Antioxidant levels of New Zealand White rabbits on Feeding of Cabbage waste

Karu. Pasupathi* and M.R.P.Purushothaman
*Assistant professor, Institute of Animal Nutrition, Kattupakkam – 603 203,
Tamil Nadu Veterinary and Animal Sciences University, Kancheepuram District
drkarupasupathy@gmail.com

Abstract— The study was carried out to evaluate the effect of cabbage waste on thyroid prifile of New Zealand White breed of Broiler Rabbits. Thirty weaned New Zealand White breed of rabbits aged between seven to eight weeks were randomized into 5 treatments with 6 replicates (one male and five females) in each. The treatments were concentrate (rabbit) feed with desmanthus (T1), concentrate with 50% cabbage and 50% desmanthus (T2), concentrate with 100% cabbage (T3), concentrate with 50% cabbage and 50% desmanthus with 100 µg per day per animal of iodine supplementation (T4), concentrate with cabbage and 100 µg per day per animal of iodine supplementation (T5). The iodine was provided as potassium iodide containing 76.9 per cent iodine. The iodine salt (130 µg per day) was mixed in the concentrate feed offered to each animal. The MDA(nmol ml^{-1}), Reduced Glutathione (µmol dl^{-1}), SOD (U dl^{-1}), Glutathione peroxide (mmol $litre^{-1}$) and Vitamin E (µg ml^{-1}) were not affected by supplementation of Iodine in cabbage waste fed rabbits.

Key words : Broiler rabbits, Antioxidant levels, cabbage waste, Desmanthus virgatus, Iodine supplementation

I. INTRODUCTION

Broiler rabbits are the alternate animal protein source for the developing countries like India because of its high prolificacy. Recent All India Livestock Population statistics showed that the rabbits population is increasing by 37.33 per cent compared to previous census on livestock though an overall reduction in livestock population of 3.33 per cent [1]. New Zealand breed rabbits are better suited to our wide varying Indian climatic condition. Most of the farmers involved in rabbit farming are landless labours feeding their rabbits with vegetable waste available at cheaper cost and throughout the year. Very few farmers are feeding their rabbits with concentrate mixture and cultivated fodders like Bajra Napier hybrid grass or *Desmanthus virgatus*. The vegetable waste predominantly consists of cabbage head and waste. In India it was estimated that 2.81 MT cabbage head and leaves are being wasted every year [2]. Since the cabbage waste contains glucosinolates,, an antinutritional factor which affect the iodine uptake and thereby the thyroid function, influence the productive and reproductive performances, the present research work was carried out to study the influence of cabbage waste with or without iodine supplementation on antioxidant levels of rabbits.

II. MATERIAL AND METHODS

A. Animals and study place

The animals for the study were taken from Rabbit Breeding Unit of Post Graduate Research Institute in Animal Sciences, Kattupakkam, Tamilnadu.

The rabbits were maintained in cage system of rearing. The male and females were housed in individual Galvanised Iron cages with the dimension of 2' x 1.5' X 1.5' kept above 3 feet from the ground level. The side walls were constructed up to 1' height and above that 1" weld mesh was placed. Fresh air was circulated in the rabbit house by using exhaust fans.

Thirty weaned New Zealand White breed of rabbits aged between seven to eight weeks were randomized into 5 treatments with 6 replicates (one male and five females) in each. The treatments were concentrate (rabbit) feed with desmanthus (T1), concentrate with 50% cabbage and 50% desmanthus (T2), concentrate with 100% cabbage (T3), concentrate with 50% cabbage and 50% desmanthus with 100 µg per day per animal of iodine supplementation (T4), concentrate with cabbage and 100 µg per day per animal of iodine supplementation (T5). The iodine was provided as potassium iodide containing 76.9 per cent iodine. The iodine salt (130 µg per day) was mixed in the concentrate feed offered to each animal.

At the end of the growth trial, the blood was collected from the ear vein of all the six rabbits in a treatment. The blood was allowed to clot and serum was separated and estimated for antioxidant profile

a. Superoxide dismutase: Serum superoxide dismutase level was measured by the method of [3] which was based on the degree of inhibition of pyrogallol auto-oxidation in an alkaline pH and read at 470 nm.

Tris buffer (containing 50 mmol of Tris buffer and 1 mmol of EDTA), 50 ml was prepared. To this, 50 ml HCl was added to adjust the pH to 8.5 and volume was made up to 100 ml. Pyrogallol (20 mmol concentration) standard was prepared with 25 mg of pyrogallol in 10 ml of distilled water.

Serum sample 0.2 ml, 2.0 ml Tris buffer, 2.3 ml water and 0.5 ml of pyrogallol was added and absorbance was read at 470 nm.

b. Glutathione peroxidase: Glutathione peroxidase activity was measured according to [4] which was based on the property of glutathione peroxidase to reduce lipid hyperoxides to their corresponding alcohols and to reduce free hydrogen peroxide (H2O2) to water by oxidizing glutathione that was measured at 412 nm.

Tris buffer 0.2 ml, 0.2 ml EDTA, 0.1 ml sodium azide and 0.2 ml of serum were added and mixed well. To this, 0.2 ml

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of GSH (2 mmol) followed by 0.1 ml of H2O2 were added. The contents were mixed and incubated at 37oC for 10 minutes, along with a control containing all reagents except enzyme. After 10 minutes the reaction was arrested by the addition of 0.5 ml of 10% TCA. The tubes were centrifuged and the supernatant were assayed for GSH.

c. Reduced glutathione: Reduced glutathione was measured according to the method as described by [5] which was based on the reaction with 5,5'- dithio tris –12-nitrobenzoic acid (DTNB or Ellman's reagent) to give an yellow colour compound that was read at 412 nm.

A known weight of tissue was homogenized in phosphate buffer. From this 0.5 ml was pipetted out and precipitate with 2.0 ml of 5 per cent TCA and centrifuged. To 1.0 ml of the supernatant, was added 1 ml of Ellman's reagents and 3.0 ml of phosphate buffer. The yellow colour was developed was read at 412nm. A series of standards were treated in a similar manner along with a blank containing 3.5 ml of buffer.

d. Serum malondialdehyde: Serum malondialdehyde (MDA) was measured according to [6]. The serum MDA content was quantified by its reaction with 2-thiobarbituric acid (TBA) which forms a yellow coloured complex with the maximum absorbance at 532 nm.

e. Serum vitamin E : Vitamin E was estimated as per the method of [7]. Plasma (0.4 ml) was pipetted into a test tube. An equal volume of purified absolute ethanol was added to the tube for protein precipitation. The contents were immediately mixed with a vortex mixer. Then 0.4 ml of xylene was added and the test tube was mixed for at least 30 seconds and centrifuged for 5-10 min at 3000 rpm. After centrifugation the upper xylene layer which contains the extracted tocopherol was collected. Plasma-xylene extract (0.2 ml) was pipetted into a test tube containing 0.1 ml of bathophenanthroline (0.4% in ethanol), 0.1 ml ferric chloride (0.06% in ethanol) was added, followed by 0.1 ml of orthophosphoric acid (0.5% in ethanol). The contents of the tube were mixed thoroughly using a vortex mixer after every addition of reagents. Absorbance was read using spectrophotometer at 536 nm after setting the instrument to zero absorbance with a blank (prepared by using 0.2 ml xylene instead of plasma-xylene extract).

The data collected were analyzed using statistical software package SPSS[17].

III. Results and Discussion

The level of melondialdehyde, reduced glutathione, super oxide dismutase, glutathione peroxidase and vitamin E were not statistically different on inclusion of cabbage at 50 and 100 per cent replacement of desmanthus. Supplementation of iodine did not decrease the melondialdehyde, nor reduce the level of reduced glutathione, super oxide dismutase, glutathione peroxidase and vitamin E. The Vitamin E level of cabbage fed group with / without iodine supplementation was higher (P>0.05) than the control group (1.06 – 1.13 *vs.* 0.99 µg ml^{-1}). This result differs with the early reports wherein cabbage extract were either subcutaneously injected or orally provided resulting in reduction in MDA and increase in super oxide dismutase and glutathione peroxidase. Though cabbage contains glucosinolates and the level of glucosinolate consumed by the animals in the 50 and 100 per cent cabbage group was 82.50 and 158.43 g still the antioxidant property of this glucosinolate as explained by Prestra *et al.* (1993) and Nilnakara *et al.* (2009) was not appreciated in the serological antioxidant parameters.

Table 1 Antioxidant levels in serum of rabbit fed cabbage waste at higher levels with / without iodine supplementation (Mean ±SE)

	Group – I	Group – II	Group – III	Group – IV	Group – V
MDA (nmol ml^{-1})	2.21± 0.06	2.22± 0.02	2.24± 0.09	2.21± 0.04	2.23± 0.03
Reduced Glutathione (µmol dl^{-1})	43.30± 0.41	44.15± 0.60	43.75± 0.31	44.71± 0.35	44.18± 0.54
SOD (U dl^{-1})	93.48± 0.23	93.77± 0.36	93.73± 0.52	93.56± 0.49	92.85± 0.37
Glutathione peroxide (mmol $litre^{-1}$)	18.47± 0.13	18.71± 0.14	18.77± 0.18	18.71± 0.09	18.15± 0.21
Vitamin E (µg ml^{-1})	0.99± 0.04	1.13± 0.06	1.11± 0.05	1.06± 0.08	1.09± 0.03

Each value is the mean of six observations
Group I – Concentrate + 100 % *Desmanthus virgatus* fed group
Group II – Concentrate + 50 % Cabbage + 50% *Desmanthus virgatus* fed group
Group III - Concentrate + 100 % Cabbage fed group
Group IV - Concentrate + 50 % Cabbage + 50% *Desmanthus virgatus* + 100 µg Iodine supplemented group
Group V - Concentrate + 100 % Cabbage + 100 µg Iodine supplemented group

IV. Conclusion

This study revealed that supplementation of iodine in cabbage fed diet did not influence the antioxidant activity in broiler rabbits.

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