAG- 3'	112	60	Huang Y.N (2015)
<i>SOD1</i>	F: 5'-AGGGGGTCATCCACTTCC- 3' R: 5'-CCCATTGTGTGTCTCTCCAA- 3'	122	57	Yarru (2009)
<i>CAT</i>	F: 5'-GGGGAGCTGTTTACTGCAAG- 3' R: 5'-TTTCCATTGGCTATGGCATT- 3'	139	56	Yarru (2009)
<i>GPX</i>	F: 5'-TTGTAAACATCAGGGGCAAA- 3' R: 5'-TGGGCCAAGATCTTTCTGTAA- 3'	140	55	Yarru (2009)

test tubes. Next, 5 mL of 0.02 M thiobarbituric acid (TBA) was added to 1 tube, and 5 mL of deionized water was added to the other tube to be used as a blank. Samples, blanks, and standards were read at 530 nm using a Bio-Rad Benchmark Plus® Microplate Spectrophotometer (Leick *et al.*, 2010).

Statistical Analysis

Significant effects of the 6 experimental diets on the meat TBARS and the mRNA of all genes were analyzed by analysis of variance, and Tukey's range test was used to compare

means. Pearson's correlation coefficients were evaluated to determine the correlations between gene expression and TBARS and among the gene expressions. Differences between the treatment means and correlations between the treatments were considered significant at $P < 0.05$.

Results and Discussion

Growth Performance

Growth performance data, including ADG, (feed intake) FI, and FCR, were reported and discussed previously (Hang *et al.*, 2018). The results showed that the dietary treatments did not affect the final BW (BW at 84 days: 1.75-1.80 kg, SE = 0.05), ADG (23.48-24.23 g/b/d, SE = 0.84), and FCR (2.84-2.94, SE = 0.08) of the chickens compared with the controls ($P > 0.05$).

Effect of The Supplemented Diets on SOD, CAT, and GPX Expressions

SOD, *CAT*, and *GPX* gene expressions did not significantly differ in the meat or livers when the chickens were fed different diets; however, *GPX* expression in the liver and *CAT* expression in the meat differed between the diets ($P = 0.07$ and 0.06 , respectively) (Table 2). Moreover, TBARS did not differ between the diets ($P > 0.05$, Figure 1).

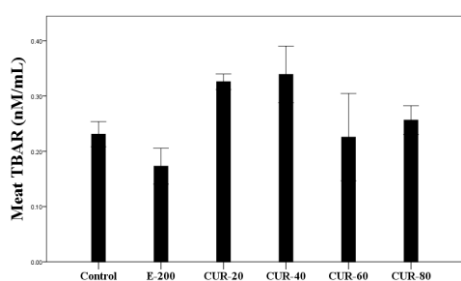


Figure 1. Meat TBARS (Mean ± SEM; n = 4/treatment; 2 samples/replicate) of chickens.
¹/CON= basal diet with 4% tuna oil; E-200 = basal diet with vitamin E 200 mg/kg; CUR-20 = basal diet with curcuminoid 20 ppm; CUR-40 = basal diet with curcuminoid 40 ppm; CUR-60 = basal diet with curcuminoid 60 ppm; CUR-80 = basal diet with curcuminoid 80 ppm

Table 2. Effect of curcuminoids combined with tuna oil on the expression of antioxidant enzyme and fatty acid transport genes

Item	¹ CON	¹ E-200	¹ CUR-20	¹ CUR-40	¹ CUR-60	¹ CUR-80	SEM	P-value
Liver								
<i>SOD</i>	1.08	0.72	0.83	0.61	0.66	0.85	0.066	0.37
<i>CAT</i>	1.22	0.76	0.88	1.14	1.31	1.60	0.131	0.53
<i>GPX</i>	1.10	1.09	0.74	0.34	1.12	0.76	0.091	0.07
<i>FATP4</i>	1.07	1.13	1.41	0.85	1.45	1.37	0.102	0.07
<i>LPL</i>	1.01 ^d	3.07 ^{ab}	4.84 ^a	4.49 ^{ab}	2.64 ^{cd}	3.03 ^{bc}	0.376	0.00
<i>FAT/CD36</i>	1.03 ^d	2.03 ^{bc}	3.77 ^a	2.84 ^b	1.04 ^d	1.28 ^c	0.177	0.00
Meat								
<i>SOD</i>	1.02	0.72	0.88	1.08	1.42	1.01	0.077	0.17
<i>CAT</i>	1.06	0.80	1.47	1.22	2.02	1.05	0.126	0.06
<i>GPX</i>	1.04	0.64	0.67	1.06	0.90	1.01	0.062	0.16
<i>FATP4</i>	1.10 ^d	1.54 ^{bc}	1.92 ^a	1.42 ^{bd}	1.98 ^a	1.86 ^{bc}	0.102	0.00
<i>LPL</i>	1.00 ^c	0.95 ^c	1.25 ^b	0.94 ^c	1.65 ^a	1.35 ^b	0.044	0.00
<i>FAT/CD36</i>	1.01 ^c	0.46 ^d	1.19 ^c	1.67 ^b	0.65 ^d	2.13 ^a	0.068	0.00

¹CON= basal diet with 4% tuna oil; E-200 = basal diet with vitamin E 200 mg/kg; CUR-20 = basal diet with curcuminoid 20 ppm; CUR-40 = basal diet with Curcuminoid 40 ppm; CUR-60 = basal diet with curcuminoid 60 ppm; CUR-80 = basal diet with curcuminoid 80 ppm; *SOD* = superoxide dismutase; *CAT* = catalase; *GPX* = glutathione peroxidase; *FATP4* = fatty acid transport protein 4; *LPL* = lipoprotein lipase; *FAT/CD36* = fatty acid translocase ^{a, b, c, d} Means in the same row with no common superscripts differ significantly ($P < 0.05$)

The rationale for this study was that feeding chickens high-fat diets can cause oxidative stress, which induces ROS. Administering curcuminoids can stimulate antioxidant enzyme gene expression, thus reducing ROS. However, no significant differences were found in the expressions of these genes or TBARS. El-Bahr (2015) studied the effects of curcumin in diabetic rats and found that administering curcumin increased antioxidant enzyme gene expression. Moreover, Yarru *et al.* (2009) found significant differences in antioxidant enzyme gene expression in chickens fed aflatoxin B1 with 4 levels of turmeric powder. These studies demonstrated that curcumin can stimulate antioxidant enzyme genes. These investigations led us to speculate that the insignificances found in our study may have been because the level of tuna oil supplementation for all experimental groups was the same and may not have been high enough to stimulate oxidative stress or induce high ROS.

Another possibility is that the genetics of slow-growing chickens may be more resistant to oxidative stress than those of commercial

broilers; therefore, under the current experimental conditions, oxidative stress may not have been induced. This idea is supported by the work of Nain *et al.* (2008), who reported that fast-growing commercial broilers in an experimental model were associated with more oxidative stress than were slow-growing chickens. Therefore, the level of tuna oil used here, which was supplemented at 4%, may have been insufficient to induce oxidative stress or high ROS levels in the slow-growing chickens.

Effect of the Supplemented Diets on *FATP4*, *LPL*, and *FAT/CD36* Expression

The results showed that supplementation with different curcuminoid levels affected the expressions of genes involved in fatty acid transport (*FATP4*, *LPL*, and *FAT/CD36*). Significant differences in these gene expressions were found in both the liver and meat tissues (Table 2).

In the liver tissues, only *FATP4* expression did not differ among the diets ($P = 0.07$), while *LPL* and *FAT/CD36* expression differed greatly ($P < 0.00$). In the meat tissues, the mRNA expressions of all genes differed

significantly ($P < 0.00$) among all curcuminoid supplementation levels. The results showed that the diets supplemented with E-200, the positive control, CUR-20, and CUR-40 yielded higher expressions of *LPL* and *FAT/CD36* than did the control group (CON) and the diets supplemented with CUR-60 and CUR-80 in the liver. In the meat, the expressions of *FATP4*, *LPL*, and *FAT/CD36* increased as the curcuminoid level increased. The gene expression results differed significantly with the levels of C18:2n-6 (linoleic acid) and total n-6 PUFA accumulation in the meat, which were previously reported by (Hang *et al.*, 2018). These results revealed that vitamin E and curcuminoids exhibited *LPL* and *FAT/CD36* expression in the meat and liver tissues.

Regarding the *LPL* gene, Kim and He (2013) found that curcuminoids can influence AMPK (adenosine monophosphate kinase), and increased AMPK levels affect *PPAR* gene expression since AMPK is a *PPAR* transcription factor. ApoC-II is cofactor of *LPL* function, which can be inhibited by *PPAR* (Gbaguidi *et al.*, 2002). Furthermore, Ricquier and Bouillaud (2000) revealed that *PPAR* function not only acts on ApoC-II, a cofactor of *LPL*, but also inhibits uncoupling protein activity involving

fatty acid uptake into cells (Schrauwen and Hesselink, 2004; Goldberg *et al.*, 2009). These studies may explain why *LPL*, *FAT/CD36*, and *FATP4* gene expressions differed significantly when chickens were fed diets supplemented with different curcuminoid levels. Kong *et al.* (2012) found no differences in *FATP4* expression in the liver ($P = 0.07$), which was lower than that in the muscle and may explain the insignificant differences found in the liver.

In addition, because the transport gene expression levels in the liver and meat tissues as well as the accumulation of fatty acids in meat monitored by Hang *et al.* (2018) were inconsistent, the current results may imply that the suitable level of curcuminoid supplementation in the diet remains unclear. Moreover, the positive or negative feedback mechanisms of the *FATP4*, *LPL*, and *FAT/CD36* genes on fatty acid accumulation in the meat when the chickens were fed the supplemented diet remain unclear.

The study's main question, however, was whether the genes can serve as gene markers to improve the antioxidant and fatty acid transport genes in slow-growing chickens. The results presented here suggest that *FATP4*, *LPL*, and *FAT/CD36* may be potential gene

Table 3. Gene expression correlation of antioxidant enzyme genes and fatty acid transport genes in liver

Item		¹ /CON		¹ /E-200		¹ /CUR-20		¹ /CUR-40		¹ /CUR-60		¹ /CUR-80	
		R ²	P-value	R ²	P-value	R ²	P-value	R ²	P-value	R ²	P-value	R ²	P-value
SOD	CAT	0.360	0.640	0.310	0.690	-0.406	0.594	0.335	0.665	0.497	0.503	0.545	0.455
	GPX	-0.710	0.290	-0.412	0.588	-0.947	0.053	0.135	0.865	-0.225	0.775	0.624	0.376
	FATP4	0.968*	0.032	0.374	0.629	-0.859	0.141	0.790	0.210	-0.108	0.892	-0.913	0.087
	LPL	-0.573	0.427	0.134	0.866	-0.292	0.708	-0.746	0.254	0.402	0.598	0.274	0.735
	FAT/CD36	-0.950	0.05	0.174	0.826	0.433	0.557	-0.985*	0.015	0.732	0.268	0.375	0.625
CAT	GPX	-0.741	0.259	-0.657	0.343	0.211	0.789	0.597	0.403	0.528	0.472	0.451	0.549
	FATP4	0.346	0.654	-0.188	0.812	-0.155	0.885	0.832	0.168	-0.882	0.118	-0.599	0.401
	LPL	-0.970	0.030	0.328	0.672	0.525	0.475	0.377	0.623	0.136	0.864	0.909	0.091
	FAT/CD36	-0.193	0.807	-0.787	0.213	-0.646	0.354	-0.264	0.128	0.582	0.418	0.044	0.956
GPX	FATP4	-0.565	0.435	0.652	0.348	0.891	0.109	0.551	0.449	-0.503	0.497	-0.286	0.714
	LPL	0.809	0.191	0.459	0.541	0.414	0.586	0.279	0.721	0.436	0.564	0.502	0.498
	FAT/CD36	0.718	0.282	0.107	0.893	-0.516	0.484	-0.220	0.780	0.424	0.576	0.907	0.093
FATP4	LPL	-0.565	0.435	0.776	0.224	-0.033	0.967	-0.191	0.809	0.28	0.72	-0.222	0.778
	FAT/CD36	-0.852	0.148	-0.03	0.97	-0.071	0.929	-0.755	0.245	-0.144	0.856	0.034	0.966
	LPL	0.40	0.60	-0.652	0.348	-0.980*	0.02	0.783	0.217	0.853	0.147	0.183	0.817

¹/CON= basal diet with 4% tuna oil; E-200 = basal diet with vitamin E 200 mg/kg; CUR-20 = basal diet with curcuminoid 20 ppm; CUR-40 = basal diet with curcuminoid 40 ppm; CUR-60 = basal diet with curcuminoid 60 ppm; CUR-80 = basal diet with curcuminoid 80 ppm; SOD = superoxide dismutase; CAT = catalase; GPX = glutathione peroxidase; FATP4 = fatty acid transport protein 4; LPL = lipoprotein lipase; FAT/CD36 = fatty acid translocase

* significant difference at $P < 0.05$

Table 4. Gene expression correlation between antioxidant enzyme genes and fatty acid transport genes in the meat

Item		¹ /CON		¹ /E-200		¹ /CUR-20		¹ /CUR-40		¹ /CUR-60		¹ /CUR-80	
		R ²	P-value	R ²	P-value	R ²	P-value	R ²	P-value	R ²	P-value	R ²	P-value
SOD	CAT	0.901	0.099	0.007	0.993	0.334	0.666	-0.061	0.939	-0.473	0.527	-0.413	0.587
	GPX	0.931	0.069	0.06	0.94	0.990*	0.01	0.346	0.654	0.993**	0.007	-0.969*	0.031
	FATP4	-0.431	0.569	-0.831	0.169	0.749	0.251	0.062	0.983	-0.567	0.433	-0.913	0.087
	LPL	0.075	0.925	-0.793	0.207	-0.447	0.553	0.022	0.972	0.693	0.307	0.274	0.735
	FAT/CD36	0.179	0.821	0.381	0.691	0.528	0.472	-0.512	0.488	0.375	0.625	-0.189	0.811
CAT	GPX	0.685	0.315	-0.987*	0.013	0.212	0.788	0.63	0.37	-0.487	0.513	-0.096	0.904
	FATP4	0.00	1.00	0.075	0.925	-0.346	0.636	0.951*	0.049	0.437	0.563	-0.877	0.123
	LPL	-0.109	0.891	-0.614	0.386	-0.679	0.321	-0.959*	0.041	-0.882	0.118	0.136	0.864
	FAT/CD36	-0.189	0.811	-0.054	0.946	0.977*	0.023	-0.911	0.089	0.221	0.779	-0.020	0.980
GPX	FATP4	-0.728	0.272	-0.209	0.791	0.815	0.185	0.837	0.163	0.661	0.339	0.384	0.616
	LPL	0.136	0.864	0.556	0.444	-0.323	0.676	0.963	0.064	0.691	0.309	-0.214	0.786
FATP4	LPL	-0.288	0.712	0.598	0.402	-0.052	0.948	0.932	0.068	-0.461	0.539	0.571	0.429
	FAT/CD36	-0.866	0.134	0.187	0.813	-0.156	0.844	-0.747	0.253	0.974*	0.026	-0.053	0.947
LPL	FAT/CD36	-0.169	0.831	-0.287	0.713	-0.727	0.273	-0.51	0.49	-0.263	0.737	-0.125	0.875

¹/CON= basal diet with 4% tuna oil; E-200 = basal diet with vitamin E 200 mg/kg; CUR-20 = basal diet with curcuminoid 20 ppm; CUR-40 = basal diet with curcuminoid 40 ppm; CUR-60 = basal diet with curcuminoid 60 ppm; CUR-80 = basal diet with curcuminoid 80 ppm; SOD = superoxide dismutase; CAT = catalase; GPX = glutathione peroxidase; FATP4 = fatty acid transport protein 4; LPL = lipoprotein lipase; FAT/CD36 = fatty acid translocase

* significant difference at P<0.05

markers; however, their polymorphisms and their effects on the mentioned traits should be investigated in further studies.

Relationships Between Gene Expressions

Knowledge of gene expression relationships is of interest and will be used to design a gene marker package to aid selection programs in improving lipid oxidation resistance and fatty acid transport in chickens fed diets with high PUFA supplementation. Significant relationships were found between *SOD* and *FATP4* ($P = 0.032$), *SOD* and *FAT/CD36* ($P = 0.050$) and *CAT* and *LPL* ($P = 0.030$) in the livers of chickens fed the control diet. A relationship was also detected between *SOD* and *FAT/CD36* in CUR-40 ($P = 0.015$). These results are presented in Table 3 and show that under the experimental conditions, the antioxidant enzyme genes functioned independently of each other in the liver, but their functions are related to those of fatty acid transport genes.

In the meat tissues, significant relationships were found between *SOD* and *GPX*, *CAT* and *GPX*, *CAT* and *FATP4*, *CAT* and *LPL*, *CAT* and *FAT/CD36* in all diet groups except the control (Table 4).

These results suggest that both antioxidant enzyme genes and fatty acid transport genes work together, and curcuminoids may be a factor that affects these significant relationships, particularly in meat.

The relationship between antioxidant enzyme genes and fatty acid transport genes may be linked with UPC, a protein involved in beta oxidation of fatty acids (Gutgesell *et al.*, 2009), by converting the fatty acids to ATP (Schrauwen and Hesselink, 2004). This mechanism generates ROS (Mailloux and Harper, 2011), which induces antioxidant enzyme gene activity.

The results of this study revealed that dietary curcuminoids combined with tuna oil did not influence antioxidant enzyme gene expression but affected fatty acid transport genes in slow-growing chickens. In addition, curcuminoids may be factors in the relationship between the gene functions of antioxidant enzymes and fatty acid transporters. Therefore, investigations of gene markers to aid in improving genetic traits in slow-growing chickens under the conditions of the supplemented diet should consider the relationship between antioxidant enzyme genes and fatty acid transport genes.

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