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# Effects of Dietary β-Cyclodextrin on Plasma Lipid and Tissue Cholesterol Content in Swine

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**ABSTRACT :** This study examined the effects of dietary β-cyclodextrin (βCD) on the cholesterol of blood and tissues of swine. Thirty six male castrated swine (Landrace×Yolkshire×Duroc) weighing 50 kg were randomly assigned to one of four dietary groups until their weight reached 110 kg. The groups were: basal diet without βCD (control) and basal diets containing 1.5%, 3.0%, or 5.0% βCD. Diets and water were offered *ad libitum*. No significant difference was found between treatments in terms of feeding performance measured by daily intake, daily weight gain, and feed efficiency. Addition of βCD to the diets significantly reduced total lipid, triglyceride and total cholesterol levels in swine blood, particularly in the group receiving 5.0% βCD, which showed decreases ($p<0.05$) of 21.9%, 55.6% and 27.7%, respectively. Cholesterol levels in back fat, loin, belly and ham portions of swine fed βCD significantly differed ($p<0.05$) from controls, especially in the 5.0% βCD-fed group, with reductions of 26.0%, 27.5%, 17.9% and 18.3%, respectively. These results suggested that the addition of βCD to the diet of swine could reduce their body cholesterol by decreasing the migration of cholesterol through the blood. (**Key Words :** Swine, β-Cyclodextrin, Plasma Lipid, Triacylglyceride, Pork Cholesterol)

## INTRODUCTION

There are many reports that frequent and abundant intake of high cholesterol food may result in high blood cholesterol, especially low density cholesterol (LDL-C) (Leaf et al., 1988). Increasing blood LDL-C results in an increased death rate due to cardiovascular disease (arteriosclerosis, cardiosclerosis). Pork has more cholesterol (95-100 mg/100 g) than other meat, grains, and fish (Stucchi et al., 1995; Dorado et al., 1999; Bragagnolo and Rodriguez, 2002), which has motivated research to reduce the amount of cholesterol in animal food sources. Recently, a hypocholesterolemic effect of βCD has been reported in rats and hamsters. βCD is a circular carbohydrate containing 7 glucopyranose units linked in the α1-4 position (cycloheptaamylose) which is synthesized by the action of cycloglycosyl transferase (E.C.2.4.1.19) on amylomaize starch (Saenger, 1984). X-ray diffraction studies reveal it to be a water-soluble cone-shaped molecule fitted with a hydrophobic cavity. As a result, βCD forms inclusion complexes with hydrophobic molecules such as cholesterol, resulting in dissoluble molecules (Horikoshi, 1979; Oakenfull et al., 1991; Abadie et al., 1994). Its chemical stability and ability to easily solubilize cholesterol make βCD a potentially effective external adsorbent to remove cholesterol from foods (Nagatomo, 1985; Okenfull et al., 1991; Yen and Chen, 2000).

βCD has been used to make hypocholesterolemic food products (≥85%), by it's adsorption of cholesterols followed by centrifugal separation of the complex, in egg yolks, cream (Okenfull et al., 1991), milk (Hwang et al., 2005; Kwak et al., 2001) and lard (Yen and Chen, 2000). This kind of technology is a useful tool for the removal of cholesterol from food products, but it is limited in its applicability to reduce cholesterol in pork. In the animal body, the inner surface of βCD is highly hydrophobic and able to bind molecules such as cholesterol, thereby making a strongly dissoluble material and inhibiting adsorption of the sterol in the body (Froming et al., 1993). It is well known that βCD is nontoxic, and its absorption by the small intestine of human and animals is minimal with partial degradation by the microflora in the hindgut (Olivier et al., 1991).

The hypocholesterolemic and low triglyceride lipid effect of βCD has been recently reported in hamsters (Ritott et al., 1993), rat (Olivier et al., 1991; Park, 2003; Kim et al., 2006), and swine (Ferezou et al., 1997). Park et

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**Table 1.** Ingredients and nutrient composition of the experimental diets

| Ingredients (%) | β-Cyclodextrin | | | |
|---|---|---|---|---|
| | Control | 1.5% | 3.0% | 5.0% |
| Yellow corn (ground) | 33.00 | 34.50 | 35.00 | 33.00 |
| Wheat (ground) | 37.00 | 34.00 | 32.00 | 32.00 |
| Wheat bran | 5.50 | 5.50 | 5.50 | 5.50 |
| Soybean oil meal | 15.20 | 15.20 | 15.20 | 15.20 |
| β-Cyclodextrin[1] | - | 1.50 | 3.00 | 5.00 |
| Limestone flour | 1.00 | 1.00 | 1.00 | 1.00 |
| Dicalcium phosphate | 0.50 | 0.50 | 0.50 | 0.50 |
| Common salt | 0.30 | 0.30 | 0.30 | 0.30 |
| Molasses | 1.50 | 1.50 | 1.50 | 1.50 |
| Tallow | 5.00 | 5.00 | 5.00 | 5.00 |
| Vitamin-min. mix[2] | 0.60 | 0.60 | 0.60 | 0.60 |
| Choline chloride | 0.10 | 0.10 | 0.10 | 0.10 |
| L-lysine | 0.30 | 0.30 | 0.30 | 0.30 |
| Total | 100 | 100 | 100 | 100 |
| Calculated nutrient content | | | | |
| Crude protein (%) | 15.00 | 15.00 | 15.00 | 15.00 |
| DE (kcal/kg)[3] | 3,400 | 3,400 | 3,400 | 3,400 |

[1] Purity 99.4%, Cavamax® w7, Wacker, USA.
[2] Contained per kg mixture: vitamin A, 5,500 IU; vitamin D3, 550 IU; vitamin E, 15 IU; riboflavin, 5 mg; pantothenic acid, 10 mg; niacin, 40 mg; vitamin B12, 0.01 mg; folic acid, 0.9 mg; biotin, 0.05 mg; pyridoxine, 3 mg; menadione sodium bisulfate, 3 mg; thiamin, 3 mg; iodine, 1 mg; manganese, 60 mg; zinc, 40 mg; copper, 4 mg; cobalt, 100 mg; iron, 40 mg; selenium, 0.09 mg.
[3] Digestible energy.

al. (2005) also reported that when laying hens were fed βCD, the amount of cholesterol in their eggs was reduced by more than 25%. The hydrophobic base of the inner core of βCD maintains a high affinity for cholesterol *in vivo*. In other words, βCD inhibits the absorption of lipids, stimulates binding of bile acids or neutral sterols in the gut, and increases fecal steroid excretion (Olivier et al., 1991; Ritott et al., 1993; Férézou et al., 1997; Park, 2003). Although many studies have been conducted on βCD, little information has been reported on the effect of a βCD-containing diet on cholesterol content in swine. Accordingly, the aim of this study was to determine the hypocholesterolemic effect of a βCD-enriched diet on pork. Swine growth performance, blood lipids, and the cholesterol content of each portion of meat were examined.

## MATERIALS AND METHODS

### Animals and experimental design

Thirty six male castrated swine (Progeny of Landrace ×Yolkshire×Duroc) weighing 50 kg were maintained for 9 weeks until they reached a weight of 110 kg. They were randomly assigned to one of the following four dietary groups: no βCD (Control), 1.5%, 3.0%, and 5.0% βCD.

### Diets and feeding

Experimental diets were prepared by mixing grains such as corn, wheat meal, and soybean meal. All experimental diets met (or marginally exceeded) nutrient requirements recommended by the National Research Council (1998). The βCD (Cavamax® w7, Wacker, USA) was highly pure (99.4% or higher). The loss of total feed weight with increasing additions of βCD was compensated by reducing the amount of ground wheat and corn, so that each group received almost the same level of crude protein and energy. The ingredient and nutrient compositions of the experimental diets are shown in Table 1. Prepared diets were stored in a cold place (4°C), and food and water were available *ad libitum*. All scientific procedures, including those on animals, were conducted according to the guidance for scientific and ethical procedures provided in the European Experimental Animal Handling Licence (SCT-w94058)

### Animal performance and plasma

Growth performances such as feed intake, weight gain, and feed efficiency were assessed every 3 weeks (3, 6, 9 weeks) until the end of the trial. Ten milliliters of blood were collected from the ear vein of the swine every 3 weeks into heparinized tubes (Franklin lakes, NJ07417, USA) using a 25-G syringe. Plasma was immediately separated from the blood by centrifugation at 4°C (20 min., 2,200×g) and then aliquoted for further analysis. Separated plasma was rapidly frozen in a LN2 tank (-196°C) and then stored at -78°C for further biochemical analysis.

### Lipid, triglyceride and cholesterol content of blood

Total blood lipid content was extracted with a chloroform/methanol (2:1; v/v) mixture (Folch et al., 1957). Triglyceride and cholesterol contents were determined

**Table 2.** Feed intake, weight gain, and feed efficiency for swine fed different levels of β-Cyclodextrin

| Weeks | β-Cyclodextrin | | | | PSE[1] |
|---|---|---|---|---|---|
| | Control | 1.5% | 3.0% | 5.0% | |
| Feed intake (kg/day) | | | | | |
| 0-3 | 2.04 | 2.14 | 2.17 | 2.15 | 0.02 |
| 3-6 | 2.27 | 2.46 | 2.45 | 2.46 | 0.02 |
| 6-9 | 3.28 | 3.30 | 3.48 | 3.32 | 0.03 |
| 0-9 | 2.53 | 2.64 | 2.69 | 2.64 | 0.02 |
| Weight gain (kg/day) | | | | | |
| 0-3 | 0.99 | 0.83 | 0.93 | 0.57 | 0.09 |
| 3-6 | 0.66 | 0.62 | 0.57 | 0.52 | 0.02 |
| 6-9 | 0.97 | 0.93 | 1.00 | 1.10 | 0.04 |
| 0-9 | 0.90 | 0.80 | 0.84 | 0.81 | 0.09 |
| FE[2] | | | | | |
| 0-3 | 2.06 | 2.58 | 2.33 | 3.73 | 0.63 |
| 3-6 | 3.43 | 3.96 | 4.29 | 4.73 | 0.23 |
| 6-9 | 3.38 | 3.54 | 3.48 | 3.01 | 0.08 |
| 0-9 | 2.81 | 3.30 | 3.20 | 3.25 | 0.15 |

[1] Pooled standard error.
[2] Feed efficiency = feed intake/weight gain.

using a commercial enzyme kit (Sigma, Co, USA) and assayed according to the manufacturer's manual.

**Cholesterol content of pork meat**

Cholesterol content was assayed with the Mannheim (1987) method using a commercial analysis kit (Cat. No. 139.5, Boehringer Mannheim, Germany) following the manufacturer's method. An accurately weighed 2.5 g sample of pork meat was placed into a 50-ml round-bottomed flask, after which 1 g of sea sand, 20 ml of freshly prepared methanolic potassium hydroxide solution (1.0 M) and 10 ml isopropanol were added. The mixture was heated under a reflux condenser for 30 min and then allowed to cool. The supernatant solution was transferred into a 25 ml volumetric flask with a pipette and the residue was boiled twice with 6 ml isopropanol, and then heated under a reflex condenser for 5 min. Solutions were collected in the volumetric flask and allowed to cool. Contents of the volumetric flask were diluted up to the mark with isopropanol and mixed. After filtration through Whatman No. 2 filter paper, 4 ml of the clear solution was used for the assay. The solution was mixed thoroughly with 5.0 ml of the cholesterol reagent mixture provided, which contained ammonium phosphate buffer, catalase, acetylacetone, stabilizers, and cholesterol oxidase. Twenty microliters of cholesterol oxidase was added to 2.5 ml of the mixture and incubated at 37°C for 60 min, then allowed to cool to room temperature. Absorbance of a sample blank followed by the sample in the same cuvette was read using a spectrophotometer (Shimadzu, UV-1201, Japan). Cholesterol content was calculated from the amount weighed as follows:

$$Content_{cholesterol} = 0.711 \times \Delta A / weight_{sample} \times 100 \text{ (g/100 g)}$$

ΔA = Absorbance of the sample-absorbance of the blank

To confirm the accuracy of this test, standard cholesterol was assayed by the same method, with a yield of 90.25%. Table 4 shows the data compensated by the yield ratio.

**Statistical analysis**

Analysis of variance was conducted on the experimental data using the GLM procedure of SAS, and the statistical significance of differences between mean values was tested ($p<0.05$) by Duncan's multiple range test (SAS, 2000).

## RESULTS AND DISCUSSION

The effects of dietary βCD on feed intake, body weight gain, and feed efficiency are shown in Table 2. All the animals remained in good health throughout the experiment. There were no significant differences in feed intake, body weight gain, or feed efficiency between treatments**.** Average feed intake was 2.62 kg/day/head. The mean body weight gain of swine receiving the control diet was 0.90 kg/day, which was a little higher than the average weight gain (0.82 kg/day) of the βCD treatment groups. Also, the average feed efficiency of the control diet group was lower than the βCD treatment groups, although the difference was not significant. This result suggested that feeding swine βCD (up to 5.0%) in the diet does not affect the growth and development of normal animals.

There have been controversial studies about the metabolic effect of β CD. Flourié et al. (1993) reported that βCD is not degraded and absorbed by the small intestine, but that microbes in the large intestine can degrade it. However, Riottot et al. (1993) found that increasing the feeding level of βCD did not affect feed intake, feed efficiency, or weight loss of rat (Olivier et al., 1991; Park, 2003). Also, Ferezou et al. (1997) proposed that, when orally administered, βCD is not toxic or genotoxic to rodents, even at high doses (up to 20% of the diet). We were concerned that βCD levels higher than in our current experiment may cause metabolic disorders or affect feeding performance. Further research will be needed.

Changes in the blood lipid and cholesterol contents of swine are shown in Table 3 and Figure 1. Total blood lipid content decreased up to the end of the experiment ($p<0.05$). The average blood lipid content on day 0 was 408.4 mg/100 ml (data not shown). At week 9, the change in blood lipids of swine fed a control diet, 1.5, 3.0, or 5.0 βCD was 403.6, 364.6, 346.2 and, 323.4 mg/100 ml, respectively. The relationship was dose-dependent and statistically significant ($p<0.05$). Compared to the control group, blood lipid content in the 5.0, 3.0 and 1.5% βCD treated groups decreased 19.9, 14.2 and 9.7%, respectively ($p<0.05$). Total

**Table 3.** Changes in total lipid, triacylglyceride, and total cholesterol levels in plasma of swine fed different levels of β-Cyclodextrin

| Item | Weeks | β-Cyclodextrin | | | | PSE[1] |
|---|---|---|---|---|---|---|
| | | Control | 1.5% | 3.0% | 5.0% | |
| Total lipid (mg/dl) | 0-3 | 391.59 | 383.38 | 382.77 | 340.09 | 9.70 |
| | 3-6 | 392.32$^{a}$ | 351.09$^{b}$ | 335.76$^{bc}$ | 327.15$^{c}$ | 8.13 |
| | 6-9 | 403.58$^{a}$ | 364.58$^{b}$ | 346.22$^{c}$ | 323.42$^{d}$ | 9.16 |
| Triacylglyceride (mg/dl) | 0-3 | 77.01$^{a}$ | 73.42$^{a}$ | 68.58$^{a}$ | 46.66$^{b}$ | 3.74 |
| | 3-6 | 85.75$^{a}$ | 79.85$^{ab}$ | 69.11$^{b}$ | 50.24$^{c}$ | 4.37 |
| | 6-9 | 111.36$^{a}$ | 81.89$^{b}$ | 54.22$^{c}$ | 49.47$^{c}$ | 7.53 |
| Total cholesterol (mg/dl) | 0-3 | 92.04$^{a}$ | 91.16$^{a}$ | 88.03$^{b}$ | 88.04$^{b}$ | 0.63 |
| | 3-6 | 100.85$^{a}$ | 86.54$^{b}$ | 86.49$^{b}$ | 83.47$^{b}$ | 2.42 |
| | 6-9 | 90.08$^{a}$ | 77.62$^{b}$ | 74.36$^{b}$ | 66.14$^{c}$ | 2.81 |

[1] Pooled standard error.
[a, b, c] Mean values within a row with unlike superscript letters were significantly different ($p<0.05$).

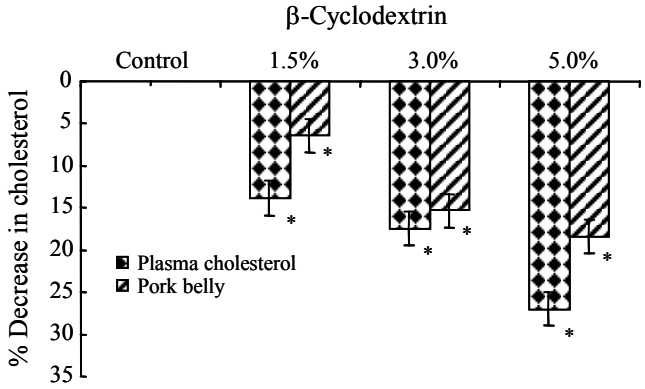


**Figure 1.** Percent decrease in plasma and pork belly cholesterol from swine fed different levels of β-Cyclodextrin. Bars represent standard error of mean values. * Significantly different than the control group ($p<0.05$).

**Table 4.** Cholesterol content in pork back fat, loin, belly and ham portions from swine fed different levels of β-Cyclodextrin (mg/100 g)

| Item | β-Cyclodextrin | | | | PSE[1] |
|---|---|---|---|---|---|
| | Control | 1.5% | 3.0% | 5.0% | |
| Back fat | 73.91$^{a}$ | 72.63$^{a}$ | 61.02$^{b}$ | 54.69$^{c}$ | 2.47 |
| | (0) | (1.73)[2] | (17.44) | (26.00) | |
| Loin | 70.52$^{a}$ | 63.77$^{b}$ | 65.92$^{c}$ | 51.07$^{d}$ | 2.19 |
| | (0) | (9.57) | (6.52) | (27.58) | |
| Belly | 82.70$^{a}$ | 77.37$^{b}$ | 70.00$^{c}$ | 67.47$^{c}$ | 1.93 |
| | (0) | (6.44) | (15.36) | (18.42) | |
| Ham | 65.38$^{a}$ | 61.42$^{b}$ | 59.50$^{b}$ | 53.46$^{c}$ | 1.33 |
| | (0) | (6.06) | (8.99) | (18.23) | |

[1] Pooled standard error.
[2] Percent rate of reduction against control group.
[a, b, c, d] Mean values within a row with unlike superscript letters were significantly different ($p<0.05$).

blood triglyceride content of the control group increased during the experiment, while that of βCD-fed groups decreased.

In week 9, the highest total blood lipid was in the control group (111.4 mg/100 ml), while groups receiving 1.5, 3.0, or 5.0% βCD had a total blood lipid content of 81.9, 54.2 and 49.5 mg/100 ml, respectively ($p<0.05$). Total blood lipid decreased by 26.5, 51.3, and 55.6%, respectively. Compared to the control group, the decrease was significantly dose-related ($p<0.05$). There was a small elevation of total cholesterol content in the control group at 6 weeks, but the level at 9 weeks was similar to that on day 0 (data not shown), while groups receiving βCD showed a marked decrease over the experimental period.

Total blood cholesterol content in the control group was 90.1 mg/100 ml, while concentrations in the βCD-fed groups at week 9 were 77.6, 74.4, and 66.1 mg/100 ml in the respective dose groups ($p<0.05$). Compared to the control group, the highest decrease in cholesterol content was 26.6% in the 5.0% βCD treatment group, compared to 17.5 and 13.8% in the 3.0 and 1.5% βCD groups, respectively. This decrease was significantly dose-related ($p<0.05$). There have been many reports that βCD-treated diets induce marked decreases in blood lipid levels in mice (Suzuki and Sato, 1985; Friilink et al., 1991), hamsters, (Riottot et al., 1993) and pigs (Juste et al., 1997; Ferezou et al., 1997; Catala et al., 2000). Generally, βCD is poorly digested in the intestines of humans and mice, and strongly binds to lipids in the body, making dissoluble material. This mechanism stimulates an increase in the fecal output of lipids (Grundy et al., 1965; Riottot et al., 1993). Thus, this mechanism eventually results in low lipid content of the blood.

βCD inhibits absorption of triglycerides and cholesterol and increases bile acid synthesis and secretion, leading to lower cholesterol content in blood (Hostmark et al., 1989; Favier et al., 1995; Park, 2003). Park (2003) reported that administration of βCD for 30 days reduced total lipid, triglyceride, cholesterol, and LDL-cholesterol levels by 87, 89, 62 and 54%, respectively ($p<0.05$). The author also suggested that these reductions occurred because of increased fecal excretion of steroids of up to 167%. Although the present study could not identify the mechanism behind the marked decrease of blood lipids and cholesterol, these data correspond to those of Park (2003).

The effect of βCD feeding on cholesterol content and its decreased ratio in back fat, loin, belly, and ham portions of pork is shown in Table 4. Cholesterol content in back fat was significantly reduced in swine fed diets containing 3.0 and 5.0% βCD; addition of 5.0% βCD in the diet decreased cholesterol content from 73.9 mg/100 g (control) to 54.7 mg/100 g, which was a significant reduction of 26.0% ($p<0.05$). Also, cholesterol content of the 3.0% βCD-fed group decreased from 73.9 mg/100 g (control) to 12.9 mg/100 g (17.4%, $p<0.05$). In pork loin, the cholesterol content was significantly decreased in the groups receiving more than 1.5% βCD ($p<0.05$). Moreover, βCD produced a dose-dependent effect in reducing cholesterol content in loin. The corresponding value for the 5.0% βCD-fed group was 51.1 mg/100 g, which was up to 19.5 mg/100 g less than the control, or a marked reduction of 27.6%. The reduction in the 1.5 and 3.0% βCD-fed groups was 9.6 and 6.5%, respectively.

Cholesterol content of belly pork of the control group was 82.7 mg/100 g, while the value in the 3.0 and 5.0% βCD-fed groups was 12.7 and 15.2 mg/100 g, respectively. The reduction was 15.4 and 18.4%, respectively ($p<0.05$).

Cholesterol content in 100 g of ham was 65.4 mg in the control group. As the βCD content of the diet increased, the cholesterol content of the ham progressively decreased ($p<0.05$). The 5.0% βCD-fed level was 18.2% (11.9 mg) less than the control. Balasubramaniam et al. (1997) indicated that administration of βCD decreases the activity of hepatic HMG-CoA reductase and inhibits cholesterol absorption with increasing fecal excretion, therefore reducing cholesterol levels in the blood (Park, 2003). Accordingly, the present data showing that higher levels of βCD in feed resulted in lower cholesterol levels in pork portions such as belly, loin, back fat, and ham, are consistent with this mechanism. In other words, blood with lowered cholesterol levels (Table 3) presumably penetrates every part of the swine, with resultant low cholesterol deposition (Table 4). Therefore, the fact that the cholesterol level in blood was markedly decreased by 26.6% in the 5% βCD-fed group may have curtailed its transfer through blood and thus led to a higher reduction of cholesterol content in the belly portion by 18.4% (Figure 1). These results suggested that the addition of βCD to the diet of swine could reduce their body cholesterol by decreasing the migration of cholesterol through the blood.

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