ANTIOXIDANT SUPPLEMENTATION EFFECTS IN ANIMALS: FINDINGS FROM PREPUBERTAL, PREGNANT, LACTATING AND NON-BREEDING RABBITS

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ABSTRACT
Antioxidant supplementation can alleviate stress arising from the environment, production systems, and animal functional metabolic activities at different physiological stages. Therefore, in this study, a microalga known as *Chlorella vulgaris* was supplemented to rabbits as an antioxidant at different physiological stages while their performances and oxidative stress attenuation mechanisms were monitored. In the prepubertal and gestation phases, forty rabbits were investigated; in the lactation phase, 35 rabbits with equal number of kits were studied while in the non-reproducing phase, forty rabbits were studied. At each phase of the study, rabbits were divided into five groups and rabbits in each of the groups were individually supplemented with 0, 200, 300, 400 and 500 mg *Chlorella vulgaris* biomass per kg bodyweight as Control, T1, T2, T3, and T4 groups respectively. There was significant effect of the supplementation on the rabbits' weight gain ($p < 0.05$), reproductive index ($p < 0.05$), feed intake ($p < 0.05$), carcass traits and meat yield ($p<0.05$), antioxidant enzymes activities and gene expression patterns ($p < 0.05$). Evaluation of these outcomes led to the conclusion that the microalga supplementation led to improved performances of the rabbits and protected the rabbits against oxidative stress at different physiological reproductive stages, hence it was recommended as suitable antioxidant supplements for rabbits while its suitability as antioxidant in other food producing animals was suggested.
Keywords: Antioxidants, *Chlorella vulgaris*, gene expression, oxidative stress and rabbits.

INTRODUCTION
Reproduction is arguably a metabolic and performances burden to animals as some group of scientists agreed it is a burden while some claimed it is not; however, either agreed or disagreed, reproduction is known to increase animals' nutrients demands in breeding animals as well as the modification of the alimentary tract in response to the increase in nutrients demand and intake (Hammond, 1997). It is also agreed upon that there is always an increase in the thermoregulatory demand of breeding animals which usually get extremely higher during lactation period due to the need by animals to produce milk for consumption of the younger ones (Heldmaier *et al*. 2004).
Reproductive specific to rabbits is that there is usually a reduction in physical activities in breeding does compared with dry does (Fernandez-Carmona *et al*. 2010). It was also reported that there is bone loss due to increased calcium supply to the foetus and offspring during gestation and lactation periods (Sarli *et al*. 2005). All these complications are almost inevitable in food producing animals due to reproduction or functional production performances; however, underlying these complications is oxidative stress a condition of imbalance between prooxidant and antioxidant in animals occasioned by rigourous energy demand and other activities which occur all the time to keep an animal suitable for production; therefore exogenous antioxidant aimed at enhancing internal antioxidant defence system become modern animal production exploit. In this study, a microalga rich in antioxidant was supplemented to rabbits in fulfillment of exogenous antioxidant supplementation hypothesis.

MATERIALS AND METHODS
In the prepubertal and gestation phases, forty rabbits were investigated; in the lactation phase, 35 rabbits with equal number of kits were studied while in the non-reproducing phase, forty rabbits were studied. At

each phase of the study, rabbits were divided into five groups and rabbits in each of the groups were individually supplemented with 0, 200, 300, 400 and 500 mg *Chlorella vulgaris* biomass per kg bodyweight as Control, T1, T2, T3, and T4 groups respectively. Approval for use of the animals and the experimental protocol was obtained from joint approval of Institutional Animal Ethics Committee (IAEC) of National Institute of Animal Nutrition and Physiology, India and Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India. The animals fed commercial rabbits feed and provided with water *ad-libitum* (Table 1); while they were supplemented with microalgae biomass intake as antioxidant supplements. The animal house was maintained at normal room temperature while electric blowing fan was provided for emission of excess heat generated and prevention of stickiness.
The prepubertal stage lasted for 60 days, gestation 35 days, lactation 21 days, and non-breeding stage 120 days; during each of the stages, blood was obtained from each animal through puncturing of auricular vein using 26G needle and 2mL hypothermic syringes (Hindustan Syringes & Medical Devices (P) Limited, India). Blood samples were collected early morning between 6.30 and 8.30 am and the animals were hand restrained while surgical spirit was used for wiping (Om Shiva Pharma Industries, India). Blood samples collected were allowed to clot at room temperature before they were transported on ice pads to the laboratory for serum separation. Clear serums were obtained by centrifuging whole blood at 2000 rpm (Remi NEYA 16R, REMI India); the centrifuge was done at 4 ^{o}C for 15 minutes period for each sample and the serums were obtained into Eppendorf tubes packed in cryo box (Tarsons Products Pvt. Ltd, India) then stored at -80 ^{o}C for downstream analysis. Concentration of malondialdehyde in the serum was used as marker of lipid peroxidation was assayed according to procedures described by (Ohkawa *et al*, 1979). Serum biochemical parameters including enzymes such as alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), creatinine, alkaline phosphatase (ALP) and bilirubin were determined using commercial kits; while antioxidant enzymes activities, gene expression patterns and other analysis were carried out as described in Sikiru *et al*, 2019a, Sikiru *et al*, 2019b and Sikiru *et al*, 2019c respectively.

RESULTS AND DISCUSSION

The supplementation of *Chlorella vulgaris* has antioxidant in this study significantly led to improvements in the rabbit production and functional performances; from feed intake, growth rate, carcass traits and oxidative stress protection via reduction of oxidative stress biomarkers and enhanced antioxidant enzymes activities as presented in the tables 2 to 6 below. The findings as revealed in this study gave a strong support to practical use of microalga *Chlorella vulgaris* as in rabbits because it improved the rabbits performance through attenuation of oxidative stress products of lipid peroxidation, enhanced antioxidant activities using its components including carotenoids and polyphenols for prooxidants scavenging, these finding agreed with earlier studies on the alga as reported (Pieretti and Meineri, 2009) who stated that it improved feed nutrients digestibility and promotion of growth rates; similarly, the microalga was also reported to improved meat qualities, reproductive performance and functional attributes of rabbits and mice models (Meineri *et al*, 2009; Kang *et al*, 2004). This study demonstrated that oxidative stress is an inevitable biochemical process in animal performance and its implications transcend beyond physiological loss to economic loss; meanwhile, supplementation of microalga *Chlorella vulgaris* in this study ameliorated the production inefficiencies as well as enhanced the animals' physiological performances and also showed molecular oxidative stress protection through up-regulation of antioxidant genes through expression modification.

Table 1: Nutritional compositions of the basal feed fed to the rabbits at all stages of the experiment

Parameters	Amount	Units
Dry Matter	90.89	%
Crude Protein	18.55	% dry matter
Total Ash	7.90	% dry matter
Crude Fibre	9.73	% dry matter
Ether Extract	2.99	% dry matter
Metabolizable Energy	2700	Kcal/kg

Parameters	Mean ± SEM	p - values
Final body weight (g)	2092.03 ± 20.74	0.01
Body weight gain (g)	1156 ± 22.54	0.003
Total feed intakes (g)	5902.84 ± 116.66	0.56
Feed conversion ratio	5.20 ± 0.01	0.003

Table 2: Effects of Chlorella vulgaris as antioxidant supplement on prepubertal rabbit performances

Table 3: Effects of *Chlorella vulgaris* supplement on oxidative stress status of the prepubertal rabbits

Parameters	Mean ± SEM	p - values
Malondialdehyde concentration (nmol/ml)	11.62 ± 2.03	0.001
Total antioxidant capacity (µmol/ml)	100.00 ± 22.4	0.003
Superoxide dismutase activity (U/ml)	79.42 ± 5.47	0.001
Catalase activity (U/ml)	28.88 ± 3.38	0.003
Reduced glutathione concentration (µmol/ml)	32.80 ± 2.04	0.001

Table 4: Effects of *Chlorella vulgaris* as antioxidant supplement on performances of the pregnant rabbits

Parameters	Mean ± SEM	p - values
Total feed intake (g)	2911.59 ± 46.78	0.08
Gestation gain (g)	362.02 ± 21.88	0.21
Litter size (count)	5.00 ± 1.00	0.67
Viability rate (%)	74.71 ± 7.14	0.20
Gestation length (days)	31.61 ± 0.27	0.03

Table 5: Effects of *Chlorella vulgaris* supplement on oxidative stress status of the pregnant rabbits

Parameters	Mean ± SEM	p - values
Malondialdehyde concentration (nmol/ml)	15.36 ± 1.23	0.003
Total antioxidant capacity (µmol/ml)	61.09 ± 10.06	0.02
Protein carbonyl (µmol/ml)	12.15 ± 13.41	0.66
Superoxide dismutase activity (U/ml)	9.06 ± 0.34	0.04
Catalase activity (U/ml)	9.59 ± 0.32	0.005
Reduced glutathione concentration (µmol/ml)	63.12 ± 1.17	0.001

Table 6: Effects of *Chlorella vulgaris* as antioxidant supplement on performances of the pregnant rabbits

Parameters	Mean ± SEM	p - values
Total feed intake (g)	2728.60 ± 53.48	0.48
Lactation loss (g)	115.54 ± 29.15	0.12
Pre-weaning litter weight (g)	574.65 ± 45.86	0.009
Milk yield (g)	1333.12 ± 77.47	0.001
Body condition score	2.30 ± 0.14	0.009

Table 7: Effects of *Chlorella vulgaris* as antioxidant supplement on performances of the non-breeding rabbits

Parameters	Mean ± SEM	p - values
Daily feed intake (g)	100.56 ± 1.22	0.01
Initial weight (g)	1060.00 ± 29.42	0.17
Final weight (g)	2383.00 ± 40.99	0.008
Feed conversion ratio	10.32 ± 0.45	0.32
Empty Carcass weight (g)	1801.74 ± 52.78	0.001
Commercial Carcass weight (g)	1419.72 ± 34.36	0.001

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