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# Effects of rice bran oil on qualitative properties of heart and breast muscle tissues in chicken embryo model

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**Abstract**

The purpose of the present study was to investigate the antioxidant properties of rice bran oil on chicken's breast and heart muscles. In this study, rice bran oil (RBO) was injected into the yolk and chorioallantoic membrane in embryonic eggs. Variables such as, thiobarbituric acid reactive substances (TBARS), glutathione (GSH) and ferric reducing antioxidant power (FRAP) were measured. Results showed that the malondialdehyde (MDA) was significantly higher ($P < 0.05$) in breast muscle of control group compared to treated groups. The level of GSH and FRAP were considerably higher either in breast or muscle of that group which has been injected by RBO into the yolk. The total carotenoid content of breast muscle was significantly ($P < 0.05$) lower in control group compared to treated groups. It was concluded that RBO can reduce lipid oxidation and improve oxidative stability in chicken's breast and heart muscles.



## Introduction

Rice bran (RB) is one of the main by products in the rice milling industry that is used as a food supplementation in poultry production (Iqbal *et al*., 2005). Rice bran contains the bran layer and germ of rice and has high fat (13-15%) content (Saunders, 1985). Rice bran Oil (RBO) is extracted by solvent extraction of rice bran. The majority of rice oil is located in the bran and germ (Zigoneanu *et al*., 2008). Antioxidant, hypoallergenic and hypocholesterolemic properties of RB and RBO have been observed in animal and human studies (Posuwan *et al*., 2013 ). The RBO antioxidants are highly effective in diminishing LDL and total cholesterol in serum (Berger *et al*., 2005). RBO contains unsaturated fat (oleic acid and linoleic acid), oryzanols, tocopherols and tocotrienols. The humans and animals do not synthesize their own vitamin E. Therefore, RBO is a good source of vitamin E for humans and animals (Minhajuddin *et al*., 2005). Furthermore several studies reported that oryzanols have cholesterol lowering property in animals and humans (Cicero and Gaddi, 2001; Berger *et al*., 2005; Wilson *et al*., 2007). The RBO is a source of energy and many nutrients for poultry. In addition, RBO is much cheaper than other energy sources and raises the profitability of the poultry industry. It seems that lipid oxidation can be prevented by RBO in poultry muscle, due to having different compounds such as: gamma-oryzanol, tocotrienols, tocopherols and squalene. (Liang *et al*., 2014). Hence RBO can be used as a feed for poultry. The objective of the present study was to determine the effect of RBO on qualitative properties of chicken's breast and heart muscles.

## Materials and Methods

### *Experimental design*

Thirty eggs were collected from a broiler breeder farm. Infertile and damaged eggs were discarded. Eggs were divided into three groups and in day 4, were candled and injected with 0.1 ml of edible grade RBO into the chorioallantoic membrane (group A) and egg yolk (group B). The injection sites were covered by paraffin and the eggs were incubated at 37.5±0.1°C and 50–60% relative humidity. The eggs were candled one day after injection and thereafter every 48 h for checking dead embryos. The experiment was terminated in day 20 of incubation. The tissues were washed with isotonic saline and were stored at -70°C until used for assessing toxicity (Seabra and Bhogal, 2010).

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*Measurement of lipid peroxidation*

The formation of thiobarbituric acid in muscle samples was assessed for the measurement of lipid peroxidation according to an original method (Sadighara and Salar-Amoli, 2009). Briefly, the supernatant of the tissue homogenate was mixed with 20% trichloroacetic acid and the mixture was centrifuged. Then, thiobarbituric acid was added to the supernatant and heated. The absorbance of the supernatant was measured at 532 nm. The values were expressed in nmoles malondialdehyde, using a molar extinction coefficient of $1.56 \times 105M^{-1}cm^{-1}$.

*Measurement of total GSH (Glutathione) assay*

The glutathione content was applied according to the previous method (Gibson *et al*., 1998). The tissues were rinsed three times by PBS. The cell solution mixed with 20% trichloroacetic acid. Samples were centrifuged. The supernatant was mixed with 4 vol of Tris. Then, 1mM DTNB was added to the sample and incubated for 30 minutes. The absorbance was read at 412 nm.

*FRAP (ferric reducing antioxidant power) assay*

The ferric reducing capacity assay measures the ferric reducing capacity. The method was based on a redox reaction in which an easily reduced antioxidant ($Fe^{3+}$) was employed in stoichiometric excess.

*Measurement of total carotenoids of muscle*

The total carotenoid concentrations were measured according to method of Gardner *et al.* (2000), with some modifications. In this method, the total muscle carotenoids were measured using β-carotene standard and spectrophotometry between 350 and 520 nm wavelength. Results were calculated as β-carotene equivalents (Gardner *et al*., 2000).

*Statistical analysis*

All results were expressed as the mean ± SD. Statically analysis was performed by employing Student's t test for unpaired data. Significance was established at the ($P < 0.05$) level. SPSS software (version 19) was used for this purpose.

**Results and Discussion**

In the current study, the major factors which can affect the quality of heart and muscles with RBO were examined. The results were shown in Tables 1 and 2. There was significant difference ($P < 0.05$) between the effects of RBO compared to the control group. Amount of MDA in muscle was significantly ($P < 0.05$) higher in control group in comparison to others groups. There was no significant difference ($P > 0.05$) between the levels of MDA in group A (RBO injected into the chorioallantoic membrane) and group B (RBO injected into the egg yolk). The RBO significantly ($P < 0.05$) reduced lipid oxidation in chicken's muscle by injection to both chorioallantoic and egg yolk Moreover, the amount of MDA in chicken heart muscle was higher in control group. The MDA in heart muscle was significantly ($P < 0.05$) lower compared with muscle group. However, the lowest amount of MDA was observed in heart where RBO injected into chorioallantoic membrane ($0.14 \pm 0.09$ nmol/ g tissue). These results are similar to previous study in restructured beef roasts (KIM and Godber, 2001). The decrease of TBARS values in chicken muscle which has been injected by rice bran oil may be due to the increase of the ratio of other components like tocopherol, tocotrienol, and oryzanol in the products that come from rice bran oil. (Kim *et al.*, 2000). Chae *et al.* (2002), reported that rancid rice bran reduced TBARS value in chicken meat.

The level of glutathione was higher either in muscle or heart in group B (RBO injected to yolk). Furthermore, the lowest level of glutathione was observed in control group. Variance analysis ($P < 0.05$) showed that muscle's glutathione was higher compared to heart group. In the other words, RBO significantly increased glutathione level in muscle. Therefore, Injection of RBO increased glutathione level in muscle and heart chicken. Glutathione is a tripeptide antioxidant that protect tissues against oxidation damages (Kidd, 1997). Oxidation damages can also be eliminated by other antioxidants. Thus, glutathione will be persevered. In this study, the level of glutathione in chicken's muscle and heart which injected by RBO were increased compared to control group. According to the results, it can be concluded that the antioxidant agents in RBO raises the level of glutathione in chicken's muscle and heart. These results also confirmed the antioxidant capacity power of RBO. These results are in accordance with Öztürk-Ürek *et al.* (2001) and Surai (1999).

FRAP assay is a method for measuring the reducing power of antioxidants (Norhaizan *et al.*, 2011). This assay is based on the measurement of the ability of the substance to reduce $Fe^{3+}$ to $Fe^{2+}$ (Firuzi *et al*., 2005). Either in muscle and heart the level of FRAP were higher in group B treatment (RBO injected to egg yolk). The lowest of FRAP were observed in control group. Moreover, variance analysis ($P < 0.05$) showed that FRAP of heart was higher compared with muscle group. The highest GSH and FRAP were significantly higher ($P <$

Table 1. Effect of RBO injected into the yolk and chorioallantoic membrane on chicken breast muscle

| Groups | MDA (nmol/ g tissue) | GSH (µmol/ g tissue) | FRAP (mmol/g tissue) | Total carotenoids (µg/g tissue) |
|---|---|---|---|---|
| Control | $1.93 \pm 0.6^{a}$ | $0.05 \pm 0.02^{a}$ | $1.39 \pm 0.39^{b}$ | $0.12 \pm 0.02^{a}$ |
| Group A | $1.31 \pm 0.26^{b}$ | $0.15 \pm 0.04^{b}$ | $1.59 \pm 0.66^{a}$ | $0.21 \pm 0.05^{b}$ |
| Group B | $1.27 \pm 0.25^{b}$ | $0.18 \pm 0.04^{b}$ | $2 \pm 0.33^{a}$ | $0.28 \pm 0.05^{b}$ |

Values are presented as Mean±SD
[a-b]Means within a column with no common superscripts differ significantly ($P < 0.05$)
Control group: without injection of RBO
Group A: RBO injected into the chorioallantoic membrane
Group B: RBO injected into the yolk

Table 2. Effect of RBO injected into the yolk and chorioallantoic membrane on chicken heart muscle

| Groups | MDA (nmol/ g tissue) | GSH (µmol/ g tissue) | FRAP (mmol/g tissue) |
|---|---|---|---|
| Control | $0.22 \pm 0.15^{a}$ | $0.06 \pm 0.02^{a}$ | $2.25 \pm 0.81^{b}$ |
| Group A | $0.14 \pm 0.09^{b}$ | $0.11 \pm 0.03^{b}$ | $2.28 \pm 0.72^{b}$ |
| Group B | $0.16 \pm 0.08^{b}$ | $0.12 \pm 0.06^{b}$ | $2.85 \pm 0.67^{a}$ |

[a-b]Means within a column with no common superscripts differ significantly ($P < 0.05$)
Control group: without injection of RBO
Group A: RBO injected into the chorioallantoic membrane
Group B: RBO injected into the yolk

0.05) found in the group B (RBO injected into the egg yolk) both in chicken's breast and heart muscle. However, RBO injected into the yolk has a huge impact on both chicken's muscle and heart. RBO is containing antioxidants such as tocopherols, gamma-oryzanol, and phenolic compounds (Chotimarkorn *et al.*, 2008). These compounds reduces $Fe^{3+}$ to $Fe^{2+}$ in muscle and heart chicken. The evidences presented in our study are in accordance with pervious findings (Gavino *et al.*, 2007).

The concentration of carotenoids pigments was calculated using the standard curve obtained by a commercial β-carotene regent. The formula used for the calculation was as follows: $y = 3240.7x + 0.1129$; $R^2 = 0.9974$

There was significant difference ($P>0.05$) between the carotenoid content of control group and treatment groups, though no significant changes were observed in carotenoids pigments of group A and B. The total carotenoid of muscle was significantly ($P > 0.05$) lower in control group. The total carotenoids content dramatically ($P < 0.05$) increased in chicken's muscle by injection RBO to both chorioallantoic and egg yolk. Carotenoids are a group of oil soluble pigments (Sachindra and Mahendrakar, 2005). Carotenoids, as neutral antioxidant pigments in RBO play a protective role on animal tissues from lipid oxidation disruptions (Sadighara *et al.*, 2013). Carotenoids pigments have been accumulated in muscle by injection of RBO into chicken's muscle. Accumulated carotenoids also increase the quality of chicken meat by improving of oxidation stability. The level of total carotenoids in our samples were similar to results of (Surai *et al.*, 1998).

## Conclusion

We have demonstrated that RBO injected either into the yolk or chicken's chorioallantoic membrane was effective in reducing the lipid oxidation of muscle and heart. However, the oxidative stability of chicken's heart has been much more improved by RBO. The injection of rice bran oil into yolk produced a higher glutathione and ferric reducing antioxidant power and lower malondialdehyde in chicken. It should be noted that RBO has a great potential to be used as a natural antioxidant in poultry feed, with this regard, meat quality will be increased. Generally, rice bran oil is a good source of antioxidants and can reduce lipid oxidation in chicken's muscles and heart.

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