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NOVEL ANTIOXIDANT CANOLOL REDUCES GLUCOCORTICOID INDUCED OXIDATIVE STRESS IN BROILER CHICKENS*

By

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Abstract: *Canolol (4-vinyl-2,6-dimethoxyphenol) a potent antioxidant extracted from canola oil was recently identified. Canolol exhibits a strong antialkylperoxyl radical activity more than well-known antioxidants like vitamin E, vitamin C and quercetin. However, the antioxidative effect of canolol has been little studied in vivo. Therefore, the objective of this study was to research the possible antioxidative effects of canolol under oxidative stress in vivo using broiler chickens. Chickens were divided into 4 groups as fellows: control group, stress group (20 mg corticosterone/ kg diet), canolol group (100 mg canolol/ kg diet) and stress + canolol group. Chicks received the dietary treatment from 15 to 29 days of age. The body weight gain was significantly decreased by stress (corticosterone treatment), but canolol retained body weight gain under the stress. The same trend was observed in relative breast muscle weight. Moreover, canolol group was superior to the control group in the non-stressed groups. Stress decreased significantly the feed efficiency, and this effect was minimized by canolol treatment, while feed consumption was not affected by stress and canolol. Canolol significantly reduced the markers of lipid peroxidation and oxidative stress as plasma total cholesterol and tissues TBARS and preserved high α-tocopherol concentrations in liver and muscles. It may be concluded that, canolol enhances the broiler performance under oxidative stress.*

INTRODUCTION

Stress susceptibility is a major problem in the modern intensive animal production. As a result of accelerated metabolic rates under stress conditions resulting from high blood circulatory levels of glucocorticoids and elevated levels of reactive oxygen species (ROS) are formed. ROS plays role in phagocytes to protect animal cells against bacteria and parasites. However, if natural antioxidants are not adequate to quench excess oxygen radicals they react with cell structures and attack proteins, lipids, carbohydrates and nucleic acid within the cell, a state referred to as oxidative stress. The destruction of ROS depends on antioxidants, by the scavenging and the reducing effects of the toxic molecules. Reduction-oxidation recycling of such antioxidants markedly increases their biological efficiency and this needs greater provision of antioxidant nutrients or non nutrients under oxidative stress conditions (Eid *et al.*, 2008).

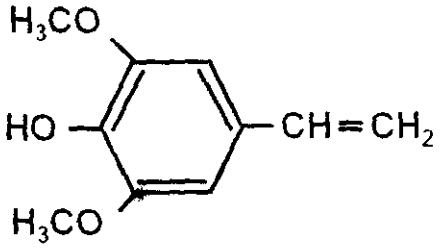

Figure 1. Chemical structure of canolol

Canolol (4-vinyl-2,6-dimethoxyphenol, Figure1) was recently identified as a potent antioxidative compound from crude canola oil (Wakamatsu *et al.*, 2005, Britta *et al*, 2010), exhibiting more potent antialkylperoxyl radical activity than well known antioxidants, like α-tocopherol, vitamin C, β-carotene, rutin and quercetin (Kuwahara *et al.*, 2009). Canolol has strong scavenging capacity against the endogenous mutagen, peroxynitrite, and exerts suppressive effect against bacterial mutation resulting from DNA damage, and prevents oxidation of lipids and proteins (Kuwahara *et al.*, 2004, Cao *et al.*, 2008, Fang *et al.*, 2009).

However, the antioxidative effect of canolol has been little studied In vivo. Therefore, the objective of this study was to research the possible antioxidative effects of canolol under oxidative stress In vivo using broiler chickens as animal model.

MATERIALS AND METHODS

Animals and schedule:

The animal experiment was conducted in accordance with the guidelines of Kagoshima University - Japan. One-day-old male broiler chickens (Chunky strain) were supplied by a commercial hatchery (Kumiai Hina Center, Kajiki, Kagoshima Prefecture, Japan). Chicks were housed in an electrically heated battery brooder. and provided with water and a commercial starter diet (22% CP and 3.000 kcal ME/kg) *ad libitum* for the first 12 day. On day 12, 24 birds of similar body weight (about 346.6 ± 3.8 g) were selected, grouped and housed in individual wire-floored aluminum cages (49 x 39 x 59 cm). The experiments were conducted in a temperature controlled room with 14 h light:10 h dark cycle. The temperature of the room was 25°C and the relative humidity 50 - 70% throughout the experiment.

Experimental diets and feeding:

The basal diet (standard corn-soybean 23% CP and 3,082 kcal ME/kg) was fed *ad libitum* during the pre-feeding period (from 12 to 15 d of age). Thereafter, chicks were divided into 4 groups (n=6): Control group, stress group (20 mg corticosterone/ kg diet) this level proved to induced physiological oxidative stress condition as described earlier (Eid *et al.*, 2003) , Canolol group (100 mg canolol/ kg diet) and Stress + canolol group. The same basal diet was fed to all groups, and the birds were given the experimental diets from 15 to 27 d of age, during this period the growth rate of whole body especially legs and breast muscles are equal, after that age the breast muscle has more accelerated growth rate than the other body parts (Eid *et al.*, 2003). Body weight was recorded at the beginning and the end of the experiment. Feed intake was recorded daily from d 16 to d 27. At the end of the experimental period, all birds were slaughtered.

Sampling:

Blood samples were collected into heparinised test tubes, quickly centrifuged at 5900g for 10 min at 4°C to separate plasma, and stored at -30°C until analysis. The birds were dissected to remove pairs of breast muscles (*Musculus pectorails profundeus* and *Musculus pectoralis superficialis*), abdominal fat and liver.

Biochemical analysis:

Lipid peroxidation in blood plasma and breast muscles was assessed as the concentration of thiobarbituric acid reactive substance (TBARS) (Ohkawa *et al.*, 1979; Richard *et al.*, 1992). Total cholesterol level and triglycerides in plasma were measured by automated Fuji DRY-CHEM 3500 (Fuji Medical Systems, Tokyo, Japan) according to the manufacturer's instructions. The α-tocopherol concentrations of tissue samples (liver and muscles) were determined by HPLC according to the method described by (Faustman *et al.*, 1989).

Statistical analysis:

The differences among treatments were statistically analyzed by general linear model using SPSS Statistics 17.0 (Statistical Packages for the Social Sciences, released 23 August 2008). The significant differences among means of treatments were compared by Duncan's new multiple-range test. $P \leq 0.05$ was set as limit of significance.

RESULTS AND DISCUSSION

Growth performance:

The effects of corticosterone (CTC) as oxidative stress inducer and canolol as antioxidant on chicken performance are shown in Table 1. The body weight gain was significantly suppressed by the stress (corticosterone group), while canolol increased the body weight gain under the same condition. It is well documented that oxidative stress induces growth inhibition; however, this was not caused by a reduction in feed consumption as shown in Table 1. Growth inhibition due to CTC may be explained by decreased muscle protein synthesis and enhanced muscle proteolysis (Hayashi *et al.*, 1994, Eid *et al.*, 2003). Skeletal muscle proteins may be damaged by active oxygen and this may cause stimulations of muscle proteolysis (Hunt *et al.*, 1988). Thus, canolol recovered growth suppression significantly under stress condition. The breast muscle weight was increased significantly by canolol under normal condition compared to control and also the same trend was observed under stress condition. Feed conversion ratio was significantly impaired by stress and canolol negated that effect of stress. As a natural result of high levels of circulatory glucocorticoids, the relative weights of liver and abdominal fat were increased under oxidative stress. The same results were obtained by (Eid *et al.*, 2003), the same trend was observed in the relative weight of liver and abdominal fat (Table 1). Canolol seemed to recover the increase in liver relative weight, but it had no significant effect on relative abdominal fat weight.

Table 1: Effects of corticosterone (CTC) and canolol on the performance of broiler chickens. Values are expressed as means ± standard error*.

	Control		Canolol	
CTC	-	+	-	+
Body weight gain (g/12day)	553.8 ± 25.7 [a]	284.2 ± 19.0 [c]	567.0 ± 24.8 [a]	342.5 ± 16.6 [b]
Feed consumption (g/12 day)	739.8 ± 43.5	706.0 ± 39.9	824.9 ± 31.7	708.4 ± 55.0
Feed conversion ratio	1.3 ± 0.03 [c]	2.5 ± 0.08 [a]	1.5 ± 0.02 [c]	2.07 ± 0.12 [b]
Breast muscle (%) **	15.0 ± 0.7 [b]	12.7 ± 0.5 [c]	17.2 ± 0.5 [a]	13.8 ± 0.5 [bc]
Liver (%) **	2.2 ± 0.06 [b]	2.6 ± 0.16 [a]	2.1 ± 0.05 [b]	2.4 ± 0.26 [ab]
Abdominal fat (%) **	0.31 ± 0.03 [b]	1.19 ± 0.09 [a]	0.32 ± 0.06 [b]	1.38 ± 0.21 [a]

*Means with different superscripts in the same row differ from each other ($P \leq 0.05$). ** Percent of live body weight.

Biochemical parameters:

The effects of stress and canolol on plasma total cholesterol and triglycerides are presented in figure 2. Glucocortiods treatment significantly increased plasma total cholesterol and triglycerides comparing to the normal group. Glucocorticoid treatment develops insulin resistance, hyperglycaemia, hypertriglyceridemia, resulting in increasing abdominal fat content, fatty liver and high level of circulatory lipids (Eid *et al.*, 2003). The same trend was observed in this study. Under stress condition canolol significantly reduced plasma total cholesterol and triglycerides comparing to control group. Surprisingly, canolol reduced plasma total cholesterol under normal condition comparing to the control. However, the author currently has no data to explain the possible mechanism for this novel finding.

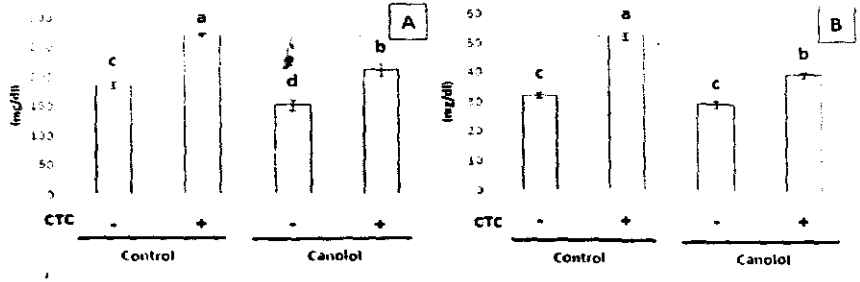

Figure 2: Effect of corticosterone (CTC) and canolol on plasma total cholesterol (A) and triglycerides (B) in broiler chickens. Values are expressed as means ± standard error; means with different superscripts differ from each other ($P \leq 0.05$).

These excessive lipids in the body are susceptible to the attacks of free radicals generated by stress. TBARS is the end product of lipid peroxidation and thus used as indicator of oxidative stress. Figure 3 shows the identical trend of both plasma and muscle TBARS as affected by stress and canolol. Canolol successfully reduced TBARS level comparing to control under both normal and stress condition Figure 3. The positive effects of canolol could be attributed to its' strong radical scavenging activity (Cao *et al.*, 2008, Fung *et al.*, 2009).

Interestingly, canolol significantly preserved high levels of α–tocopherol in both liver and muscle tissues (Figure 4) either under normal or stress condition. This may be due to the strong antioxidative properties of canolol to quench the free radicals generated by oxidative stress condition. The potent antioxidative properties of canolol in this study could be explained by two mechanisms. First, canolol has a high scavenging activity against ROS. Secondly, canolol spare α–tocopherol utilization in the tissues which may help to exert more potent antioxidative properties against ROS attacks.

From these results, it is expected that canolol can improve the quality of poultry meat because lipid oxidation leads to meat spoilage and cause adverse changes in meat quality (Kennedy *et al.*, 2005). The quality of chicken meat can be improved by antioxidant in feed. The pressure to reduce artificial additives use in foods has led to attempts to increase meat quality and stability by dietary strategies in recent years (Morrissey *et al.*, 1998). Thus dietary ingredients which have strong antioxidative capacity with minimal or no negative effects on production or on the quality of the product have received much attention from researchers. Consequently, it has been found that many feed additives and antioxidants, such as green tea polyphenols, vitamin E and vitamin C are effective in decreasing lipid peroxidation and oxidation (Eid *et al.*, 2003, 2008, Young *et al.*, 2003; Brenes *et al.*, 2008). Canolol is a natural antioxidant extracted from crude canola oil and thus using canolol could be a successful strategy in this aspect.

It could be concluded that, enhanced broiler performance, decreased in plasma lipids and lipid peroxidation, reserved tissue contents of α–tocopherol were observed with canolol treatment under oxidative stress condition. However, further studies are needed to clarify the most effective dose and the possible methods of application.

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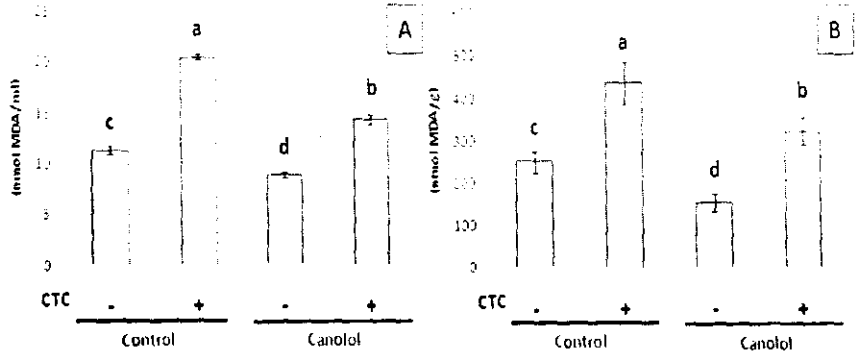

Figure 3: Effect of corticosterone (CTC) and canolol on TBARS in plasma (A) and muscles (B) in broiler chickens. Values are expressed as means ± standard error; means with different superscripts differ from each other ($P \leq 0{\cdot}05$).

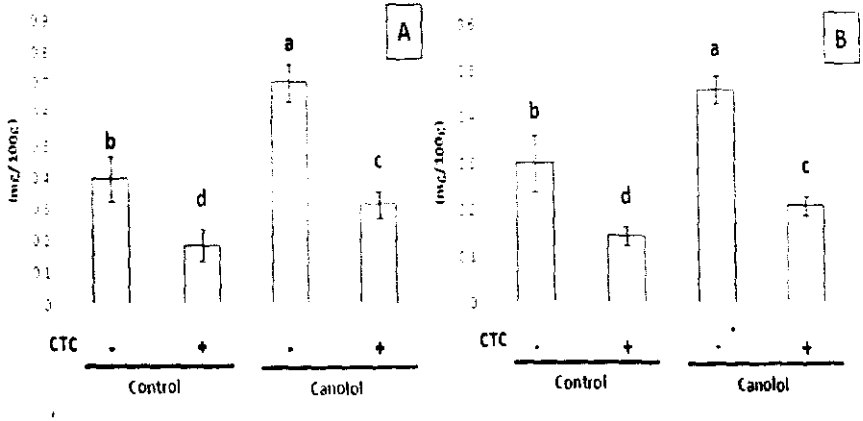

Figure 4: Effect of corticosterone (CTC) and canolol on α-tocopherol concentrations in liver (A) and muscles (B) in broiler chickens. Values are expressed as means ± standard error; means with different superscripts differ from each other ($P \leq 0{\cdot}05$).

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الملخص العربى

مضاد الأكسدة المكتشف حديثا (كانولول) يقلل من الإجهاد التأكسدي المستحدث بالجلوكوكورتيكويدز في دجاج التسمين

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الكانولول مضاد قوي وفعال للتأكسد مستخرج من زيت الكانولا تم إكتشافه حديثا. يعتبر الكانولول في صفاته الضد تاكسديه أكثر فاعليه من معظم مضادات الأكسدة المعرفة مثل فيتامين هـ و سي و كيرسيتين. و ثبت أنه يثبت بقوه الطفرات البكتيرية و يحمي الحامض النووي و يمنع البروتينات و الدهون من الأكسدة. ولكن التأثيرات الضد تأكسديه للكانولول لم يتم دراستها بصوره كافيه داخل الجسم الحي ككل. لذلك فان الهدف من هذه الدراسه هو بحث التأثيرات الضد تأكسديه المحتمله للكانولول داخل الجسم الحي تحت ظروف الإجهاد التأكسدي بإستخدام دجاج التسمين. تم تقسيم الدجاج إلى أربع مجموعة ، مجموعة المقارنه و مجموعة الإجهاد (تمت معاملتها بي ٢٠ مج كورتيكوستيرون /كجم علف) و مجموعة الكانولول (تمت معاملتها بي ١٠٠ مج كانولول /كجم علف) و المجموعة الأخيره كانت خليط بين الإجهاد والكانولول. تلقت الطيور العليقة المعاملة من عمر ١٥ حتى ٢٩ يوم وكان أهم النتائج المتحصل عليها انخفاض معنوي في وزن الجسم المكتسب قابلها زياده في مجموعة الكانولول تحت ظروف الإجهاد. و كان هذا هو الاتجاه الملاحظ بالنسبة لعضلات الصدر بل علاوة على ذلك كانت مجموعة الكانولول متفوقة على المجموعة المقارنة تحت الظروف الطبيعيه. قلل الإجهاد من الكفاءه الغذائية لكن الكانولول عدل من هذا التاثير بينما الاستهلاك الغذائي لم يتاثر سواء بالإجهاد أو الكانولول. حد الكانولول معنويا من نسبة الكوليسترول الكلي في الدم و نسبة TBARS في الدم والعضلات وحافظ على مستويات مرتفعة من فيتامين هـ في العضلات و الكبد كادلائل على الحالة الضد تأكسديه في الجسم. و يمكن إيجاز النتائج المتحصل عليها في أن استخدام الكانولول حسن من أداء دجاج التسمين تحت ظروف الإجهاد التأكسدي.