Enhanced curcumin solubility by solid dispersion technique reduces fat accumulation in pigs

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Abstract

Lifestyle-related diseases such as diabetes mellitus, arteriosclerosis and cardiac diseases are important illness for people around the world. Therefore, there is attempt to produce quality pork with high red meat and low fat content. In this study, the solid dispersion (SD) technique was used to enhance the solubility of curcumin from Turmeric Oleoresin (TO) for use in an animal diet. The SD technique was prepared by mixing TO with carrier (Polyethylene glycol, PEG400) and adsorbent (Magnesium oxide, MgO) at the ratio of 1:1:3 (TOPM). Curcumin was determined using high performance liquid chromatography (HPLC). The result showed that solubility rate of crude curcumin was increased with carrier and adsorbent ($p<0.05$). This mixed curcumin was used to reduce fat in pig. Twenty crossbred castrated male pigs (Large White x Landrace x Duroc) with an average weight of 30±3.2 kg were randomly allotted to receive basal diet with curcumin from TOPM at 0, 0.5, 1.0, and 1.5 g/ kg of the diet. Blood samples were collected on days 0, 30, and 60. Each pig was raised to 100 kg and all pigs were slaughtered in the slaughter house. *Longissimus dorsi* (LD) muscles were collected from each pig. Lipid profiles in blood and muscle were determined. At 60 days of feeding, plasma lipid profile of pigs fed curcumin had higher high-density lipoprotein cholesterol (HDL-C), lower low-density lipoprotein cholesterol (LDL-C), and lower LDL-C: HDL-C ratio than those pigs fed no curcumin. Pigs fed curcumin at 0.5 g/kg diet reduced total cholesterol (TC), and triglycerides (TG) in LD muscles ($p<0.05$). It concluded that TO mixed with carrier and adsorbent by the SD technique can increase crude curcumin solubility. Top-dressing TOPM in pig diets tend to reduce plasma TC, TG, LDL-C, VLDL-C, and LDL-C: HDL-C ratio.

Key words: Curcumin, Fat reduction, Pigs, Solid dispersion

1. Introduction

Pork is one of the main protein source for people around the world. For last decade, feeding pigs in the view of animal production for industries has been increasingly developed. With these changes, high protein with high fat meat was produced to the market. However, much consumption oil and fat was resulted in lifestyle-related diseases such as diabetes mellitus, arteriosclerosis and cardiac diseases (Lakatta and Levy, 2003; Minamino and Komuro, 2007). Therefore, there is attempt to produce pig meat with high red meat and low fat content instead.

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Curcumin is the principle form of curcuminoid isolated from the rhizome of turmeric plant (*Curcuma longa*), and various member of the ginger family (Zingiberaceae). There is an evidence that curcumin may regulate lipid metabolism by increased $5'$AMP-activated protein kinase phosphorylation (AMPK), reduce glycerol-3-phosphate acyl transferase-1(GPAT-1), and increase carnitine palmitoyltransferase-1 (CPT-1) expression. These enzymes influence oxidation and reduce fatty acid esterification. In addition, curcumin had been shown to reduce total cholesterol (TC), triglycerides (TG) and free fatty acids in the plasma, hepatic, and body fat of high-fat-fed mice (Asma *et al*., 2009; Ejaz *et al*., 2009). Dietary supplementation of curcumin are somewhat limited because of low solubility in alkaline pH and being subject to hydrolysis when exposed to light which result in poor absorption in animals (Kochhar, 2008). Research showed that curcumin was water insoluble, had poor over-all solubility and wettability, and had poor bioavailability. The solid dispersion (SD) technique had been used to increase the solubility and absorption of poorly soluble drugs by dispersing the drug in a highly water soluble carrier in a solid state (Lefebvre *et al*., 1985). The objectives of this research were to 1) enhance curcumin solubility by mixed Turmeric Oleoresin (TO) with carrier (Polyethylene glycol 400, PEG400) and adsorbent (Magnesium oxide, MgO) at the ratio of 1:1:3 (TOPM) following the method of Pornanek and Uriyapongson (2014) and 2) determine the appropriate level of TOPM for inclusion in diets to reduce adiposity in the pig.

2. Materials and Methods

2.1 Materials

Curcumin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). TO was obtained from Government pharmaceutical organization (Thailand). PEG400, MgO, Ethyl acetate and Hydrochloric acid were purchased from chemical agent Co., Ltd. (Shanghai, China).

2.2 Solid Dispersion (SD) Preparations

Crude curcumin from Turmeric Oleoresin (TO) was mixed with carrier (ethyl acetate, PEG400), and adsorbent (MgO) at the ratio of 1:1:3 (TOPM) using SD technique following the method of Pornanek and Uriyapongson (2014). Ethyl acetate was removed from all mixed samples in hot air oven at 70°C for 30 minutes and dried under hot air oven at 40°C for 6–12 h. The samples were pulverized 0.05–0.25 mm particle size fractions using mortar and pestle. All samples were analyzed for solubility, curcumin quantity, and curcumin recovery.

2.3 Solubility of curcumin

Ten milligrams of curcumin from TO and TOPM were transferred into a 10 ml volumetric flask. The samples were dissolved in 0.1 N hydrochloric acid dissolution and water.

A magnetic stirrer (New York, ARE, Alfa Medical) with paddles rotated for 200 rpm at 5, 15, 30, 60, and 120 minutes at a temperature of 37±0.5ºC. The supernatants were filtered through a 0.2 μm pore size millipore membrane filters at the same temperature. An aliquot of 20 μl was injected into the HPLC. Curcumin quantity (before, (Cb) and after, (Ca) incubation in hot air oven) was determined by HPLC (Shimadzu, Kyoto, Japan) with photo diode array detector (SPD-M20A, Shimadzu, Kyoto, Japan) using C_{18} (250×4.6 mm, 5 μm particle size, J.T. Baker) as an analytical column. Methanol, 2% (w/v) acetic acid and acetonitrile at the ratio of 23:36:41(v/v) were used as mobile phase with 420 nm UV detection. All experiments were determined in triplicates. The quantity of curcumin was determined by using a standard curve plotted as a plot of absorbance versus concentration. The curcumin recovery after incubation was calculated by the following equation: % curcumin recovery = (Ca)/Cb) ×100

2.4 Animals and diets

Twenty crossbred castrated male pigs (Large White x Landrace x Duroc) with an average weight of 30±3.2 kg were randomly allotted to receive the basal diet containing 16 % crude protein and 3.6 % fat. Sources of curcumin from TOPM were supplemented in the pig diet at 0 (TOPM0), 0.5 (TOPM0.5), 1.0 (TOPM1), and 1.5g (TOPM1.5) curcumin/kg of diet. There were five replications (5 pigs) per each treatment. The basal diet and water were fed to the pigs at ad libitum. Blood samples were collected on days 0, 30, and 60. Lipid profile was estimated by enzymatic colorimetric method (Jung *et al.,* 1957). Pigs were raised in an individual pen until the final weight of 100 kg. At the end of feeding trial, all pigs were randomly selected to slaughter in the slaughter house. The LD muscles were collected from each pig and were determined for TC and TG (Folch *et al*., 1957).

2.5 Statistical analysis

Curcumin quantity, curcumin recovery and curcumin solubility from TOPM samples were compared using PROC TTEST. Data obtained from lipid profiles of blood samples and LD muscles were subjected to analysis of variance in a completely randomized design experiment with SAS (2001) program (SAS Institute, Inc., Cary, NC, USA). Treatment means were compared using Duncan's New Multiple Range Test. The values were displayed as least square means (±SE).

3. Results and Discussion

3.1 Quantity, recovery and solubility of curcumin

Crude curcumin samples were sticky and had black color. Added carrier and adsorbent changed crude curcumin color to light yellow powder as shown in Figure 1. Crude turmeric oleoresin contained 17 mg/100 mg curcumin content. Quantity and recovery of curcumin in TOPM were 2.7% and 81.4% (Table 1). The chromatogram of curcumin using HPLC had shown in Figure 2. Curcumin from TOPM showed higher ($p<0.05$) solubility than crude TO (Figures 3A and 3B). The SD technique increased the solubility and maximizing the surface area of curcumin. This might be due to the PEG 400 and MgO increasing active molecular carriers on the surface of curcumin. This was similar to other studies which suggested that the solubility of curcumin increased linearly because the PEG400 was highly hydrophilic (Modasiya1and Patel, 2012). The higher solubility of curcumin was also showed by excellent wettability, which could be observed clearly from the solid dispersion as it rapidly left the surface and was dispersed in the bulk of dissolution media (Tonnesen, 2002). Moreover, wetting property of the TOPM was responsible for the improved solubility enhancement (Craig, 2002; Leuner and Dressman, 2009).

Table 1 Quality of curcumin and cucurmin in recovery from Turmeric Oleoresin (TO) and TOPM.

Ratio TO:PEG400:MgO	Curcumin (mg/100mg)	Curcumin recovery (%)
1:0:0 (crude)	17±0.01[a]	99.98±0.92[a]
1:1:3 (TOPM)	2.7±0.01[b]	81.47±0.92[b]

Note: Mean in the same column with different lowercase letters differ ($p<0.05$).
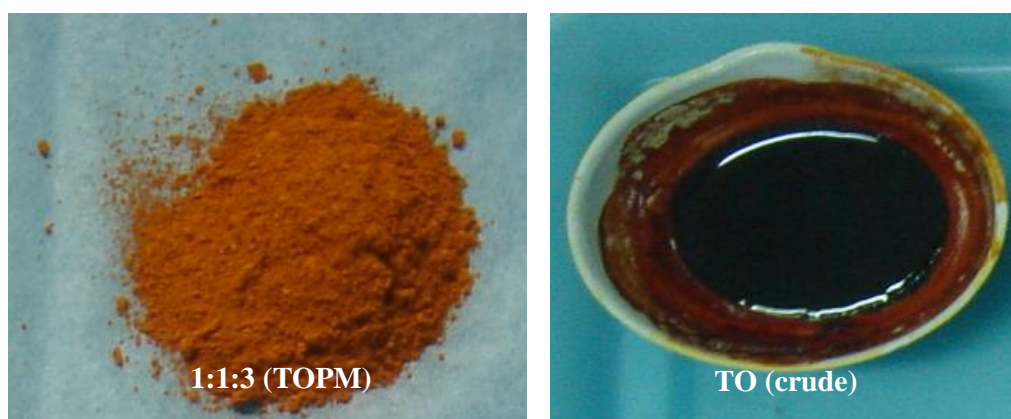

Figure 1 Characteristic of Turmeric Oleoresin (TO) and TOPM by solid dispersion (SD) technique.
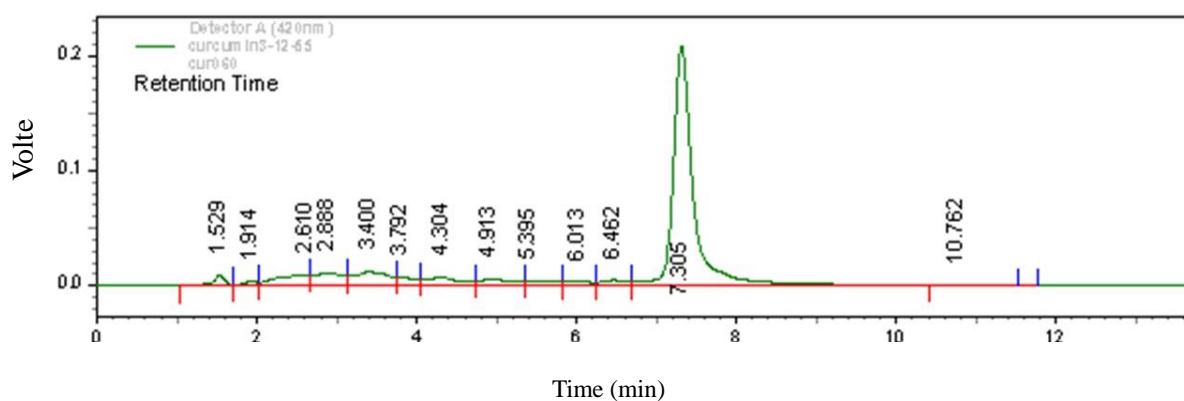
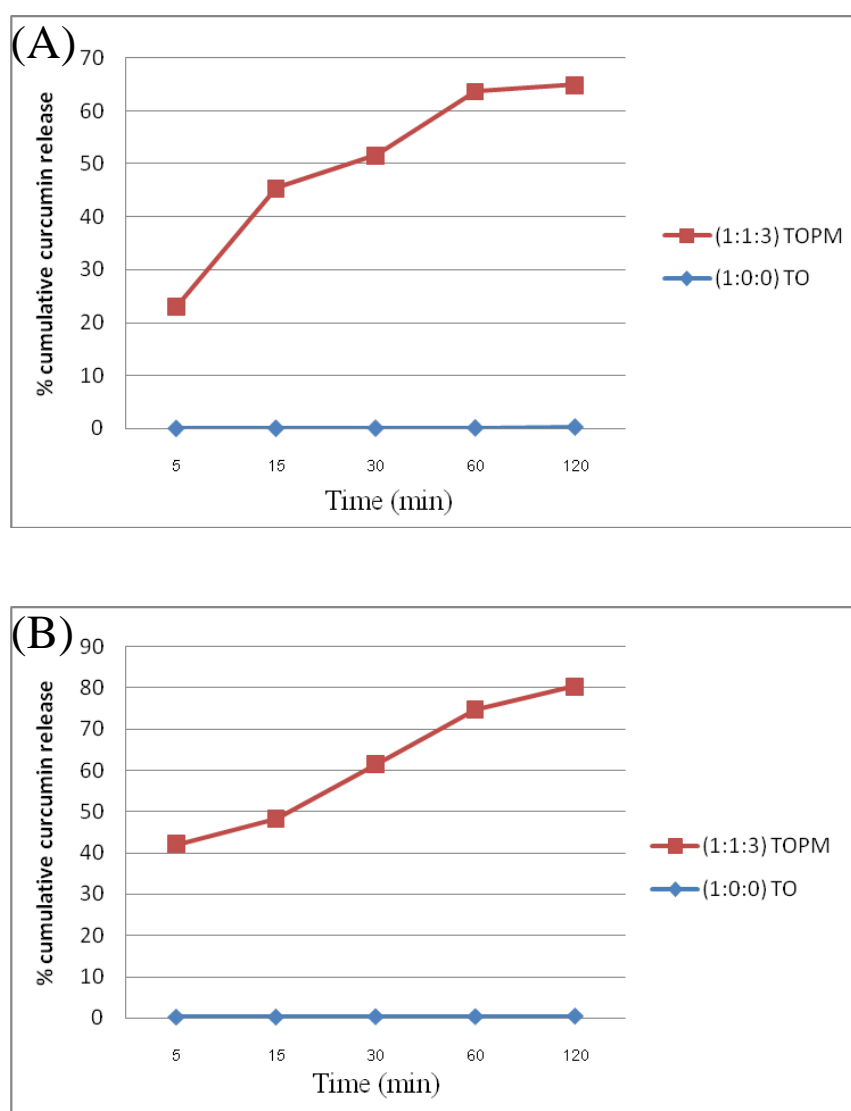**Figure 2** Chromatogram of curcumin by HPLC

Figure 3 Solubility profiles of curcumin from Turmeric Oleoresin (TO) and TOPM in water (A) and in 0.1 N hydrochloric acid solutions (B).

3.2 Lipid profiles of blood sample

Pigs fed dietary curcumin had significantly higher concentration of HDL- C, but lower concentrations of LDL-C and LDL-C: HDL-C ratio in the plasma than those pigs fed without curcumin (p<0.05) as shown in Table 2. Supplementation of curcumin tended to reduce concentrations of TC, TG, LDL-C and VLDL-C in plasma from day 0 to day 30 and day 60 of pig as shown in Figure 4. From day 0 to day 60 of feeding trial, concentrations of plasma lipid profile of pigs had higher HDL-C, but lower TC, TG, LDL-C and VLDL-C than those pigs fed curcumin from day 0 to day 30. It may be described by day 60 pig had more matured intestinal tract and absorbed more curcumin than day 30. Furthermore, the higher solubility of curcumin may increase the adsorption of curcumin to the animals. This study was similar to other studies which suggested that curcumin suppresses the hepatic enzymes HMG-CoA reductase and acyl CoA cholesteryl acyl transferase (ACAT). Fed curcumin reduced hepatic cholesterol, TC, and non-HDL-C levels (Jang *et al*., 2008) in animal. Jang *et al*. (2008) also showed the hypolipidemic effect of curcumin reduced both TG and free fatty acids in the plasma of hamsters. In another study, dietary curcumin effectively reduced hepatic TG concentration in rats (Munjunatha *et al*., 2006). In addition, curcumin significantly reduced TC, TG, and free fatty acids in the plasma, hepatic and body fat of mice (Asma *et al*., 2009). Ejaz *et al*. (2009) showed that mice fed 500 mg curcumin/ kg diet decreased adiposity, fatty liver, blood lipids, and glucose. Similarly to those reported by Kim and Kim (2010) who showed that rats fed high fat diet with curcumin reduce TC, TG, and LDL-C due to the higher cholesterol 7α-hydroxylase (CYP7A1) expression which is the key enzyme in bile acid formation. However, using curcumin at 1500 mg/ kg in the diet had less effect than lower doses (Hasan *et al*., 2014).

Table 2 Plasma lipid profiles (mg/dl) of pigs.

Items	0 day				30 days				60 days			
	TOPM 0	TOPM 0.5	TOPM 1	TOPM 1.5	TOPM 0	TOPM0.5	TOPM 1	TOPM 1.5	TOPM 0	TOPM 0.5	TOPM 1	TOPM 1.5
TC	103.5 ± 3.52^{ab}	105.50 ± 3.52^{ab}	107.00 ± 3.52^{ab}	106.75 ± 3.52^{ab}	106.00 ± 3.52^{ab}	97.66 ± 4.54^{abc}	94.66 ± 4.54^{bc}	100.66 ± 4.54^{ab}	110.25 ± 3.52^{a}	97.33 ± 4.54^{abc}	86.66 ± 4.54^{c}	97.00 ± 3.93^{abc}
TG	47.50 ± 2.48^{b}	47.25 ± 2.51^{b}	44.28 ± 2.51^{b}	43.00 ± 2.51^{b}	48.81 ± 2.80^{ab}	48.50 ± 3.97^{ab}	46.00 ± 3.97^{b}	45.33 ± 3.24^{b}	57.61 ± 2.51^{a}	39.33 ± 3.24^{b}	40.33 ± 3.24^{b}	42.00 ± 3.92^{b}
HDL	39.00 ± 1.57^{b}	39.00 ± 1.75^{b}	38.00 ± 1.75^{b}	39.00 ± 1.57^{b}	40.00 ± 1.57^{b}	38.66 ± 2.03^{b}	49.66 ± 2.03^{a}	51.25 ± 1.57^{a}	42.00 ± 1.57^{b}	49.00 ± 2.03^{a}	52.33 ± 1.75^{a}	52.00 ± 1.57^{a}
LDL	54.75 ± 4.10^{a}	52.00 ± 4.58^{ab}	51.33 ± 4.58^{ab}	53.33 ± 4.58^{a}	44.33 ± 5.29^{abc}	47.33 ± 5.29^{abc}	34.33 ± 5.29^{c}	31.33 ± 5.29^{c}	44.25 ± 4.58^{abc}	40.33 ± 5.29^{abc}	36.33 ± 5.29^{bc}	42.33 ± 5.29^{abc}
VLDL	9.50 ± 0.49^{abc}	9.45 ± 0.49^{abc}	8.857 ± 0.49^{bc}	8.60 ± 0.49^{bc}	9.76 ± 0.55^{abc}	9.70 ± 0.78^{abc}	9.20 ± 0.78^{bc}	10.40 ± 1.10^{ab}	11.52 ± 0.49^{a}	7.86 ± 0.63^{c}	8.06 ± 0.63^{c}	8.4 ± 0.78^{bc}
LDL:HDL	1.40 ± 0.14^{a}	1.33 ± 0.15^{ab}	1.35 ± 0.15^{ab}	1.36 ± 0.15^{ab}	1.15 ± 0.18^{abc}	1.37 ± 0.18^{ab}	0.68 ± 0.18^{c}	0.60 ± 0.18^{c}	1.06 ± 0.15^{abc}	0.82 ± 0.18^{bc}	0.69 ± 0.18^{c}	0.82 ± 0.18^{bc}

Note: Means in the same row with different lowercase letters differ ($P<0.05$). TOPM 0 diet with no curcumin, TOPM 0.5 diet with 0.5 g curcumin /kg diet, TOPM 1 diet with 1.0 g curcumin /kg diet, TOPM 1.5 diet with 1.5 g curcumin /kg diet. TC total cholesterol TG Triglyceride HDL high-density lipoprotein cholesterol LDL low-density lipoprotein cholesterol VLDL very low-density lipoprotein cholesterol.
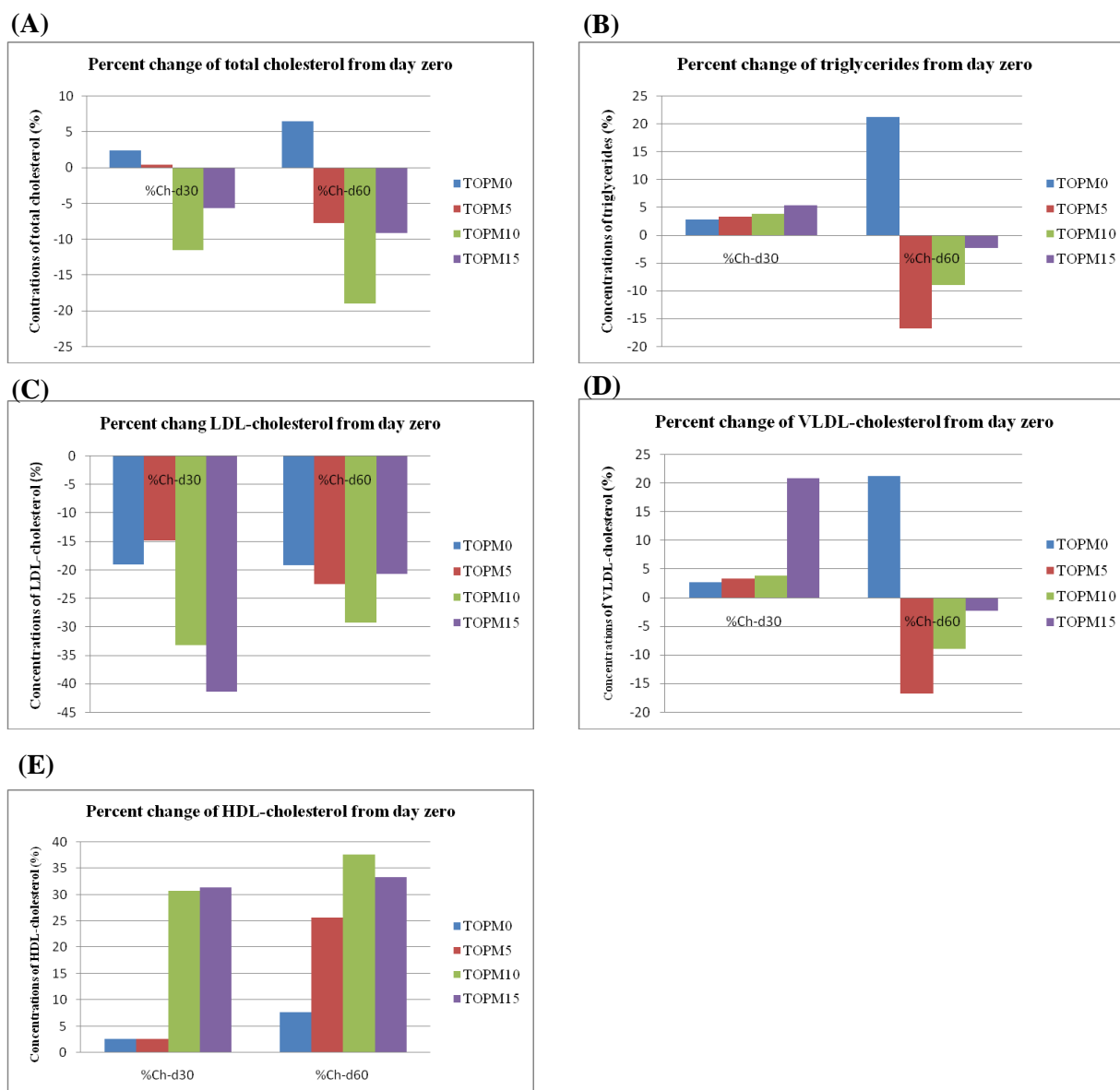
(A)

(B)

(C)

(D)

(E)

Figure 4 Percent change concentrations of total cholesterol (A), triglyceride (B), LDL-cholesterol (C), VLDL-cholesterol (D) and HDL-cholesterol (E) from day zero today 30 and day 60 in plasma of pig after curcumin supplementation. %ch-d30 = percent change of TC from day 0 to day 30, %ch-d60 = percent change of TC from day 0 to day 60.

3.3 Muscle fat content

The TC, and TG level in LD muscles were significantly reduced in pigs fed curcumin at 0.5 g/ kg compared to control ($p<0.05$). In addition, TC, and TG level in LD muscles were lowered in pig fed curcumin than non-supplemented pigs (Table 3). Smith (1994) reported that curcumin supplementation inhibited hepatic fatty acid synthase (FAS) activity and increased beta oxidation of fatty acids, leading to an effective decrease in fat storage.

Dietary curcumin reduced lipid storage in adipocytes by increase 5AMP-activated protein kinase (AMPK), acetyl CoA carboxylase activities and increase phosphorylation reaction which resulted in a suppression of the conversion of acetyl CoA to malonyl CoA. The lower levels of malonyl CoA increases carnitine palmitoyltransferase-1 (CPT-1) expression, which increases fatty acid oxidation. The phosphorylated AMPK also suppresses expression of glycerol-3-phosphate acyltransferase-1 (GPAT-1), which results in reduced fatty acid esterification (Asma *et al.*, 2009; Ejaz *et al.*, 2009; Kim *et al.*, 2011).

Table 3 Lipid profiles in pig meat supplemented with different levels of curcumin solid dispersion from TOPM.

Item	Curcumin solid dispersion from TOPM (g/kg diet)			
	TOPM 0	TOPM 0.5	TOPM 1	TOPM 1.5
Animal, head	5	5	5	5
Cholesterol, mg/100 g meat	49.54±6.16[a]	31.78±5.03[b]	33.65±5.95[ab]	34.24± 5.03[ab]
Triglyceride, g/100 g meat	2.43±0.34[a]	1.29±0.20[b]	1.61±0.20[ab]	1.77±0.20[ab]

Note: Means in the same row with different lowercase letters differ ($p<0.05$). TOPM0 diet with no curcumin, TOPM0.5 diet with 0.5 g curcumin/ kg diet, TOPM1 diet with 1 g curcumin /kg diet, TOPM1.5 diet with 1.5 g curcumin/ kg diet,

4. Conclusion

The aqueous solubility of curcumin was improved by adding TO into PEG400 and MgO at the ratios of 1:1:3 using the SD technique. The treated curcumin had a higher solubility rate than crude curcumin from TO. Supplementation of the TOPM in pig diet increased plasma HDL-C, while plasma TC, TG, LDL-C, VLDL-C and LDL-C: HDL-C ratio tended to reduce. In addition, TC, and TG in LD muscles seemed to decrease in the pig fed diet with TOPM compared to control group.

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