

# Kaempferitrin improves meat quality of broiler chickens

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**ABSTRACT:** Angiotensin-like protein 3 (ANGPTL3) may promote adipose formation. The objective of this study was to investigate the effect of kaempferitrin, a 3,7-diglycosyl flavone, on meat quality in broiler chickens and the mechanisms involved. One thousand two hundred broiler chickens were offered commercial diet that was supplemented with 0.0 (control), 0.1, 0.3, or 0.9% kaempferitrin, respectively. After 42 days, kaempferitrin (0.3 or 0.9%) treatment significantly increased the lightness of meat colour. Kaempferitrin (0.3 or 0.9%) supplementation decreased breast muscle drip loss, breast muscle crude fat, breast muscle malondialdehyde level, and hepatic ANGPTL3 mRNA expression. The present results suggest that kaempferitrin improves meat quality by decreasing expression of ANGPTL3 in broiler chickens.

**Keywords:** 3,7-diglycosyl flavone; meat trait; angiotensin-like protein 3; broiler

Angiotensin-like protein 3 (ANGPTL3) is a hormone-like protein expressed primarily in the liver. ANGPTL3 inhibits lipoprotein lipase activity and decreases very low density lipoprotein clearance (Shimizugawa et al., 2002). Recent experiments have shown that ANGPTL3 may promote adipose formation (Feng et al., 2006; Fujimoto et al., 2006). Excessive fat in the carcass reduces meat quality and feed efficiency of chickens (Oyedeji and Atteh, 2005). These findings suggest that increased expression of ANGPTL3 promotes fat deposition to impair meat quality.

Oxidative stress can impair the meat quality of animal (Zhang et al., 2011). A high oxidative stability of muscle-based food avoids or delays rancid products or warmed-over flavour. Improved antioxidative status in the living animal and increased oxidative stability of the raw product are beneficial to the consumer and the processing industry (Jiang

et al., 2007). Besides, oxidative stress induces the expression of ANGPTL3 (Marcil et al., 2006; Moon et al., 2007; Miida et al., 2008). Therefore, the impairment of ANGPTL3 on meat quality might be related to oxidative stress. ANGPTL3 may be a useful target for improvement of meat quality.

Feed additives influence the oxidative stability of meat (Jiang et al., 2007). Polyphenol derivatives can improve meat quality (Oshida et al., 2002; Rehfeldt et al., 2007), however, the mechanism has not been fully defined yet. Kaempferitrin, also known as 3,7-diglycosyl flavone, is isolated from the leaves of *Hedyotis verticillata* (Hamzah et al., 1994). It has potent antioxidant activity. *In vitro*, kaempferitrin inhibits myeloperoxidase activity, lipid peroxidation induced by ascorbyl radical either in microsomes or in asolectin and phosphatidylcholine liposomes (de Sousa et al., 2004). Thus, we hypothesized that kaempferitrin

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improves meat quality by decreasing ANGPTL3 gene expression. In the present study, therefore, we tested the beneficial effect of kaempferitrin on meat quality in broiler chickens.

## MATERIAL AND METHODS

### Reagents

Kaempferitrin (purity 98.0%) was purchased from Shanghai Tong Tian Technology Development Co., Ltd. (Shanghai, P.R. China). Other reagents were obtained from Sinopharm Chemical Reagent (Shanghai, P.R. China).

### Experimental protocols

One thousand two hundred male 1-day-old healthy AA broiler chickens were obtained from Hunan Agricultural University. Feed and water were freely available to all birds. The experiments complied with established standards of the Institute of Animal Science, Hunan Agricultural University. Broilers were divided into 4 groups with 6 replicates of 50 birds per replicate pen for each group. Birds were offered commercial diet that was supplemented with 0.0 (control), 0.1, 0.3, or 0.9% kaempferitrin, respectively (Table 1). After 6 weeks, all chickens were fasted overnight. They

were slaughtered by bleeding the left jugular vein. Breast muscles and liver were sampled.

### Measurement of lipid oxidation

Eight birds per treatment were killed. Breast meat of carcasses was immediately dissected. 1 g of samples of breast muscle was homogenized within 9 ml of 1.15% KCl. From the solution, 100 µl of homogenate were obtained and incubated at 37°C in 80mM Tris maleate buffer (pH 7.4) with 5mM FeSO<sub>4</sub> (to catalyze lipid peroxidation) in a total volume of 1 ml. The 2-thiobarbituric acid was used to measure lipid oxidation according to a previous method (Salih et al., 1987). The 2-thiobarbituric acid reactive substance was expressed in mg of malondialdehyde (MDA) per kg of meat.

### Analysis of hepatic ANGPTL3 mRNA expression

Real-time PCR was performed to quantify ANGPTL3 mRNA expressions according to the previous methods (Niki and Yokouchi, 2009). Total RNA was extracted from chicken liver using TRIzol reagent. Primers for ANGPTL3 (GenBank Accession Nos. NW\_001471740.2) were as follows: 5'-GCGCAGGAGATGAACCTGAAGA-3' and 5'-GTCGAAAGCTTCTGAGCCGTT-3'.

Table 1. Composition of diets in the experiments

Ingredients (g/kg)	Days 1–14	Days 15–42	Nutrient levels	Days 1–14	Days 15–42
Soya oil	25.0	20.0	ME (MJ/kg)	14.3	14.7
Soya HP	312.5	296.0	crude fat (%)	7.6	8.4
Corn	579.3	597.5	crude protein (%)	22.1	21.5
Megafat	12.5	25.0	Na (%)	0.3	0.3
Lysine	3.8	1.5	P (%)	0.9	0.8
Premix <sup>1</sup>	62.5	60.0	Ca (%)	1.6	1.4
Threonine	1.3	-	Mg (%)	0.3	0.3
Methionin	0.8	-			
Monocalciumphosphate	2.5	-			

<sup>1</sup>content per 1 kg diet: sodium 30 g, calcium 196 g, phosphorus 64 g, magnesium 6 g, zinc 1200 mg, iron 2000 mg, copper 400 mg, cobalt 20 mg, manganese 1200 mg, iodine 40 mg, selenium 8 mg, vitamin A 60 mg, thiamin 35 mg, riboflavin 135 mg, pantothenic acid 10 mg, nicotinic acid 1340 mg, pyridoxine 100 mg, biotin 3.350 µg, folic acid 34 mg, cobalamin 670 µg, vitamin C 1300 mg, vitamin D<sub>3</sub> 2 mg, vitamin E 1600 mg, vitamin K 0.50 mg, choline chloride 8400 mg, methionine 30 g

Primers for GAPDH (GenBank Accession Nos. NW\_003763490.1) were as follows: 5'-ACGCCAT-CACTATCTTCCAG-5' and 5'-CAGCCTTCAC-TACCCTCTTG-5'.

### Measurement of meat quality

Chicken carcasses were fabricated to remove both breasts (*pectoralis major* and *pectoralis minor*) to calculate breast yield. Fresh colour, drip loss, shear force, pH 45 min postmortem (pH<sub>45</sub>), crude protein, and crude fat of breast muscle were measured using previously described methods (Bianchi et al., 2007; Jiang et al., 2007; Tavárez et al., 2011). Meat colour was determined using Chroma Meter CR-410 (Konica Minolta Co. Ltd., Osaka, Japan) to assess CIE LAB values. Warner-Bratzler shear force was measured with Instron model 4210 (Instron Corp., Canton, USA), crude fat (CF) was measured with extraction in petroleum ether after acidification with 4N HCl solution (Wiseman et al., 1992). After a six-hour extraction of the samples by ethyl ether, crude protein was determined using Kjeldahl method (Zvonimir, 2008). The pH value was determined in the right *pectoralis major* by a PHSB-260 Portable PH Meter (Shboqu Co. Ltd., Shanghai, P.R. China).

### Statistical analysis

Results are expressed as means ± SE. All statistical analyses were computed using the GLM procedures

of SPSS software (Version 15.0, 2012). A software program using Duncan's multiple range tests to compare treatment means was used. Correlations between parameters were examined using Pearson's correlation analysis. The significance level was chosen as  $P < 0.05$ .

### RESULTS

Table 2 summarizes muscle MDA production. Kaempferitrin (0.1%) treatment did not significantly attenuated breast muscle MDA generation in broiler chickens. Breast MDA level in broiler chickens was decreased by kaempferitrin (0.3 or 0.9%) administration ( $P < 0.05$  or  $P < 0.01$ ).

Expression of hepatic ANGPTL3 mRNA is presented in Figure 1. After treatment with kaempferitrin (0.3 or 0.9%), ANGPTL3 mRNA expression in the liver from broiler chickens was markedly down regulated ( $P < 0.05$  or  $P < 0.01$ ).

Meat quality parameters are given in Table 2. Kaempferitrin (0.3 or 0.9%) decreased drip loss of breast muscle, reduced crude fat of breast muscle, increased the lightness of meat colour in broiler chicken ( $P < 0.05$  or  $P < 0.01$ ) (Table 2). However, kaempferitrin (0.1, 0.3, 0.9%) treatment had no significant effect on the values of shear force, crude protein, crude fat, and pH<sub>45</sub> ( $P > 0.05$ ).

Pearson's correlation analysis showed a significantly positive correlation between the drip loss and expression of ANGPTL3 mRNA in broiler chickens ( $r = 0.689$ ,  $P = 0.007$ ), whereas the light-

Table 2. Meat quality of breast muscle in chickens

Item <sup>1</sup>	Kaempferitrin (%)			
	0.0	0.1	0.3	0.9
Lightness	48.54 ± 1.42	50.61 ± 2.52	53.99 ± 1.87*	57.25 ± 1.70**
Redness	1.50 ± 0.72	1.51 ± 0.87	1.56 ± 0.67	1.49 ± 0.87
Yellowness	11.83 ± 0.05	12.11 ± 0.06	12.22 ± 0.07	12.36 ± 0.08
Drip loss (%)	1.75 ± 0.02	1.62 ± 0.04	1.42 ± 0.03*	1.28 ± 0.02**
Shear force (kg)	2.68 ± 0.29	2.71 ± 0.32	2.73 ± 0.33	2.66 ± 0.27
Crude protein (%)	22.76 ± 6.46	22.56 ± 6.21	23.99 ± 7.22	24.13 ± 6.62
Crude fat (%)	1.38 ± 0.19	1.26 ± 0.17	1.16 ± 0.22	1.02 ± 0.23*
pH <sub>45</sub>	5.63 ± 0.69	5.56 ± 0.62	5.62 ± 0.63	5.48 ± 0.49
Malondialdehyde (mg/kg tissue)	0.22 ± 0.03	0.19 ± 0.01	0.16 ± 0.03*	0.12 ± 0.02**

<sup>1</sup>lightness, redness, yellowness measured 24 h postmortem; dip loss is expressed as a percentage of *Pectoralis major* muscle weight 24 h postmortem, pH<sub>45</sub> = muscle pH 45 min postmortem

data presented as means,  $n = 120$  per treatment

\* $P < 0.05$ , \*\* $P < 0.01$  compared with control

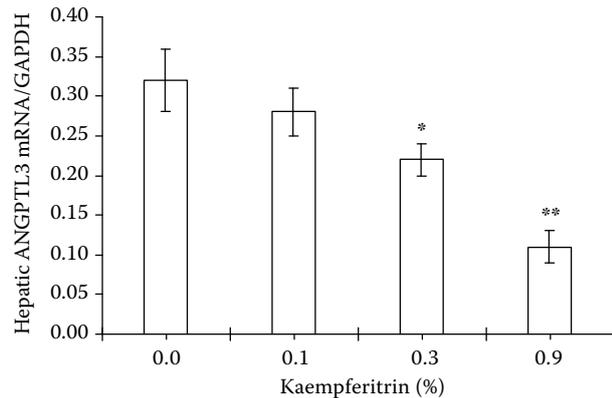


Figure 1. Effect of kaempferitrin on ANGPTL3 mRNA expression

ANGPTL3 = Angiopoietin-like protein 3, GAPDH = Glyceraldehyde 3-phosphate dehydrogenase. Values are mean  $\pm$  SE,  $n = 60$ . Compared with control, \* $P < 0.05$ , \*\* $P < 0.01$

ness of meat colour was inversely correlated with expression of ANGPTL3 mRNA ( $r = -0.512$ ,  $P = 0.004$ ).

## DISCUSSION

Meat quality is dependent on its fat content and composition. Much fat deposition can depress feed efficiency in poultry (Jiang et al., 2007). Here we showed that mRNA expression of ANGPTL3 was decreased concomitantly with decreased breast muscle crude fat in broiler chickens; there was a positive correlation between the drip loss and expression of ANGPTL3 mRNA, whereas the lightness of meat colour was inversely correlated with expression of ANGPTL3 mRNA. Similar to this, feeding high-fat/high-calorie diet to ANGPTL3-null mice reduces epididymal adipose tissue weight (Fujimoto et al., 2004; Feng et al., 2006). Hepatic ANGPTL3 mRNA in white adipose tissue is higher in genetically obese pigs than in their lean counterparts (Feng et al., 2006). These findings supported the hypothesis that increased expression of ANGPTL3 promotes fat deposition to impair meat quality.

Meat quality of animals can be impaired by oxidative stress. On the one hand, dietary oxidized oil induces oxidative stress in live birds, increases lipid and protein oxidation, and increases drip loss in broiler breast muscles (Zhang et al., 2011). Our results showed that MDA level was decreased accompanied by increased fresh colour and decreased

drip loss in broiler chicken muscle. On the other hand, antioxidants can improve meat quality. Vitamin E prevents lipid deterioration in meat (Bartov and Bornstein, 1997). Apple polyphenol decreases cholesterol in meat (Rehfeldt et al., 2007). Supplemental daidzein increases the proportion of fast-twitch glycolytic fibres in semitendinosus muscle of pregnant sows (Oshida et al., 2002). Moreover, a high oxidative stability of muscle-based foods is crucial for delaying or avoiding development of rancid products or warmed-over flavour (Jiang et al., 2007).

Kaempferitrin is a natural antioxidant product. It is notable that kaempferitrin administration increased the lightness of meat colour, and decreased drip loss concomitantly with decreased expression of ANGPTL3 mRNA and breast muscle MDA level and breast muscle crude fat in broiler chickens. ANGPTL3 is transcriptionally regulated by liver X receptors. Oxidative stress limits cholesterol outflow by inhibiting its gene expression (Marcil et al., 2006). Antioxidant drug probucol can decrease the level of ANGPTL3 (Moon et al., 2007). It is probable that kaempferitrin decreased expression of ANGPTL3 by inhibiting lipid peroxidation, which in turn resulted in the beneficial effect of kaempferitrin on meat quality. These findings support the hypothesis that the beneficial effect of kaempferitrin on meat quality is related to decrease ANGPTL3 gene expression by inhibiting of lipid peroxidation.

## CONCLUSION

The present results suggest that kaempferitrin improves meat quality by decreasing expression of ANGPTL3 in broiler chickens.

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