Blood and reproductive indices of rabbit does fed supplemented algal biomass diets

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ABSTRACT

Background and Objective: The study was designed to determine the effect of dietary inclusion of algal biomass on the blood and reproductive performance of rabbit does.

Methodology: The total of 20 nulliparous rabbit does, and 8 bucks were arranged into 4 treatment groups of 5 does and 2 bucks per group in a completely randomized design. The algal biomass was included in the basal diet at 0, 0.5, 1.0, and 1.5%. The animals were kept in individual cages and had access to feed and water *ad libitum*. Blood samples for analyses were taken via ear vein from the animals after 2 weeks of feeding trial. Data generated on hematological, serum biochemical, feed intake, and reproductive performance indices (doe weight, conception, gestation period, litter size, kit weight, and percent survivability of the kit) were subjected to a one-way analysis of variance.

Main Results: All the hematological and serum biochemical parameters were similar ($P > 0.05$) across the groups. The litter size (7.33 ± 0.58 kits) and kit weight (50.59 ± 2.65 g) were significantly ($P < 0.05$) highest in does fed 1.5% dietary inclusion level of the algal biomass. Conception, kindling period, and kit survivability tended to increase ($P > 0.05$) with the increased level of biomass. The correlation analysis showed that litter size was significantly ($P < 0.05$) positively correlated with kindling period ($r = 0.589$). The doe weight also had a positive ($P > 0.05$) relationship with conception, litter weight, feed intake, and kit survivability. The gestation period had a negative correlation ($P > 0.05$) with all other reproductive parameters except conception with $r = 0.100$.

Conclusions: The inclusion of algal biomass at a 1.5% level of inclusion in the diet of rabbit does did not pose any health challenge but improved reproductive performance with respect to litter size and kit weight.

Keywords: Survivability, nulliparous rabbit, kindling period, serum biochemical, hematology

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INTRODUCTION

Food security is very important in this dispensation where the human population is increasing geometrically, and the source of livelihood needs to be enhanced. Livestock as a source of animal protein is a panacea to combat the scourge of the population explosion. Prolific animals such as swine and rabbits have been looked at as a means of enhancing animal protein intake. Ajala and Balogun (2004) reported that rabbit production could be one of the ways of alleviating animal protein deficiency in Nigeria. This is attributed to the immense potential the animal possesses, which was suggested to be a high growth rate, high efficiency in converting forage to meat, short gestation period and high prolificacy, relatively low cost of production, the high nutritional quality of rabbit meat, which includes low fat, sodium, and cholesterol levels. It also has a high protein level of about 20.8%, and its consumption is bereft of cultural and religious biases.

Researchers have started concentrating efforts on the supplementation of the maternal diet (pigs; sow) with polyunsaturated fatty acids, particularly the long-chain docosahexaenoic acid (DHA; Edwards *et al.,* 2003; Adeleye *et al*., 2011; 2014) in a bid to enhance vitality and growth including survival of piglets in the first few days of life. DHA-Gold® is a dry algae powder containing 530 mg g^{-1} crude lipid on a dry-weight basis, 460 mg g^{-1} of which is DHA, 22:6n-3. The benefit of this long-chain polyunsaturated fatty acid source (alga biomass) in human and animal nutrition has been investigated (Spolaore *et al.,* 2006). Investigations have shown that newborn kit vigor in various species could be enhanced by including long-chain omega-3 fatty acids in the diet of the dam, especially during late pregnancy (Capper *et al.,* 2005; Pickard *et al.,* 2006). Birberg-Thornberg *et al.* (2006) and Edwards (2002) all reported that long-chain fatty acids had been shown to be crucial to the survival of neonates through the development of brain, eye, and neural tissue function and have been found to play a major role in human and animal health in relation to their anti-atherogenic, anti-thrombotic, anti-carcinogenic, and anti-inflammatory activities; they also contribute to improvements in cardiovascular functions, mental health, and reproductive functions (Simopoulos, 2009) as reported by Adeleye *et al.* (2011). The inclusion of this omega-3 fatty acid (PUFA) in the diets of livestock animals may be of immense benefit to the livestock industry in modulating mediators of humoral and cellular immunity (Rossi *et al.*, 2010), as reported by Adeleye *et al.* (2011). Research also confirmed the reduction of cholesterol with diets rich in omega-3 fatty acids (Simopoulos, 2002). The dietary source of DHA for livestock animals can either be from micro-algae, seafood, or fishmeal and most of the time, they are regarded as algal biomass products manufactured for a specific function. DHA is essential for the proper development and functioning of the brain (Innis, 2000) and the development of the animal nervous system and visual abilities during the early months of life.

Moran *et al*. (2018) reported that supplementation of DHA had no negative effects on the mortality, blood parameters, or productivity of broiler chickens in a study that investigated the tolerance of broilers to dietary supplementation with the unextracted biomass of a DHA-rich microalgae *Aurantiochytrium limacinum*. The effects of these polyunsaturated fatty acids on rabbits have not been studied or less studied, leading to a dearth of information even though they are prolific animals as well. Hence, this study was designed to monitor the influence of dietary inclusion of DHA on blood parameters and the reproductive performance of rabbit does.

MATERIALS AND METHODS

The experiment was carried out at the rabbitry unit of the College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Nigeria. The site is located in the rainforest vegetation zone of South West Nigeria on latitude 7°10’N and longitude 3°2’E and altitude of 76 m above sea level. The climate is humid, with a mean annual rainfall of about 1,037 mm and mean temperature and humidity of 30.7°C and 83%, respectively.

Source and Composition of the Algal Biomass

The test ingredient was supplied by DSM Animal Nutrition and Health, the Netherlands. The proximate composition of the algal biomass is 90.24% dry matter, 30.82% fat, 10.91% ash, 6.72% crude fiber, 15.77% crude protein, and 26.02% nitrogen-free extract.

Experimental Design and Management

Twenty matured rabbit does, and 8 bucks crossbred rabbits (Chinchilla × Dutch) with an average weight of 1.8 kg were sourced from a reliable rabbit farm and divided into 4 dietary treatment groups of 5 does based on weight equalization. Two bucks were assigned to each treatment group. The treatments were arranged in a completely randomized design with the algal biomass supplementation at 0, 0.5, 1.0, and 1.5%. The animals were acclimatized for 2 weeks, after which they were subjected to the experimental diets for another 2 weeks before mating. Natural mating was used by carrying does to bucks. The experimental rabbits were kept in a hutch equipped with concrete feeders and earthen drinkers. Bucks were individually housed in cages (similar to that of does). Feed and water were provided *ad libitum* throughout the experimental period. The ingredient composition of the experimental diets is presented in Table 1.

Table 1 Basal composition of the experimental diets

Composition	Inclusion level of algal biomass (%)			
	0	0.5	1.0	1.5
Ingredients				
Maize	45.00	45.00	45.00	45.00
Soya bean meal	24.00	24.00	24.00	24.00
Fish meal	1.00	1.00	1.00	1.00
Rice husk	25.00	25.00	25.00	25.00
Salt (Nacl)	0.25	0.25	0.25	0.25
Oyster shell	1.50	1.50	1.50	1.50
Bone meal	3.00	3.00	3.00	3.00
Vitamin/mineral premix	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Algal biomass	-	0.5	1.0	1.5
Calculated analysis				
Crude protein (%)	16.04	16.11	16.19	16.27
Crude fiber (%)	12.41	12.44	12.48	12.51
Fat (%)	4.20	4.37	4.52	4.67
Ash (%)	7.57	7.62	7.67	7.73
Calcium (%)	2.17	2.17	2.17	2.17
Phosphorus (%)	0.80	0.80	0.80	0.80
Metabolizable energy (kcal/kg)	2,602	2,602	2,602	2,602

Data Collection

The animals (does) were weighed before subjecting them to the experimental diets before mating. A pregnancy test through palpation was carried out 14 days after mating. The does that were not pregnant were re-mated (second mating). Those that refused to conceive were mated a third time (third mating) after the pregnancy test was re-carried out. Average feed intake was determined by subtracting leftover feed from the amount of feed given to the animals. Other indices that were measured are conception rate (this is the number of times mating/service was taken place before conception/pregnancy was established), gestation period (number of days from the first day of conception to the day of kindling), litter size (number of young ones (kits) at birth per doe), kit weights (average weight of the kits at birth) and percentage of surviving kits (number of kit(s) that survived from birth till the period of weaning). Kits were left with their dams for 8 weeks before weaning. The kindling period was recorded with the aid of an 8-unit CCTV camera. The experiment lasted for 20 weeks.

The conception rate was expressed as 100% for those that conceived at first mating, 50% for those that were mated twice while 33.33% for those that were mated three times before conception was established.

$$\text{Kit weight (g)} = \frac{\text{Weight of all kits at birth}}{\text{Number of kits at birth}}$$

$$\text{Kit survivability (\%)} = \frac{\text{Number of kits survived at weaning}}{\text{Number of kits at birth}} \times 100$$

Five mL of blood was collected from 3 does in each treatment after mating via the ear vein using a needle and syringe. Half of the blood sample was dropped into a sample bottle containing ethylene diamine tetra-acetic (EDTA) as an anti-coagulant for hematological parameters determination while the remaining half was put in another set of plain bottles without EDTA for serum analysis. The blood samples were analyzed for total protein, albumin, creatinine, aspartate transaminase (AST), alanine transaminase (ALT), and glucose using analytic test kits (Benjamin, 1978). The following hematological parameters were determined; hemoglobin concentration was determined by diluting the blood samples with dracking solution and then incubating them for 10 min. Then, the result was read under a spectrophotometer (Mitruka and Rawnsley, 1977). White blood cells were counted under a microscope (Mitruka and Rawnsley, 1977). Red blood cells were determined from an anticoagulated fresh blood sample which had been diluted with 0.09% NaCl and shaken thoroughly (Baker and Silverton, 1985). The diluted blood was mounted on a hemacytometer, and the number of erythrocytes in a circumscribed volume of 0.01 m^3 was counted microscopically (Aiello, 1998). Hematocrit (pack cell volume) was analyzed by the use of a hematocrit centrifuge (Mitruka and Rawnsley, 1977). Neutrophil (differential leucocyte count) was estimated by counting 100 cells (leucocytes) under oil immersion through a microscope on a blood film prepared from fresh blood and fixed with methanol for 3 min. All the white blood differential counts (basophil, lymphocytes, eosinophils, platelets, and monocytes) were analyzed through blood stains (Hodges, 1974). mean corpuscular volume, white cell differential count (WCDC), and mean corpuscular hemoglobin concentration (MCHC) were estimated by calculation using a standard formula (Samour, 2008)

Statistical Analyses

Data collected were subjected to a one-way analysis of variance (ANOVA). Significant means were separated at a 95% confidence interval by the Tukey test in a statistical software package (SAS, 2002). The ANOVA results were presented as means ± standard deviation, while correlation statistics result was presented as matrixes in the table.

RESULTS AND DISCUSSION

The effects of dietary supplementation of algal biomass on the hematological indices of rabbit does are presented in Table 2. It was revealed that algal biomass did not influence (P > 0.05) any of the parameters. The results indicated that the inclusion of DHA gold algal biomass did not pose any health challenge or threat to the animals. These results are in contrast with the finding of Abdelnour *et al*. (2019), who indicated that dietary microalgae biomass supplementation had a significant effect on all the blood hematological traits except for hemoglobin and red blood cell count. El-Ratel (2017) indicated that the treatment of rabbit does with *Spirulina platensis* significantly increased the hemoglobin concentration count of red blood cells and hematocrit value. The variations in the observations reported in this present study compared with previous studies on other species of algal biomass could be connected to the different nutrient compositions present in the biomass and the level of dietary inclusion.

Table 2 Effects of algal biomass supplemented diets on hematological parameters (mean ± standard deviation) of rabbits at mating

Parameters	Inclusion level of algal biomass (%)				P-value
	0	0.5	1.0	1.5	
Pack cell volume (%)	26.67 ± 6.02	33.33 ± 7.09	35.33 ± 2.51	29.67 ± 6.02	1.38
White blood cell (×10^6/mm^3)	4.30 ± 1.21	10.83 ± 9.47	5.55 ± 1.35	9.50 ± 1.60	1.22
Lymphocytes (%)	54.33 ± 4.16	41.33 ± 22.01	44.67 ± 6.43	41.00 ± 2.65	0.85
Neutrophil (%)	34.67 ± 3.06	51.33 ± 21.01	44.67 ± 12.74	51.33 ± 2.08	1.21
Monocytes (%)	7.67 ± 3.78	5.33 ± 0.57	5.67 ± 2.88	3.67 ± 1.15	1.33
Basophil (%)	3.67 ± 1.52	2.00 ± 1.00	1.67 ± 2.08	4.00 ± 2.00	1.41
Red blood cell (×10^6/mm^3)	3.13 ± 0.68	3.63 ± 0.71	4.03 ± 0.71	5.90 ± 3.74	1.13
Hemoglobin (g/dL)	9.33 ± 2.51	10.00 ± 3.00	11.67 ± 0.57	9.67 ± 2.08	0.64
MCV (fL)	85.00 ± 4.58	87.00 ± 8.54	85.67 ± 4.72	81.67 ± 1.52	0.52
MCH (pg)	30.33 ± 2.31	26.67 ± 2.52	27.33 ± 0.58	31.33 ± 3.78	2.34
MCHC (g/dL)	31.67 ± 4.51	31.67 ± 1.15	32.67 ± 0.57	30.67 ± 1.15	0.34

Note: MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentrate.

The effect of dietary supplementation of algal biomass on the serum biochemical indices of rabbit does is presented in Table 3. It was observed that none of the measured parameters was influenced (P > 0.05) by the introduction of the algal biomass. These results corroborate the report of Abdelnour *et al*. (2019), who observed that most of the serum parameters were non-significantly influenced by *Chlorella vulgaris* microalgae supplementation in rabbit diets. Similarly, Seyidoğlu and Galip (2014), Khanna *et al*. (2016), and Salim *et al*. (2019) reported that there were no significant changes in serum biochemical indices as a result of supplemented rabbit diets with microalgae (*Spirulina platensis*).

The values of serum proteins in the present study were similar to the values reported by Salim *et al*. (2019). The results indicated that the inclusion of DHA gold algal biomass did not pose any health challenge or threat to the animals. Long-chain omega-3 PUFA, namely docosahexaenoic acid (DHA; 22:6ω3) and eicosapentaenoic acid (EPA; 20:5ω3), have been shown to offer an array of health benefits (Tocher *et al*., 2019).

The reproductive performance of the rabbit does fed varying levels of algal biomass is presented in Table 4. The results showed that only litter size and kit weights were significantly ($P < 0.05$) influenced by the dietary inclusion of the algal biomass. It was revealed that the highest litter size (7.33 ± 0.58 kits) was obtained among does fed 1.5% of algal biomass. It was also observed that the least kit weight (37.33 ± 3.78 g) was recorded among the does on the least inclusion of algal biomass. This observation could be attributed to the effect of algal biomass on the fertility of animals. It has been reported by Badawy *et al*. (2019) that the most commanding indicators of rabbit reproductive performance are litter size and kit survivability among others. The effect of DHA in the diets of treated groups on sperm quality could be responsible for the numerical high conception rate recorded in does subjected to DHA diets. Ragab *et al.* (2019) reported improvement in the sperm quality of rabbit fed *Spirulina platensis.* The highest litter size and kit weight observed in the present study negates the findings of Ragab *et al.* (2019), who reported similarities in the two indices of reproductive performance of rabbit does subjected to varying levels of *Spirulina platensis*. The variation in these reports could be attributed to the quality and importance of omega-3 fatty acids in the algal biomass in the diets used in the present study. The algal biomass fed to rabbits in this study consists of 100% docosahexaenoic acid (DHA gold). Nehra *et al.* (2012) established that diets containing omega-3 fatty acid improves reproductive performance and oocyte quality in rodents. Similarly, Colin *et al.* (2017) also reported that the dietary inclusion of algae rich in docosahexaenoic acid improves the viability in rabbits because the presence of omega-3 fatty acid in the diet improves the immunity of the animal (Boudour *et al*., 2020).

Table 3 Effects of algal biomass supplemented diets on serum biochemical parameters (mean ± standard deviation) of rabbits does at mating

Parameters	Inclusion level of algal biomass (%)				P-value
	0	0.5	1.0	1.5	
Total protein (g/L)	6.37 ± 2.33	5.40 ± 0.95	7.53 ± 0.71	5.90 ± 0.82	1.32
Albumin (g/L)	3.70 ± 1.65	3.97 ± 0.49	5.17 ± 1.50	4.17 ± 0.92	0.81
Globulin (g/L)	2.67 ± 0.72	1.43 ± 0.59	2.37 ± 0.83	1.73 ± 0.55	2.06
Alanine aminotransferase (U/L)	45.00 ± 15.71	34.00 ± 15.59	37.00 ± 19.00	45.33 ± 42.15	0.15
Aspartate aminotransferase (U/L)	54.33 ± 38.99	35.33 ± 6.66	62.33 ± 15.31	57.00 ± 47.65	0.41
Glucose (mg/dL)	70.00 ± 18.82	52.33 ± 20.60	64.00 ± 9.54	42.33 ± 3.51	2.24
Urea (mg/dL)	26.00 ± 5.56	26.67 ± 6.81	27.00 ± 7.81	29.33 ± 12.50	0.09
Creatinine (mg/dL)	0.87 ± 0.45	1.00 ± 0.61	1.33 ± 0.75	1.00 ± 1.12	0.20
Platelets (10^5 mL)	1.89 ± 1.50	1.44 ± 0.66	1.38 ± 1.07	1.94 ± 1.81	0.14

Table 4 Effects of algal biomass supplemented diets on reproductive performance (mean ± standard deviation) of rabbits does

Parameters	Inclusion level of algal biomass (%)				P-value
	0	0.5	1.0	1.5	
Initial weight (kg)	1.77 ± 0.17	1.67 ± 0.08	2.11 ± 0.28	1.89 ± 0.17	0.09
Conception (%)	77.79 ± 38.46	83.33 ± 28.87	100.00 ± 0.00	100.00 ± 0.00	0.64
Gestation period (days)	31.33 ± 0.57	33.00 ± 2.64	31.33 ± 1.15	31.33 ± 0.57	0.47
Litter size (kit)	5.67 ± 1.15[ab]	5.33 ± 0.57[ab]	5.00 ± 1.00[b]	7.33 ± 0.58[a]	0.04
Kit weight (g)	45.63 ± 2.25[a]	37.33 ± 3.78[b]	45.33 ± 2.51[a]	50.59 ± 2.65[a]	0.04
Daily feed intake (g)	89.08 ± 11.65	94.38 ± 11.64	115.64 ± 30.68	102.84 ± 22.89	0.47
Kindling period (s)	9.00 ± 1.73	9.00 ± 1.00	9.33 ± 2.88	11.67 ± 1.52	0.32
Survivability (%)	40.95 ± 1.64	43.33 ± 40.41	50.00 ± 50.00	61.31 ± 44.62	0.92

Note: [ab] Means with different superscripts along the same row are significantly ($P < 0.05$) different.

The heavier birth weight of kits recorded in the group with larger litter size negates the report of Olateju and Chineke (2022), who reported that kit weights are indirectly proportional to litter size, which may be contributed by sufficient intra-uterine nourishment available to enhance the growth and development of the fetuses, unlike the largest litters where there will be competition for nutrients. So, kits in the least litter develop more rapidly than those in the largest litter, even in their post-uterine life, due to easier access to a large amount of nutritious milk. The heavier weight recorded in the larger litter size in the study could also be connected to the importance of algal biomass in the diet.

Adarme-Vega *et al*. (2014) and Stramarkou *et al*. (2021) reported that DHA plays a key role in the healthy development and growth of the fetal brain and retina. Thus, it is commonly included in infant-oriented food products and supplements. Therefore, the heavier the weights recorded from the kits of the does on a diet with higher inclusion of the DHA. It was also reported that the algal biomass contains essential amino and fatty acids, photosynthetic pigments, vitamins, minerals, carotenoids, chlorophyll, pigments, and essential polyunsaturated fatty acids (El-Ratel, 2017). Pyle *et al*. (2008) reported that DHA algal biomass is rich in lysine and cysteine when compared with many common feedstuffs. Lysine is important for normal growth and muscle turnover and is used to form carnitine, a substance found in most cells of the body. This could also be responsible for greater survivability in litters from doe fed the algal biomass diets.

The numerical increase ($P > 0.05$) in the feed intake of the does could be attributed to the fishy flavor of the algal biomass (Lafarga, 2019), which induces the appetite of the animals on the diets with the algal biomass. The trend of the feed intake was similar to the report of El-Ratel (2017) who included *Spirulina* algae in the diets of rabbit does. Though conception percentage, kindling period, and kit survivability were similar ($P > 0.05$), it is worth noting that there was an increase in the value of the indices as the level of algal biomass increased. This showed that algal-supplemented rabbit does seem to have better colostrum and milk yield and composition, as well as enhanced fertility traits. Colostrum is a special type of milk formed during the last days of pregnancy and the first few days after birth. The main components of colostrum in rabbits are proteins and fats (El-Desoky *et al*., 2022). Hence, the nutritional requirements for these components are elevated at the late pregnancy period and early stage of lactation to support the survivability of kits. The numerical increase in the conception rate (77.79, 83.33, 100.00, and 100.00% recorded from does on 0. 0.5, 1.0, and 1.5% DHA, respectively) confirmed the superior reproductive performance of the DHA-supplemented rabbit does, which had better fertility and pregnancy outcomes than the non-supplemented rabbit does.

It has been reported in some studies that fatty acid-rich diets improve fertility and pregnancy outcomes in rabbits (Rebollar *et al*., 2014; Rodríguez *et al*., 2019). In fact, fatty acids are an important element for many reproductive events through various modes of action. de Mattos *et al*. (2000) reported that numerous fatty acids could positively influence reproduction by altering the ovarian follicles and corpus luteum function via improved energy status and increasing precursor levels for the synthesis of reproductive steroids and prostaglandins. They also improve the competence of oocytes and boost embryo development (Cerri *et al*., 2009) and pregnancy and fetal development (Volpato *et al*., 2008). The higher litter size obtained at the highest inclusion level of the algal biomass could be the reason for the longer kindling period recorded in the treatment group. It was reported that litter size has influenced a farrowing period in sows (Schild *et al*., 2020; Ju *et al*., 2022). It was observed that the higher the litter size, the longer the farrowing period.

The Pearson's correlation coefficient among the reproductive indices showed that it was only litter size and kindling period had a positive significant ($P < 0.05$) relationship ($r = 0.589$) (Table 5). Doe weight had a negative relationship with gestation period, litter size, and kindling period but a positive correlation relationship with conception, litter weight, daily feed intake, and kit survivability ($P > 0.05$). The positive correlation relationship observed in this study negates the findings of Fadare and Fatoba (2018), who observed a strong negative correlation ($r = -0.697$) between litter size and kit weight at birth. The doe weight also had a positive ($P > 0.05$) relationship ($r = 0.515$) with feed intake. This was similar to the report of Lewis and Emmans (2020), who reported a positive relationship between feed intake and live weight in domestic animals.

Table 5 Pearson's correlation coefficient of the relationship among reproductive performance indices

Parameters	Doe weight	Conception	Gestation period	Litter size	Average litter weight	Daily feed intake	Kindling period
Conception	0.320						
Gestation period	-0.459	0.100					
Litter size	-0.309	-0.285	-0.180				
Average litter weight	0.196	0.461	-0.291	0.417			
Daily feed intake	0.515	0.313	-0.004	-0.350	0.234		
Kindling period	-0.147	0.205	-0.267	0.589*	0.443	-0.402	
Kit survivability	0.431	-0.133	-0.532	0.146	-0.141	-0.222	0.414

Note: * Correlation is significant at the 0.05 level (2-tailed).

CONCLUSION

The dietary inclusion of the DHA-gold® algal biomass at 1.5% supplementation in the diet of rabbit does improve the reproductive performance of rabbits which could be a great benefit to the farmer as a means to increase their profit margin. In the same vein, the study revealed that the algal biomass has no deleterious effect on the health of the animals since the blood indices were not significantly affected across the groups.

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