Original Research

Effects of *Chlorella vulgaris* Supplementation on Performances of Lactating Nulliparous New Zealand White Rabbits Does and Their Kits

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Abstract

Thirty-five lactating New Zealand white rabbits were divided into five experimental groups (n = 7) each with equal number of kits (n = 5). Control group was not supplemented while treatment groups were supplemented with 200, 300, 400 and 500 mg Chlorella vulgaris biomass per kg body weight daily. Daily feed intakes, weekly body weight changes and body condition scores were recorded. Body conditions scores was 2.30±0.14 (p < 0.009), milk yield 1333±77.47 g (p < 0.001), litter weight at 21 days was 574.65±45.86 (p < 0.001) while feed intake was 97.45±1.91 (p < 0.48). We concluded from the results that supplementation of the microalgae improved performances of the lactating rabbits and their kits; hence, it was recommended for supplementation at 400 mg per kg body weight in lactating rabbits for promotion of improved performances in both the rabbit does as well as their kits.

Key words: Body Condition Score, *Chlorella vulgaris*, Litter Weight Gain, Milk Yield

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Introduction

Reproduction is necessity for continuous existence of animals and maintenance of their economic importance in food production and supply for human consumption; but reproduction is a physiological burden on their performances and welfare. Lactation is an example of reproduction burdens on animals; in rabbits, physiology of reproduction supports both gestation and lactation at the same time and this is common breeding system employed in rabbit production in most commercial rabbit farms around the world (Latu *et al.*, 2017). Meanwhile, this system of breeding is a potential source of stress because it causes negative energy balance resulting from mobilization of body energy reserves for milk production for the kits; hence, deliberate and special nutritional management such as antioxidant supplementation was suggested as way out especially in young rabbit does (Abdel-Aal *et al.*, 2016).

Lactation causes either positive or negative energy balances; and extreme negative energy balance reduces animal performance as well as impact negatively on the animals' welfare (Hall *et al.*, 2012). Oxidative stress positively correlate with lactation via increased formation of malondialdehyde (MDA) in high milk producing cattle (Zachut *et al.*, 2016); while at different physiological stages under different environmental conditions in rabbits, lactation was reported to have a positive correlation with oxidative stress; hence, supplementation of antioxidant become essentially necessary in rabbit to increase productive capacity especially during lactation period (Meineri *et al.*, 2017; and Jimoh *et al.*, 2017). In this study we supplemented *Chlorella vulgaris* – a green microalgae with enormous antioxidant compounds to lactating nulliparous rabbit does with a view of improving performance of the rabbits by preventing weight loss; and improve their welfare by promoting optimum body conditions as well as improved performance of their kits at pre-weaning stage when the kits depends entirely on the does milk yield.

Materials and Methods

Thirty-five nulliparous New Zealand white rabbits were used for this study; the animals were divided into five experimental groups (n = 7); each group comprises of four nulliparous rabbit does who kindled on the same day and standardized to have equal number of kits (n = 5) per doe. The rabbits in control were not supplemented while the rabbits in T1, T2, T3 and T4 were supplemented with 200, 300, 400 and 500 mg of *Chlorella vulgaris* biomass per kg body weight. The animals were housed individually in cages; water was provided *ad-libitum* while commercial pelletized rabbit feeds (Krishna Valley Agrotech LLP; India) was provided as basal diet, the nutrients composition of the feed is presented in Table 1.

Table 1: Nutritional composition of basal diet fed to the rabbits during the experiment

Parameters	Amount	Unit
Dry matter	90.89	% dry matter
Crude protein	18.55	% of dry matter
Total ash	7.9	% of dry matter
Metabolizable energy	2700	kcal/kg
Crude fibre	9.73	% of dry matter
Ether extract	2.99	% of dry matter

Source: Laboratory analysis

Daily records of feed intakes per animal were taken, body weight changes were recorded weekly by determination of differences in weight of the animals for the current week and the previous week; body condition scorings were done weekly as well for each animals throughout the lactating period of eight weeks; parameters used for body condition characteristics scoring of the animals is presented in Table 2.

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Table 2: Body condition score characteristics of the nulliparous lactating rabbits

Score	Description	Characteristics of the animals' body conditions
1	Very thin	Rabbits with more than 20 % body weight less than normal body weight of lactating and non-lactating rabbits. Sharp hip bones, ribs and spine; curved rump area.
2	Thin	Rabbits with 10 - 20 % body weight less than normal body weight of lactating and non-lactating rabbits. Flat rump area; easily felt ribs, spine and hip bones.
3	Ideal	Rabbits with normal body weight of lactating and non-lactating; the animals have flat rump without sharp spine.
4	Overweight	Rabbits with 10 – 15 % above normal body weight of lactating and non-lactating rabbits; easily felt hip bones, ribs and spine.
5	Obese	Rabbits with 10 – 15 % above normal body weight of lactating and non-lactating rabbits; very hard to feel spine and hips, ribs cannot be felt and rump area bulges out.

Source: Prebble, J. L., Shaw, D. J., & Meredith, A. L. (2015)

Milk yield production per does in gram was determined according to procedures described by Fortun-Lamothe and Sabater (2003) as reported by Maertens *et al.* (2006); it involves estimation of the kits growth rates due to weight changes in relation with their milk consumption quantity within the first 21 days of the kits lifetime using the formula below:

$$MY = 1.69 \text{ X } WGL + 362 \text{ …. (Equation i)}$$

Where
MY = Milk Yield in g for lactation period 0 – 21 days
WGL = Weight Gain of the Litter in g for lactation period 0 – 21 days

Data Analysis

Data obtained were subjected to one-way analysis of variance using SPSS version 20.0 (IBM, USA), significant differences among means were determined at $p < 0.05$ and mean separation was done using Duncan test of post-hoc tools in the software. Non-parametric analysis of variance was conducted in order to determine relationship between the supplementation and panel of lactation performances parameters for ranking of the rabbits' overall performance during the lactation period as results of the microalgae supplementation using Kruskal-Wallis test.

Results

There was significant difference in body condition scores ($p < 0.009$) of the rabbits; Mean body condition score was 2.30±0.14 while minimum and maximum body condition scores were 1.00 and 3.00 respectively. There was also significant difference in milk yield capacity of the rabbit does ($p < 0.001$); Mean milk yield was 1333.12±77.47 g, while minimum and maximum milk yields were 584.07 and 1802.73 respectively. However, there was no significant difference ($p < 0.124$) in lactation loss or gain of the rabbit does mean

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lactation gain/loss was 115.54±29.15 g; minimum gain/loss was -208.40 g while maximum gain/loss was 462.60 g. There was significant difference ($p < 0.009$) in litter weight gain at 21 days post kindling when the rabbit kits are exclusively dependants on the does milk yield; mean litter weight gain was 574.65±45.86 g, while minimum and maximum litter weight gains were 131.00 and 853.00 g respectively. There was no significant difference ($p < 0.477$) in the rabbit does feed intake during the lactation period, mean feed intake was 97.45±1.91 g while minimum and maximum feed intakes were 75.59 g and 106.410g respectively (Table 3).

Table 3: Effects of *Chlorella vulgaris* supplement diets on lactation performance of the rabbit does and kits

Parameters	Control	T1	T2	T3	T4	SEM	p - value
Body conditions score	1.50^{a}	2.00^{ab}	2.75^{b}	2.75^{b}	2.50^{b}	0.14	0.009
Milk yield (g)	1118.00^{a}	1533.25^{b}	1570.00^{b}	1565.25^{b}	879.25^{a}	77.47	0.001
Litter weight at 21 days (g)	447.50^{a}	693.00^{b}	715.00^{b}	712.00^{b}	305.75^{a}	45.86	0.001
Daily feed intakes (g)	102.87	97.92	92.27	99.56	94.64	1.91	0.48

[a,b,c] Means with different superscript in the same row are different (p < 0.05) for the parameters measured; Control-group without supplement diet; T1-group supplemented with 200 mg/kg BW Chlorella vulgaris biomass; T2-group supplemented with 300 mg/kg BW Chlorella vulgaris biomass; T3-group supplemented with 400 mg/kg BW Chlorella vulgaris biomass; T4- group supplemented with 500 mg/kg BW Chlorella vulgaris biomass

Kruskal-Wallis relational non-parametric analysis of variance for determining interaction between the supplementation of the *Chlorella vulgaris* and cumulative performance of the rabbit does and their kits indicated there was no significant differences for feed intake ($p < 0.839$); but significant differences for body condition scores ($p < 0.031$); milk yield ($p < 0.009$) and litter weight gain (0.009). The relational significant differences in the performance of the rabbits and their kits between the groups showed that treatment groups ranked better compared with control (Fig. 1).

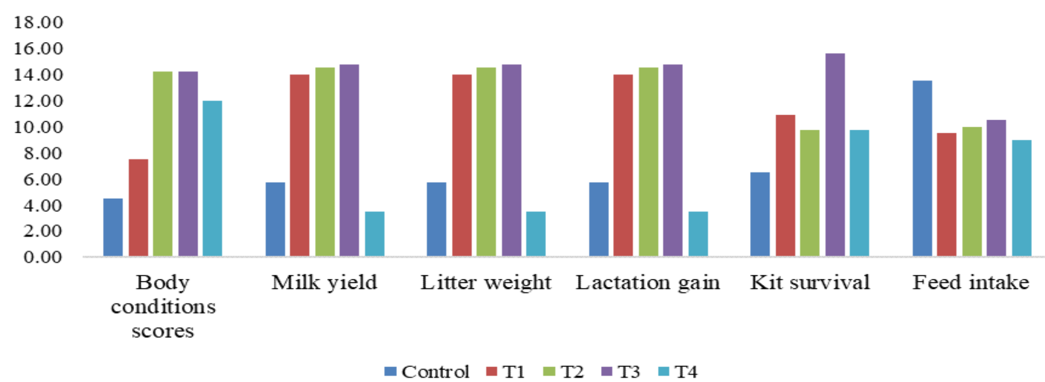

Fig. 1: Comparative effects of interactions between *Chlorella vulgaris* supplementation and performance of the rabbit does and their kits. Control - group without supplement diet, T1- group supplemented with 200 mg/kg BW *Chlorella vulgaris* biomass, T2 - group supplemented with 300 mg/kg BW *Chlorella vulgaris* biomass, T3 – group supplemented with 400 mg/kg BW *Chlorella vulgaris* biomass, T4 - group supplemented with 500 mg/kg BW *Chlorella vulgaris* biomass.

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Discussion

This study indicated that supplementation of *Chlorella vulgaris* at different levels improved performances of the rabbit does as well as their kits. Patterns of milk yield, litter weight gain, kits survival and body conditions are similar in relation with body condition scores and kits survival rates; therefore, it can be deduced that body condition scores, and kits survival rates are underlying factors influenced by the supplementation of *Chlorella vulgaris* which lead to increased milk yield and litter weight gain. However, lower milk yield in T4 where milk yield was similar to the control had higher kits survival rate compared with control group, this implies that the quality of milk in the T4 was better due to the supplementation of the micro algae unlike the control group where kits survival rate was lower (Fig. 2).

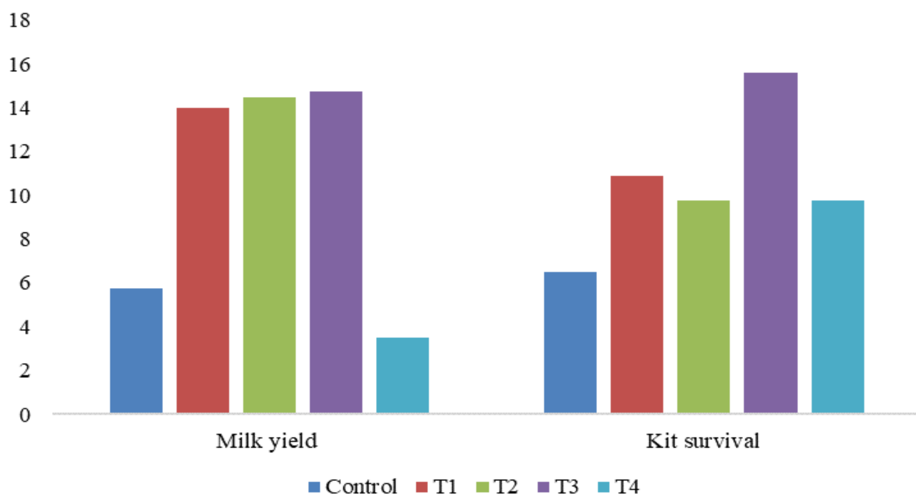

Fig. 2: Effects of *Chlorella vulgaris* supplementation on milk yield and kits survival rates. Control - group without supplement diet, T1- group supplemented with 200 mg/kg BW *Chlorella vulgaris* biomass, T2 - group supplemented with 300 mg/kg BW *Chlorella vulgaris* biomass, T3 – group supplemented with 400 mg/kg BW *Chlorella vulgaris* biomass, T4 - group supplemented with 500 mg/kg BW *Chlorella vulgaris* biomass.

From the explanation above, this study has shown that body conditions of the lactating rabbits, milk yield and litter weight gains are leading factors influenced by the dietary supplementation of *Chlorella vulgaris* in the rabbits. The physiology behind this performance is that *Chlorella vulgaris* supplementation prevented stress in the rabbits and hence the rabbit had enough energy reserve to support milk production for kits consumption. These observations are in agreement with submission of Szendro *et al.* (2002) which confirmed that survival of the kits is a determining factor in rabbit milk yield while quality of the milk produced is a factor affecting the kits performances.

This study also confirmed that number of kits per doe is a major factor affecting milk yield because since the rabbit does at the beginning of the lactation were standardized with equal number of kits; it was however

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observed during the course of the study that does which lost their kits had reduction in milk yield. This observation agreed with reports of previous authors who submitted that number of kits per rabbit doe is a major factor affecting milk yield; some of these authors including Partridge and Allan (1982), who reported 24.1 % increase in milk yield when number of kits was doubled in rabbits does. Mohamed and Szendrö (1992) also reported 3.3% and 5.4% increase in milk yield by additional increase of kits per does from 6 – 10 kits respectively. Apart from the milk yield, kits number per doe was also reported as a factor affecting litter weight gain; this could be linked with outcomes of this study which showed that does with a smaller number of kits had lower litter weight gain.

Higher significant milk yield, higher significant litter weight gain and better significant body conditions of the rabbits in *Chlorella vulgaris* supplemented groups in this study can be summarily refer to as a nutritional manipulation of both biological and economic importance because milk production which has been reported to got little attention in rabbits selection programmes is key in kit survival and post-weaning performance. Therefore, nutritional supplementation for its increase has shown in this study with *Chlorella vulgaris* demonstrated nutritional approach for increased rabbits' productivity during lactation (Maertens *et al.*, 2006; Kunnath *et al.*, 2018).

The supplementation of *Chlorella vulgaris* also led to improvement of body condition which is another production performance advantage; all the rabbit does in the treatment groups had better body condition scores compared with control despite lower feed intake. These observations are in agreement with findings of Pascual *et al.* (2002) who observed that rabbits with higher body conditions produced more milk compared with rabbits with poor body conditions because production of milk is negatively correlated with deletion of body fat reserves.

Body condition scoring is an indicator of well-being and optimum productivity as well as optimum animal reproductive performance; it is directly related to energy balance and it is an underlying factor affecting lactation, animal capacity to return to breeding and long live animal existence (Sadiq *et al.*, 2017). During lactation, energy from body reserves is mobilized into the mammary glands for milk production; this mobilization often left animals in either negative or positive energy balance. Since rabbit reproductive rhythm supports both gestation and lactation, concurrent gestation and lactation poses much burden on energy demand and hence rabbits are most liable to poor body condition score during lactation.

However, this study has demonstrated that supplementation of *Chlorella vulgaris* is capable of improving body conditions of lactating rabbits because despite higher milk production in the treatment groups, the body conditions score falls within the range of ideal body condition of matured rabbits. Antioxidant composition of the microalgae could be identified as the major biochemical mechanisms behind the better body condition in the treatment groups because the antioxidant composition provided additional protection complementing antioxidant enzymes activities of the rabbits against oxidative stress effects of energy

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partitioning between mammary glands for milk production and other body tissue during lactation (Jamali *et al.*, 2017). Antioxidant superiority of the microalgae for promotion of animal productivity during lactation through free radicals scavenging is in agreement with studies in lactating rabbits which reported that higher body condition scores reflected decrease in oxidative stress because it is closely related to energy generation process at the cellular levels and when its markers are reduced it signifies less occurrence leading to improved body condition (Dobbelaar *et al.*, 2010; Jimoh and Ewuola, 2017).

In this study, it was also discovered that kits of the supplemented groups has higher survival rates compared with control group; carotenoids present in the supplemented *Chlorella vulgaris* could be responsible for this improvement because in neonate animals, carotenoids present in milk is a major source of vitamin A and also a source for protection against oxidative stress and hence better immunity. In agreement with this our observation, Nagayama *et al.* (2014) reported that presence of carotenoids following maternal *Chlorella* supplementation in pregnant women improved neonatal performances of the babies an observation linked with higher concentrations of carotenoids in the milk of the supplemented nursing mothers. According to Zielińska *et al.* (2017), carotenoids are essential nutrients for newborns and infant who depends on carotenoids concentration of the mother's milk but unfortunately carotenoids is always present in limited amount in the mother; however, supplementation of carotenoids to the mother both during pregnancy and lactation can increase the concentration and hence the availability to the new-born. In response and agreement with this submission, outcomes of this our present study has demonstrated that dietary supplementation of *Chlorella vulgaris* during lactation can serve as a way of improving carotenoids nutrition and availability for at neonatal stages in animals and humans.

In addition to better survival, early opening of the kits eyes and more activeness were observed in the kits of supplemented groups; the better vision could be as result of antioxidant free radical scavenging potential of the microalgae in the macula of the rabbit kits. In agreement with this observation, carotenoids and antioxidant vitamins were reported to protect the retina from oxidative damage initiated in part by absorption of light as well as retardation of destructive activities associated with macula (Snodderly, 1995 and Sommerburg *et al.*, 2000).

Conclusion

Conclusively, we submit that supplementation of *Chlorella vulgaris* in lactating rabbits improved both the performance of the rabbits does and their kits; we also submit that antioxidant, anti-inflammation, neuro-protective, visual improvement and immune protection properties of bioactive compounds present in the microalgae could be responsible for these improvements because the microalgae is a rich source of carotenoids, phycobilin, lutein, minerals and vitamins usually accumulated as secondary metabolites by the microalgae during cultivation. The supplementation positively improved lactating rabbits' milk yield; body

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conditions; litter weight gain and rates of kits survival. We therefore recommend supplementation of the microalgae within the range used in this study as supplements for improving performance of lactating rabbits while we recommend further studies into elucidation of underlying mechanisms associated with carotenoids transfer to neonates through lactation.

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Conflict of Interest

The authors declared no conflicts of interest for this study

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