Prawarun Agr. J. Volume 15(2) 2018, Pages 319-324

Research Article

Effect of dietary *Piper samentosum* (Wild betal) leaves on activity of antioxidant enzymes in chicken meat

Umaporn Pastsart*

Faculty of Science and Industrial Technology, Prince of Songkla University, Surat Thani Campus, 84000, Thailand

Abstract

The purpose of this experiment was to investigate the effect of *Piper samentosum* leaves (PSL) in broiler diets on antioxidant activities in meat. The meat samples (drumsticks) were taken from 60 broilers at 42 days old which were treated with 5 different levels of PSL; 1) fed by basal feed (8 birds per cage) 2) fed by basal feed (10 birds per cage) 3) fed by basal feed supplemented with 1% PSL (10 birds per cage) 4) fed by basal feed supplemented with 2% PSL (10 birds per cage) and 5) fed by basal feed supplemented with 3% PSL (10 birds per cage). Then the activities of superoxide dismutase (SOD) and catalase (CAT) were analyzed. The results showed that the activity of SOD were significantly different among all groups ($P < 0.01$). The supplementation of 3% PSL in broiler diets increased the activities of SOD compared to 0 and 1% PSL but no difference from 2% PSL. There was no significant difference in activity of CAT in meat ($P > 0.05$). However, the CAT activity from 2% PSL group tended to be higher than other groups ($P = 0.15$). Therefore, it can be concluded that the supplementation of PSL 2 and 3 % in broiler diets can be positive effect on the activities of antioxidant enzymes in meat.

Keywords: *Piper samentosum,* Chicken, Antioxidant enzymes

* Corresponding author : E-mail : umaporn.p@psu.ac.th

Prawarun Agr. J. Volume 15(2) 2018, Pages 319-324

ผลของการเสริมใบชะพลูในอาหารต่อกิจกรรมของเอนไซม์ต้านอนุมูลอิสระในเนื้อไก่

อุมาพร แพทย์ศาสตร์*

คณะวิทยาศาสตร์และเทคโนโลยีอุตสาหกรรม มหาวิทยาลัยสงขลานครินทร์ วิทยาเขตสุราษฎร์ธานี จังหวัดสุราษฎร์ธานี 84000

บทคัดย่อ

วัตถุประสงค์ของการทดลองนี้เพื่อศึกษาผลของการเสริมใบชะพลูในอาหารไก่เนื้อต่อกิจกรรมของเอนไซม์ต้านอนุมูลอิสระในเนื้อไก่ โดยทำการสุ่มตัวอย่างเนื้อไก่ส่วนน่องของไก่เนื้ออายุ 42 วัน จำนวน 60 ตัว ที่ทำการเลี้ยงโดยการเสริมใบชะพลูในอาหารไก่ที่ระดับต่างๆ 5 กลุ่ม กลุ่มละ 12 ตัว ดังนี้ 1) อาหารควบคุมเลี้ยงจำนวน 8 ตัวต่อกรง 2) อาหารควบคุมเลี้ยงจำนวน 10 ตัวต่อกรง 3) อาหารควบคุมที่เสริมด้วยใบชะพลูผง 1% เลี้ยงจำนวน 10 ตัวต่อกรง 4) อาหารควบคุมที่เสริมด้วยใบชะพลูผง 2% เลี้ยงจำนวน 10 ตัวต่อกรง และ 5) อาหารควบคุมที่เสริมด้วยใบชะพลูผง 3% เลี้ยงจำนวน 10 ตัวต่อกรง จากนั้นนำเนื้อมาทำการวิเคราะห์ค่ากิจกรรมของเอนไซม์ superoxide dismutase (SOD) และ catalase (CAT) ผลการทดลองพบว่าการเสริมใบชะพลูในอาหารไก่เนื้อ มีผลต่อค่ากิจกรรมของเอนไซม์ SOD อย่างมีนัยสำคัญทางสถิติ ($P<0.01$) โดยการเสริมใบชะพลูในอาหารไก่เนื้อที่ระดับ 3% มีค่ากิจกรรมของเอนไซม์ SOD สูงที่สุดเมื่อเทียบกับระดับ 0 และ 1% แต่ไม่แตกต่างจากระดับ 2% สำหรับค่ากิจกรรมของเอนไซม์ CAT ในเนื้อไก่ทุกกลุ่มไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($P>0.05$) อย่างไรก็ตามเนื้อไก่ที่เสริมใบชะพลูที่ระดับ 2% มีแนวโน้มค่ากิจกรรมของเอนไซม์ CAT สูงที่สุด ($P=0.15$) ดังนั้นจึงสรุปได้ว่าการเสริมใบชะพลูในอาหารไก่เนื้อที่ระดับ 2 และ 3% ช่วยส่งเสริมให้ค่ากิจกรรมของเอนไซม์ต้านอนุมูลอิสระในเนื้อไก่สูงขึ้น

คำสำคัญ : ใบชะพลู เนื้อไก่ เอนไซม์ต้านอนุมูลอิสระ

* ผู้เขียนให้ติดต่อ : E-mail : umaporn.p@psu.ac.th

Introduction

Wild betel (*Piper sarmentosum*) is an edible plant and widely found in tropical and subtropical countries, including Thailand. Prior studies have shown that extracts of this plant exhibit antimicrobial, antioxidant and antimutagenic activities *in vitro* (Hafizah *et al*., 2010; Lee *et al*., 2014; Boonla *et al*., 2014). The natural antioxidant from *Piper sarmentosum* leaves (PSL) is a superoxide scavenger, naringenin (Subramaniam *et al*., 2003; Chanwitheesuk *et al*., 2005), which is supposed to protect cells from oxidative stress by inhibiting production of reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2) (Hafizah *et al*., 2010).

Oxidative deterioration in meat leads to a loss of nutritional value and reduced sensory quality. There are several mechanisms to protect muscle *in vivo* and post-mortem against oxidative processes, including the endogenous antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) (Chan and Decker, 1994). SOD is the first line antioxidant enzyme catalysing the dismutation of the highly reactive superoxide anion to O_2 and to the less reactive species H_2O_2. The resultant H_2O_2 is detoxified to O_2 and H_2O by CAT or GSH-Px (Matés *et al*., 1999). Activity of these enzymes in muscle post-mortem has been measured in several studies (Renerre *et al*., 1996; Qwele *et al*., 2013). Moreover, Pradhan *et al*. (2000) demonstrated that CAT seems to play a role in modulating lipid oxidation in post-mortem muscle from different species.

The objective of the present study was to determine the influence of *Piper samentosum* leaves with an abundance of antioxidant activities, in broiler diets on antioxidant activities (CAT and SOD) of muscles post-mortem.

Materials and methods

1. Animals

In the experiments, totally 144 commercial broiler chicks at 1 day old (about 50:50 mixtures of males and females) were randomly assigned to 5 treatment groups (3 replicate cages/group). The cage size was 1x1 m. The treatment groups were: 1) CONTROL. Basal feed supplemented with 0% *Piper sarmentosum* leaves (PSL) powder (8 birds/cage), 2) Control. Basal feed supplemented with 0% PSL powder (10 birds/cage), 3) Basal feed supplemented with 1% PSL powder (10 birds/cage), 4) Basal feed supplemented with 2% PSL powder (10 birds/cage) and 5) Basal feed supplemented with 3% PSL powder (10 birds/cage). Feed and water were supplied for consumption *ad libitum*. Basal starter (23% protein) (1 to 3 week) and finisher (20% protein) (4 to 6 week) diets were formulated according to the nutrient requirement recommenddations of NRC (1994) for broiler chickens. The duration of dietary treatments was 42 days.

2. Meat samples

At the end of the rearing period (42 days), 4 birds (2 females and 2 males) with body weights near the group average from each replicate were slaughtered and cooled according to common practices. Then leg muscle (drumstick) samples were collected for determining the antioxidant activities. The meat samples were stored frozen until use.

3. Antioxidant enzyme activity assays

During the analysis, the muscle samples were kept on ice. A 5 g sample was homogenized in 10 ml of 0.05 M phosphate buffer (pH 7.0) and centrifuged at 4°C for 20 min at 7000g. The supernatant fraction was filtered through glass wool before determining enzyme activities.

The SOD activity assay was performed as described by Marklund and Marklund (1974) by measuring the inhibition of pyrogallol autoxidation. A unit of enzyme activity was defined as the amount of sample needed to inhibit the reaction by 50%.

The CAT activity was determined according to the method of Aebi (1983). One unit of CAT activity was defined as the amount of sample required to decompose 1 μmol of H_2O_2 per min at room temperature.

4. Statistical analysis

The data were analyzed by General Linear Model procedures to test for the effect of treatment according to completely randomized experimental design (CRD). Post-hoc tests were performed at a significance level of $P<0.05$ using Duncan's new multiple range test. The analyses were done using SPSS version 21.0 for Windows.

Results and Discussion

As shown in Fig. 1, the treatment 5 (3% of PSL) had a higher SOD activity than treatment 3, 1 and 2, respectively ($P<0.01$), but had no difference from treatment 4. The present results indicate that SOD activity was affected by PSL supplementation. For the CAT activity in broiler meat, it was found that there was no significant difference in CAT activity ($P>0.05$) as can be seen in Fig. 2.

The above findings illustrated that supplementation of 2 and 3% PSL could give benefit effect on antioxidant enzymes, especially SOD and CAT in broiler meat. However, the relationships between enzyme activities and meat quality traits including lipid, protein and myoglobin oxidations in meat need to be investigated.

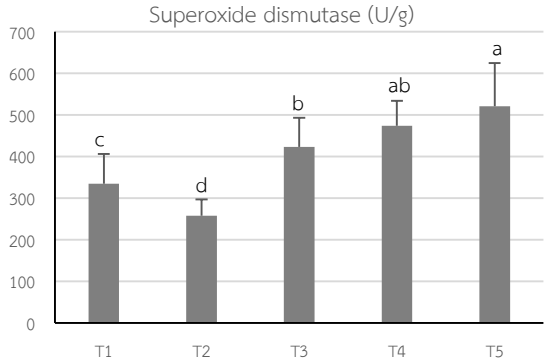

Fig. 1 Mean values of superoxide dismutase (SOD) activity by dietary treatment with *Piper sarmentosum* leaves (PSL) supplementation (T1: 0% PSL (8 birds/cage), T2: 0% PSL (10 birds/cage), T3: 1% PSL (10 birds/cage), T4: 2% PSL (10 birds/cage) and T5: 3% PSL (10 birds /cage). Error bars represent standard deviations. $^{a-d}$Mean values with a different superscript are significantly different at $P=0.00$.

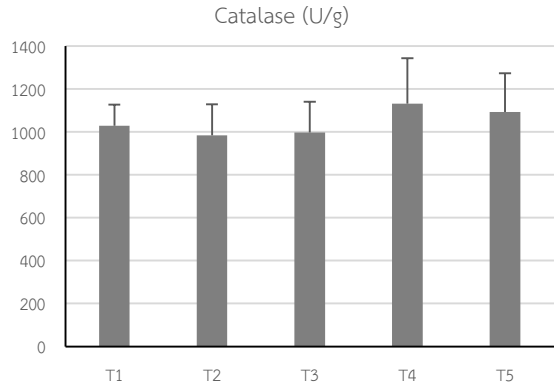

Fig. 2 Mean values of catalase (CAT) activity by dietary treatment with *Piper sarmentosum* leaves (PSL) supplementation (T1: 0% PSL (8 birds/cage), T2: 0% PSL (10 birds/cage), T3: 1% PSL (10 birds/cage), T4: 2% PSL (10 birds/cage) and T5: 3% PSL (10 birds /cage). Error bars represent standard deviations. $P=0.15$.

SOD plays an important role in protecting cells against ROS by lowering the steady state of superoxide anions and reducing the level of cellular damage. It is one of the most important enzymes in the antioxidant defense system (Oyedemi *et al*., 2010). The consumption of PSL by the animals significantly (P>0.05) increased the activity of SOD which can be indicated that its ability to protect the animal body/cells from cellular damage by quenching free radicals and may result in maintaining the meat quality and protecting meat from oxidations. Catalase is one of the enzymatic antioxidants widely distributed in all animal tissues. In our study, the levels of CAT were not different in the meat sample from chickens supplemented with PSL (P>0.05). However, the study of Qwele *et al*. (2013) found that *Moringa oleifera* leaves, higher amount of polyphenols, has more the significant antioxidant potential especially SOD and CAT in the meat samples from goats.

Conclusion

Supplementation of broiler diets with up to 3% of *Piper sarmentosum* leaves had remarkable potential to improve meat quality in broilers subjected to a 6-week dietary treatment, specifically chicken drumsticks, in terms of antioxidant enzyme activities. Consequently, if the antioxidant enzymes analysed in the present study play a role in maintaining meat oxidative stability, one can expect protection of meat from oxidations and longer shelf-life of meat.

Acknowledgements

The authors acknowledge the financial support for this study from Prince of Songkla University, Thailand.

References

Aebi, H.E. 1983. Catalase. In: Bergmeyer, H.U., Ed. Methods of Enzymatic Analysis. Weinhem : Verlag Chemie. p273- 286.

Boonla, P., Phadungkit, M., Mahaweerawat, U., and Somdee, T. 2014. Antioxidant and antimutagenic activities of *Piper sarmentosum* Roxb. leaf extracts. *Isan Journal of Pharmaceutical Sciences*. 10 : 283-294.

Chan, K.M., and Decker, E.A. 1994. Endogenous skeletal muscle antioxidants. *Critical Reviews in Food Science and Nutrition*. 34 : 403-426.

Chanwitheesuk, A., Teerawutgulrag, A., and Rakariyatham, N. 2005. Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. *Food Chemistry*. 92 : 491-497.

Hafizah, A. H., Zaiton, Z., Zulkhairi, A., Ilham, A. M., Anita, M. M. N., and Zaleha, A.M. 2010. *Piper sarmentosum* as an antioxidant on oxidative stress in human umbilical vein endothelial cells induced by hydrogen peroxide. *Journal of Zhejiang University Science B*. 11: 357-365.

Lee, J. H., Cho, S., Paik, H. D., Choi, C. W., Nam, K. T., Hwang, S. G., and Kim S. K. 2014. Investigation on antibacterial and antioxidant activities, phenolic and flavonoid contents of some Thai edible plants as an alternative for antibiotics. Asian-Australas. *Journal of Animal Science*. 27: 1461-1468.

Marklund, S., and Marklund, G. 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*. 47 : 469-474.

Matés, J. M, Pérez-Gómez, C., and De Castro, I. N. 1999. Antioxidant enzymes and human diseases. *Clinical Biochemistry*. 32 : 595-603.

NRC. 1994. Nutrient requirements of poultry. Washington, D. C. : National Academy Press.

Oyedemi, S. O., Bradley, G., and Afolayan, A. J. 2010. *In vivo* and *in vitro* antioxidant activities of aqueous stem bark extract of *Strychnos henningsii* (Gilg). African Journal of Pharmacy and Pharmacology. 4 : 70-78.

Pradhan, A. A., Rhee, K. S., and Hernandez, P. 2000. Stability of catalase and its potential role in lipid oxidation in meat. *Meat Science*. 54 : 385-390.

Qwele, K., Hugo, A., Oyedemi, S. O., Moyo, B., Masika, P. J., and Muchenje, V. 2013. Chemical composition, fatty acid content and antioxidant potential of meat from goats supplemented with Moringa (*Moringa oleifera*) leaves, sunflower cake and grass hay. *Meat Science*. 93 : 455-462.

Renerre, M., Dumont, F., and Gatellier, P. 1996. Antioxidant enzyme activities in beef in relation to oxidation of lipid and myoglobin. *Meat Science*. 43 : 111-121.

Subramaniam, V., Adenan, M. I., Ahmad, A. R., and Shahdan, R. 2003. Natural antioxidants: *Piper sarmentosum* (Kadok) and *Morinda elliptica* (Mengkudu). *Malaysian Journal of Nutrition*. 9 : 41-51.