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Icariin Changes ANGPTL3 Expression and LPL Activity to Improve Meat Quality in Swine

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Abstract: Excessive cholesterol and fatty acids in the diet can lead to cardiovascular disease. Recent studies have reported that elevated Angiopoietin-Like Protein 3 (ANGPTL3) expression contributes to dyslipidemia. The aim of the study was to examine the beneficial effect of icariin, an active flavonoid glucoside, on meat quality in swines and the mechanisms involved. About 36 Duroc x Landrace x Yorkshire swines with an initial average body weight 65 kg were randomly divided into 3 groups: control swines group, two groups of icariin-treated swines (0.1 or 0.3%). About 42 days after, icariin (0.1 or 0.3%) treatment significantly decreased fat meat percentage, drip loss rate, plasma and hepatic total cholesterol levels, plasma and hepatic triglyceride concentrations, expression of ANGPTL3 mRNA and increased meat color, lean meat percentage, plasma high-density lipoprotein cholesterol level, activity of Lipoprotein Lipase (LPL). The present results suggest that icariin changes ANGPTL3 expression and LPL activity to improve meat quality in swine.

Key words: Icariin, meat quality, angiopoietin-like protein 3, lipoprotein lipase, swine

INTRODUCTION

It became obvious that dietary cholesterol played a important role in regulating serum cholesterol levels and excessive cholesterol and fatty acids in the diet can lead to cardiovascular disease. The development of human cardiovascular diseases is strongly associated with the dietary intake of saturated fatty acids and cholesterol (Wang *et al*., 2005; Jalali-Khanabadi *et al*., 2006). Thus, regulating the level of lipid in one's diet has become an prominent concern for patients with cardiovascular diseases.

Dyslipidemia is the condition of raised levels of plasma Triglycerides (TG) and/or Cholesterol (TC) or low concentration of High-Density Lipoprotein (HDL). Angiopoietin-Like Protein 3 (ANGPTL3) is an angiopoietin-like protein. Recent experiments have showed that ANGPTL3 contributes to dyslipidemia by suppressing Lipoprotein Lipase (LPL) (Koishi *et al*., 2002). Excessive fat in the pig muscle is one of the problems faced by the pig industry since it reduces meat quality and feed efficiency (Oyedeji and Atteh, 2005). Therefore, increased ANGPTL3 expression and decreased LPL activity may impair meat quality due to dyslipidemia.

It was apparent to all that oxidant stress can impair meat quality of animal. Improved oxidative stability of the raw product and ameliorated antioxidative status in the living animal are beneficial to the consumer and the processing industry (Jiang *et al*., 2007). A group of researchers once released a study showing that oxidative stress may induce the expression of Angptl3 and supress the expression of LPL (Miida *et al*., 2008; Yang *et al*., 2006). Therefore, researchers hypothesized that impairment of elevated *ANGPTL3* gene expressions on meat quality may be related to oxidant stress. As previously said increased ANGPTL3 expression and decreased LPL expression may impair meat quality. Therefore, ANGPTL3 and LPL may be a effective target for improving meat quality.

Improving meat quality with feed additives is interesting in swine production (Jiang *et al*., 2007). There some evidences that some flavonoids can improve meat quality (Rehfeldt *et al*., 2007; Oshida *et al*., 2002); however, the mechanism responsible for these findings is not yet fully defined. As mentioned, ANGPTL3 and LPL may be a useful target for improving meat quality. There are some evidence that oxidative stress could induce the ANPTL3 expression and supress LPL activity (Miida *et al*., 2008; Yang *et al*., 2006). An active flavonoid glucoside, icariin is the prenyl acetylation of kaempferide 3, 7-O-diglucoside, gained from several species of plants in the epimedium family. Previous investigations have shown that icariin has potent antioxidant activity (Pan *et al*., 2005; Wang and Huang, 2005). On the basis of

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the antioxidant properties of icariin, we postulate that icariin may have the favorable effect on meat quality which is related to changed ANGPTL3 expression and LPL activity. Now a days, the swine industry is focused on improving meat quality. In the present study therefore, we examined the beneficial effect of icariin on meat quality in swine.

MATERIALS AND METHODS

Reagents: Icariin (purity: 98.0%) was obtained from Yingxuan Chempharm Co., Ltd. (Shanghai, China). Other reagents were purchased from Sinopharm Chemical Reagent (Shanghai, China).

Experimental protocols: About 36 Duroc x Landrace x Yorkshire swine (65.8±7.26 kg of average initial body weight) were randomly divided into 3 groups, 3 replicates for each group and 4 for each replicate. There are three groups including swine control receiving commercial diet, two groups of icariin-treated swine receiving commercial diet plus (0.1 or 0.3%) icariin. The experiment lasted 42 days at which time all swines were fasted overnight. Samples of meat, plasma, liver and adipose tissue were obtained at the end of study.

Determination of lipid oxidation: In brief, 1 g of samples of longissimus muscle was homogenized with 9 mL of 1.15% KCl. From the solution, 100 μL of homogenate was removed and incubated at 37°C in 80 mM Tris maleate buffer (pH 7.4) with 5 mM $FeSO_4$ (to catalyse lipid peroxidation) in a total volume of 1 mL. At fixed time (60, 120, 200 and 300 min), aliquots were taken for determinations of TBARS according to a previous method (Corino *et al*., 2003). The TBARS values are expressed as nanomoles of malondialdehyde per gram of muscle tissue.

Analysis of adipose Lipoprotein Lipase activity: The activities of Lipoprotein Lipase (LPL, EC 3.1.1.34) were analyzed in subcutaneous adipose tissue homogenates. Approximately 1 g adipose tissue was homogenized in 3 mL 10 mM HEPES buffer containing 0.25 M sucrose, 1 mM dithiothreitol and 1 mM EDTA and then centrifuged at 100,000 g for 30 min at 4°C. Supernatants were isolated and used for the enzyme assays. LPL activity was analyzed using a commercial kit (Nanjing Jiancheng Biochemical Reagent Co.) according to the manufacturer's recommendations (Rush *et al*., 2000).

Analysis of hepatic ANPTL3 mRNA expression: Briefly, primers for GAPDH were as follows: (forward) 5'-AGGGGCTCTCCAGAACATCATCC-5' and 5'-TCGCGTG CTCTTGCTGGGGTTGG-5' (reverse). Primers for ANPTL3 were as follows: (forward) 5'-TTGGGAGGCT TGATGGAGA-3' and 5'-TGTAGCGTATA GTTGG TTTCGTG-3' (reverse). PCR was performed as described previously methods (Feng *et al*., 2006).

Determination of plasma lipid concentrations: Fasting blood samples were placed in precooled EDTA-containing tubes (final concentration 4 mmol L^{-1}) and centrifuged at 2500×g for 20 min at 4°C. Plasma aliquots were stored at -70°C. Plasma TC, TG and HDL-C concentrations were determined using the enzymatic method (Luci *et al*., 2007) by an automated analyzer (Type 7170A, Hitachi) according to the manufacturer's instructions.

Measurement of hepatic lipid levels: Lipids were extracted from liver using a mixture of isopropanol and n-hexane (2:3, vol/vol). After lipid extracts were dried, aliquots were dissolved in Triton X-100 (Luci *et al*., 2007). As described previously, hepatic cholesterol and triglycerides levels were measured with enzymatic kits.

Measurement of meat quality parameters: Fat meat percentage, lean meat percentage, drip loss rate, meat color value, meat marbling and $pH_{45\ min}$ were measured according to the method as previously described (Kouba *et al*., 2001).

Statistical analysis: Results are expressed as mean±SD. Statistical analysis was performed using ANOVA followed by the unpaired Student's t-test for multiple comparisons. The level of significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Muscle MDA production: At fixed time (300 min), MDA generation was were elevated in icariin (0.1 or 0.3%) treated swine when compared with control swine ($p<0.05$ or $p<0.01$; Table 1).

Table 1: MDA production and Lipid levels in pig

MDA production and Lipid levels	Control	Icariin (L)	Icariin (H)
Muscle TBARS (nmol MDA g^{-1})	22.09±2.62	16.35±1.89^{+}	13.15±1.56^{++}
Plasma TG (mmol L^{-1})	0.70±0.06	0.58±0.06^{+}	0.42±0.05^{++}
Plasma TC (mmol L^{-1})	2.86±0.17	1.99±0.22^{+}	1.62±0.28^{++}
Plasma HDL-C (mmo L^{-1})	0.52±0.06	0.68±0.07^{+}	0.82±0.09^{++}
Hepatic TG (μmol g^{-1} liver)	94.80±8.80	79.60±8.60^{+}	62.50±7.20^{++}
Hepatic TC(μmol g^{-1} liver)	69.20±7.20	58.00± 6.20^{+}	52.10±6.10^{++}

Values are mean±SD. n = 12. +Icariin (L): 0.1% icariin; +Icariin (H): 0.3% icariin. Compared with control, $^{+}p<0.05$ or $^{++}p<0.01$. Malondialdehyde: MDA; Total Cholesterol: TC; Triglycerides: TG; High-Density Lipoprotein Cholesterol: HDL-C

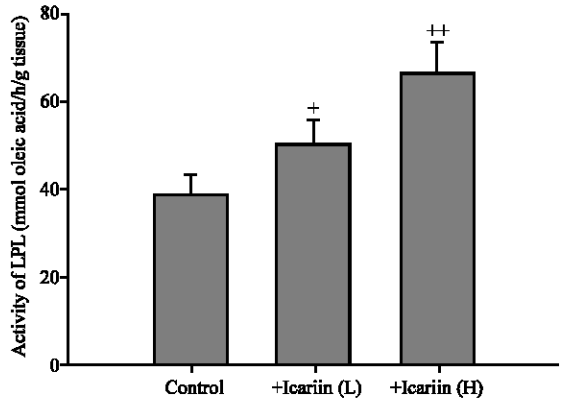

Fig. 1: Effect of icariin on the activity of LPL. Values are mean±SD. n = 12. +Icariin (L): 0.1% icariin; +Icariin (H): 0.3% icariin. Compared with control, $^{+}p<0.05$, $^{++}p<0.01$. Lipoprotein Lipase: LPL

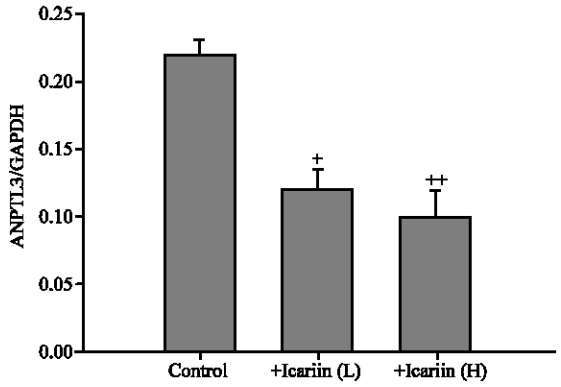

Fig. 2: Effect of icariin on ANPTL3 mRNA expression. Values are mean±SD. n = 12. +Icariin (L): 0.1% icariin; +Icariin (H): 0.3% icariin. Compared with control, $^{+}p<0.05$, $^{++}p<0.01$. Angiopoietin-Like Protein 3: ANPTL3; Glyceraldehyde 3-Phosphate Dehydrogenase: GAPDH

Activity of LPL and expression of and ANPTL3: Icariin (0.1 or 0.3%) treatment up-regulated activity of LPL, down-regulated expression of ANPTL3 in icariin-treated swine ($p<0.05$ or $p<0.01$) (Fig. 1 and 2).

Plasma and hepatic lipid concentrations: Icariin (0.1 or 0.3%) treatment significantly resulted in a decrease in plasma and hepatic TG concentrations, a drop in plasma and hepatic TC levels and an increased in plasma HDL-C concentration in swine ($p<0.05$ or $p<0.01$; Table 1).

Meat quality: Fat meat percentage and drip loss in icariin (0.1 or 0.3%) treated swine were decreased compared with the control group. Lean meat percentage and fresh color were higher in icariin (0.1 or 0.3%) treated than in control swine ($p<0.05$ or $p<0.01$; Table 2). However, researchers found no significant differences in the values of $pH_{45\ min}$ and meat marbling between icariin-treated swine and control swine ($p>0.05$).

Table 2: Meat quality traits of muscle in pig

Characteristics	Control	Icariin (L)	Icariin (L)
Drip loss rate (%)	16.82±1.98	13.32±2.26^{+}	12.01±1.27^{++}
Meat color	3.20±0.38	3.99±0.36^{+}	4.12±0.42^{++}
Meat marbling	3.11±0.33	3.09±0.38	3.16±0.41
$pH_{45\ min}$	6.29±0.66	6.33±0.71	6.38±0.56
Lean meat percentage (%)	54.49±2.16	66.12±7.25^{+}	72.86±8.26^{++}

Values are mean±SD n = 12. +Icariin (L): 0.1% icariin; +Icariin (H): 0.3% icariin. Compared with control, $^{+}p<0.05$ or $^{++}p<0.01$

Pearson's correlation analysis showed adipose LPL activity positively correlated with lean meat percentage ($r = 0.498$, $p = 0.005$), meat color ($r = 0.516$, $p = 0.002$) inversely correlated with fat meat percentage ($r = -0.487$, $p = 0.011$), drip loss rate ($r = -0.525$, $p = 0.006$). Hepatic ANGPTL3 mRNA positively correlated with fat meat percentage ($r = 0.472$, $p = 0.006$) and drip loss rate ($r = 0.504$, $p = 0.003$), hepatic ANGPTL3 mRNA negatively correlated with lean meat percentage ($r = -0.512$, $p = 0.009$) and meat color ($r = -0.515$, $p = 0.013$).

Majority of dyslipidemias are usually associated with diet in many countries. The worldwide diets tend to contain more meat products. Meat quality is highly dependent on its fat content and composition. In this respect, much fat deposition can suprress feed efficiency in swines. To improve the meat quality of animal origin, the search for new procedures is an indis-putable tendency in swine production. The present results showed that meat quality was improved concomitantly with increased plasma HDL-C concentration and decreased TG and TC plasma levels in swine. These findings suggest that regulation of lipid metabolism may be looked upon as potentially attractive aims for improving meat quality.

Swine ANPTL3 mRNA was entirely expressed in liver (Feng *et al*., 2006). There is much evidence that ANPTL3 directly contribute to dyslipidemia. There are elevated levels of plasma ANPTL3 in leptin-deficient obese mice (Shimamura *et al*., 2004). In human clinical investigations have found that the ANPTL3 level correlates with hypertriglyceridemia (Stejskal *et al*., 2007). ANPTL3 can inhibite LPL to regulate TG metabolism (Shimizugawa *et al*., 2002). LPL hydrolyzes TG-rich lipoproteins and leads to various changes in lipoprotein metabolism (Shimizugawa *et al*., 2002). In the present study, the results showed that LPL production was elevated, ANPTL3 level was decreased concomitantly with increased TG and TC concentrations in icariin-treated swine. As mentioned earlier, regulation of lipid metabolism may be viewed as potentially attractive targets for

improving meat quality. Previous investigations have shown that dyslipidemia can impair meat quality of animal (Oyedeji and Atteh, 2005). The present results showed that fat meat percentage and backfat thickness were positively correlated with the ANPTL3 mRNA expression in swine. Therefore, ANPTL3 and LPL may be a useful target for improving meat quality.

ANPTL3 is transcriptionally regulated by LXR. Oxidative stress suppresses cholesterol outflow through a molecular cascade by inhibiting *LXR* gene expression (Marcil *et al*., 2006). There is evidence that (-)-eswineallocatechin-3-gallate can decrease expression of LXR (Moon *et al*., 2007). Recent studies reported that antioxidant drug probucol inhibited hyperlipidemia involving inhibition of ANPTL3 level (Miida *et al*., 2008). Previous data indicated that the high-fat diet induced abnormal increases in lipid peroxidation, decreased activity of LPL concomitantly with a depressed antioxidant defense system was observed in HFD-fed rats (Yang *et al*., 2006). These findings suggest that oxidative stress may induce the ANPTL3 expression and suppress LPL activity. Therefore, researchers hypothesized that increased *ANPTL3* gene expressions may impair meat quality due to oxidant stress.

Interestingly in the present study, icariin treatment significantly increased lean meat percentage, meat color, expression and activity of LPL; decreased fat meat percentage, drip loss rate, expression of ANPTL3 mRNA. There are many evidences suggest oxidative stress is closely associated with meat quality. On the one hand, oxidant stress can impair broiler meat quality. On the other hand, feed additives could alleviate the oxidative stress to improve meat quality in broiler chickens (Zhang *et al*., 2011). In recent years, icariin has received much attention because of its antioxidant (Pan *et al*., 2005; Wang *et al*., 2005).

The present results also show that chronic administration of icariin effectively reduces muscle MDA concentration, fat meat percentage, drip loss rate and expression of ANGPTL3 mRNA and increased meat color, lean meat percentage and LPL activity. Based on antioxidant properties of icariin, we postulated that icariin increased ANGPTL3 expression and decreased LPL activity by inhibiting oxidative stress which in turn led to the beneficial effect of icariin on meat quality. These findings support the hypothesis that icariin have the favorable effect on meat quality which is related to changed ANGPTL3 expression and LPL activity.

CONCLUSION

The present results suggest that icariin changes ANGPTL3 expression and LPL activity to improve meat quality in swine.

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REFERENCES

Corino, C., S. Magni, G. Pastorelli and R. Rossi, 2003. Effect of conjugated linoleic acid on meat quality, lipid metabolism and sensory characteristics of dry-cured hams from heavy porcines. J. Anim. Sci., 81: 2219-2229.

Feng, S.Q., X.D. Chen, T. Xia, L. Gan and H. Qiu *et al*., 2006. Cloning, chromosome mapping and expression characteristics of porcine ANGPTL3 and -4. Cytogenet Genome Res., 114: 44-49.

Jalali-Khanabadi, B.A., H. Mozaffari-Khosravi, M. Rafiei, S.M. Ghoreishian and F. Darabi, 2006. Association of cholesterol-rich lipoproteins with coronary artery disease in subjects who referred to yazd cardiovascular research center for coronary angiography. Pak. J. Biol. Sci., 9: 2777-2780.

Jiang, Z.Y., S.Q. Jiang, Y.C. Lin, P.B. Xi, D.Q. Yu and T.X. Wu, 2007. Effects of soybean isoflavone on growth performance, meat quality and antioxidation in male broilers. Poult. Sci., 86: 1356-1362.

Koishi, R.Y., M. Ando, M. Ono, H. Shimamura, T. Yasumo and H. Fujiwara, 2002. Angptl3 regulates lipid metabolism in mice. Nat. Genet., 30: 151-157.

Kouba, M., D. Hermier and J. Le Dividich, 2001. Influence of a high ambient temperature on lipid metabolism in the growing porcine. J. Anim. Sci., 79: 81-87.

Luci, S., B. Giemsa, H. Kluge and K. Eder, 2007. Clofibrate causes an upregulation of PPAR-{alpha} target genes but does not alter expression of SREBP taret genes in liver and adipose tissue of pigs. Am. J. Physiol-Reg., 1: 70-77.

Marcil, V., E. Delvin, A.T. Sane, A. Tremblay and E. Levy, 2006. Oxidative stress influences cholesterol efflux in THP-1 macrophages: Role of ATP-binding cassette A1 and nuclear factors. Cardiovasc. Res., 72: 473-482.

Miida, T., U. Seino, O. Miyazaki, O. Hanyu, S. Hirayama and T. Saito, 2008. Probucol markedly reduces HDL phospholipids and elevated prebeta1-HDL without delayed conversion into alpha-migrating HDL: Putative role of angiopoietin-like protein 3 in probucol-induced HDL remodeling. Atherosclerosis, 200: 329-335.

Moon, H.S., C. Chung, H.G. Lee, T.G. Kim, Y.J. Choi and C.S. Cho, 2007. Inhibitory effect of (-)-eporcineallocatechin-3-gallate on lipid accumulation of 3T3-L1 cells. Obesity Silver Spring, 15: 2571-2582.

Oshida, T., R. Sakata, S. Yamada, K. Horiguchi, S. Itoh and H. Matsumura, 2002. Effects of apple polyphenol on porcine production and meat quality. Bull. Anim. Hygiene, 7: 77-83.

Oyedeji, J.O. and J.O. Atteh, 2005. Response of broilers to feeding manipulations. Int. J. Poult. Sci., 4: 91-95.

Pan, Y., L. Kong, X. Xia, W. Zhang, W. Xia and F. Jiang, 2005. Antidepressant-like effect of icariin and its possible mechanism in mice. J. Pharmacol. Biochem. Behav., 82: 686-694.

Rehfeldt, C., I. Adamovic and G. Kuhn, 2007. Effects of dietary daidzein supplementation of pregnant sows on carcass and meat quality and skeletal muscle cellularity of the progeny. Meat Sci., 15: 103-111.

Rush, J.W., M. H.Laughlin, C.R. Woodman and E.M. Price, 2000. SOD-1 expression in porcine coronary arterioles is increased by exercise training. Am. J. Physiol. Heart. Circ. Physiol., 279: 2068-2076.

Shimamura, M., M. Matsuda, Y. Ando, R. Koishi, H. Yasumo, H. Furukawa and I. Shimomura, 2004. Leptin and insulin down-regulate angiopoietin-like protein 3, a plasma triglyceride-increasing factor. Biochem. Bioph. Res. Co., 322: 1080-1085.

Shimizugawa, T., M. Ono, M. Shimamura, K. Yoshida, Y. Ando, R. Koishi and K. Ueda, 2002. Angptl3 decreases very low density lipoprotein triglyceride clearance by Inhibition of Lipoprotein Lipase. J. Biol. Chem., 277: 33742-33748.

Stejskal, D., M. Karpisek, V. Humenanska, P. Solichova, P. Stejskal, 2007. Angiopoietin-like protein 3: Development, analytical characterization and clinical testing of a new ELISA. Gen. Physiol. Biophys., 26: 230-233.

Wang, J.J., T.M. Pan, M.J. Sheih and C.C. Hsu, 2005. Effect of red mold rice supplements on serum and meat cholesterol levels of broiler chicken. Applied Microbiol. Biotechnol., 71: 812-818.

Wang, Y.K. and Z.Q. Huang, 2005. Protective effects of icariin on human umbilical vein endothelial cell injury induced by H2O2 *in vitro*. Pharmacol. Res., 52: 174-182.

Yang R., G. Le, A. Li, J. Zheng and Y. Shi, 2006. Effect of antioxidant capacity on blood lipid metabolism and lipoprotein lipase activity of rats fed a high-fat diet. Nutration, 22: 1185-1191.

Zhang, W.H., F. Gao, Q.F. Zhu, C. Li, Y. Jiang, S.F. Dai and G.H. Zhou, 2011. Dietary sodium butyrate alleviates the oxidative stress induced by corticosterone exposure and improves meat quality in broiler chickens. Poult. Sci., 90: 2592-2599.