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The dietary effects of coconut oil on the leucocytes and neutrophils count in rabbits

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Abstract: *This study investigated the dietary effect of Cocos nucifera oil on the leucocytes and neutrophils level in rabbits. Fifteen (15) young and healthy white rabbits were fed for 8 weeks on daily basis with a 0.5ml/ kg body weight using coconut oil meal together with normal animal feed stock and water. A baseline blood sample was collected from the animals prior to feeding them with the coconut extract. Thereafter, blood samples were collected from them in weeks 1, 4 and 8 respectively. The samples were analyzed for total white blood cell (TWBC, leucocytes) count and differential white blood cell (lymphocytes and neutrophils) count using Sysmmex KX 24 Machine. The biochemical analysis of the coconut oil was carried out using Clark's Vitros 250 Analyzer, Model 5546, USA. The results showed no significant mean difference ($p>0.05$) in the lymphocytes count between the baseline and the mean values for the four weeks interval experiment. However, a significant increase ($p<0.05$) in the neutrophils count was observed in the 8th week (15.5 %) and for leucocytes level in the 4th and 8th weeks (977 and 2700 µL respectively). The significant increase in the neutrophils and leucocytes level could be attributed to the extrinsic effect of the Cocos nucifera oil used as food supplement in the animal feed stock.*

Key Words: Rabbits, *Cocos nucifera* oil, Leucocytes, Neutrophils, Lymphocytes.

Introduction

Neutropenia is a hematological disorder characterized by an abnormally low number of neutrophils. The relationship between a low neutrophil count and increased risk of infection was first demonstrated in patients with leukemia (Bodey *et al.*, 1966). Hsieh (2007) reported that in the United States of America, neutrophil counts were lower in the black than in the white persons and that neutropenia was more prevalent among the blacks. Asymptomatic reduction in neutrophil levels were also observed in individuals of all ethnic backgrounds but may be more common in those of African descent, hence a serious ethnic neutropenia has been described among the Africans (Ezello, 1974). The lack of robust increase in leucocytes or neutrophil is also consistent with several reports showing that leucocytes and neutrophils were mobilized to a lesser extent in black participants than in white participants after corticosteroid administration (Mason *et al.*, 1979; Shoenfeld *et al.*, 1982; and Jumean and Sudah, 1983). Leucocytes are non-pigmented white blood corpuscles concerned with body defense mechanism against infections. The neutrophils, amoeboid in shape, constitute 70 % of the TWBCs and are manufactured in the bone marrow, (Roberts, 1976). They are the white blood corpuscles that combat most bacterial infections, (Horn, 2004). The lymphocytes are but 24 % of the TWBCs with kidney-shaped nucleus and are produced in the lymphatic system (Roberts, 1976). They are the key WBCs involved in immune responses by the production of antibodies.

Previous researchers have shown that coconut oil has medium chain triglycerides (MCTs) with antiviral, antibacterial and antifungal properties (Karaba, 1978). Lauric acid has also been identified as the most predominant medium chain triglycerides found in coconut oil (Vickery and Vickery, 1979; Enig, 1996). Studies also showed some anti-microbial effects of free lauric acid in coconut oil on the viral load of HIV patients (Dayrit and Conrado, 2000). Traditional Asian culture that have significant amount of coconut oil in their diet are not known to suffer from these modern diseases as seen presently in the western cultures, hence coconut has been classified as a functional food, which has health benefits over and beyond the basic nutrients (Enig, 1993). In this study, animal model has been used to investigate the dietary effect of coconut oil as food supplement on the leucocytes (TWBC), neutrophils, and lymphocytes count in white rabbits.

Materials and Methods

The materials include ethylenediamine - tetraacetic acid, EDTA containers, butterfly needle, syringes, xylene, New Zealand white rabbits, animal blood, coconut oil, cotton wool.

Fifteen New Zealand white rabbits with initial mean weight of 2.25kg on delivery were purchased from Adamawa Cattle Market in Benin City, Nigeria. The rabbits were housed in a stainless steel animal cage with wire mesh floor. The animals were acclimatized on growers mash (Bendel Feed and Flour Mill Limited, Ewu Nigeria) for two weeks before commencing the study. Food and water were given *ad libitum*. At the end of the adaptation period, a baseline blood sample was collected from each of the animals before feeding them routinely with both the conventional animal feed and supplemental coconut oil meal. Animal management and experimental procedures were carried out in accordance with the requirements of the National Research Council's guide for the use of laboratory animals (NRC, 1985).

The baseline blood samples were drawn from the rabbit's ear vein using butterfly needle and 21- gauge syringes. This served as the control for the subsequent blood samples collected after feeding the animals with the coconut oil meal. The blood samples were put into the EDTA container for laboratory analysis. These blood samples were collected on monthly basis to avoid possible anaemia and leucopenia in the animals.

Traditional method of extraction of coconut oil was employed in this study. The freshly harvested coconut seeds were grated and the milk was expressed mechanically into a container. It was allowed to ferment for 24 to 36 hours. During this period the water separated from the oil and then removed. The oil was heated slightly for 5 minutes to remove excess moisture, and then filtered. The realised coconut oil was clear and retained the distinct scent and taste of coconut.The total white blood cell (TWBC), neutrophil and lymphocyte levels were determined using the Sysmmex KX 24 Machine available at the University of Benin Teaching Hospital, Edo State, Nigeria while CD4 Count was analyzed using Cyflow Slgreen Machine, Wolf gang Gohde Make, Germany. The coconut oil was analyzed for nutrient contents using Clark's Vitros 250 Analyzer (Model 5546, USA).

The data obtained were analyzed using Students' t-test at 95 % confidence limit to compare independent variables and results were expressed as mean ± standard error of the mean (SEM). P- Values < 0.05 were indicative of significance difference. All analyses were done with the Microcal 5.0 Statistical Package and Excel Microsoft Office, 2007.

Results and Discussion

The biochemical analysis of the coconut oil shows the presence of total protein < 1.0 g/dl and albumin < 0.5g/dl, while glucose, cholesterol and triglyceride levels were below the detection limits of the analyzer. Although these essential food nutrients are in trace amounts, previous studies showed that coconut oil is a functional food which has health benefits over other basic nutrients (Enig, 1993).

The results of the total white blood cell count (leucocytes) and the differential white blood cell count (neutrophils, lymphocytes and the helper T-cell, CD4 Count) are shown in Table 2. It is evident from Figs. 1 and 3 that there were remarkable increase in the leucocytes (Fig. 1) and neutrophils count (Fig. 3) throughout the experimental periods as an incremental mean difference of 1320 µL and 6.76 % were observed in leucocytes and neutrophil levels respectively, (Table 2). This was statistically significant ($p<0.05$) at the 4th and 8th weeks for the leucocytes count and only at the 8th week for the neutrophils count. In the 4th and 8th weeks monitoring period, an increase of 977 µL and 2700 µL were observed for the leucocytes level, while the neutrophils level increased by 15.5 % in the 8th week only compared with the baseline results. The lymphocytes count increased by only a 0.19 % in the 1st week and then decreased progressively by 1.76 % and 1.95 % in the 4th and 8th weeks respectively (Fig. 2). The results were tested for significant difference at 95 % level of confidence between the control and the experimental blood samples. Significant differences ($P < 0.05$) existed between the control and the test samples in weeks 4 and 8 for the leucocytes count and in week 8 for the neutrophils count. The variation in the lymphocytes level were however not significant ($P > 0.05$) since only a marginal difference of 1.17 % was observed (Table 2). The variation between the mean levels of lymphocyte and neutrophil in the experimental animals over the eight weeks of study is shown in Fig. 4. While the lymphocytes decreased minimally (1.17 %), the neutrophils count increased by a reasonable margin (6.76 %).

The observed increase in the neutrophils count probably implies anticipatory immune response for the fall in the lymphocytes count. But the cluster differential cell, CD4, which is also referred to as a Helper T-cell increased progressively (5. 6). The fall in the CD4 observed in Week 4 could be due to skin infection that attacked the animals in the initial environment they were kept, which probably affected their immune responses. The animals were immediately transferred to a disinfected prototype pen and a remarkable increase in their average CD4 count was then observed in week 8. The two period forecast trend line shown in Fig. 5 clearly indicates the expectation

Table 1. The Mean ± S.E.M of the immunological white blood cell counts in test rabits.

Parameters	Control	Test wk. 1	Test wk. 4	Test wk. 8	Mean of means	Mean diff.
TWBC(µL) *Sig. Level*	6350 ± 850	6633±1534 p>0.05	7327±829 p<0.05	9050±1496 p<0.05	7670±1244	1320(µL)
Lymphocyte count (%) *Sig. Level*	50.61± 8.50	50.80 ±6.40 P > 0.05	48.85±3.06 P > 0.05	48.66 ±4.05 P > 0.05	49.44±1.18	1.17 %
Neutrophil count (%) *Sig. Level*	46.6± 9.45	48.76± 6.76 P > 0.05	49.28 ± 3.14 P > 0.05	62.1± 6.14 P < 0.05	53.41±7.60	6.76 %
CD4 (µL)	15.00 ± 1.00	17.00 ± 0.58	13.92 ± 3.64	21.75 ±8 .92	17.56+4.38	14.58 µL

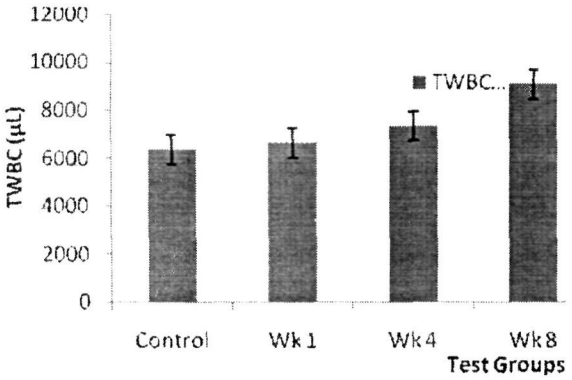

Fig .1 The variation of the mean leucocytes level between the control and the test groups

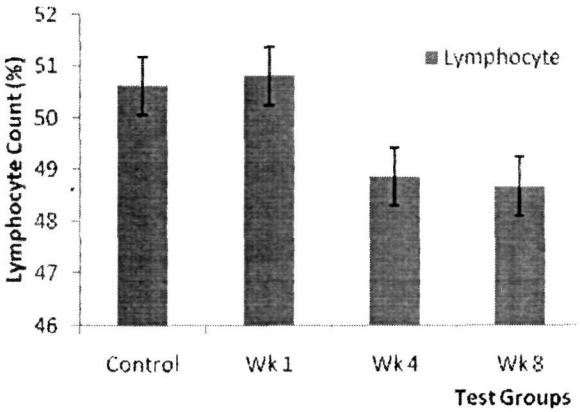

Fig. 2 The variation of the mean lymphocyte level between the control and the test groups

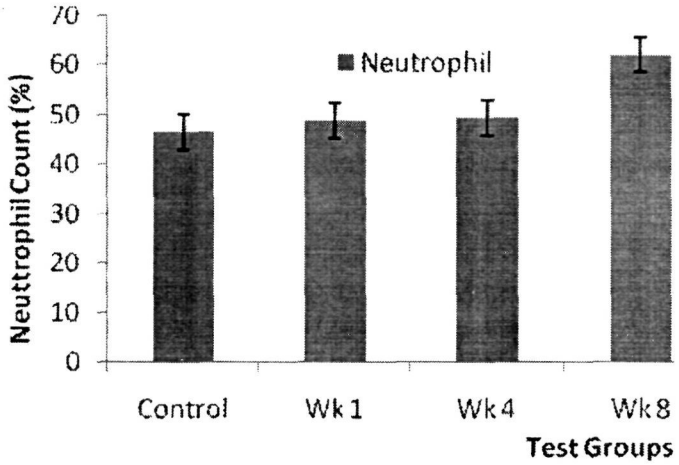

Fig .3 The variation of the mean neutrophils count between the control and the test groups.

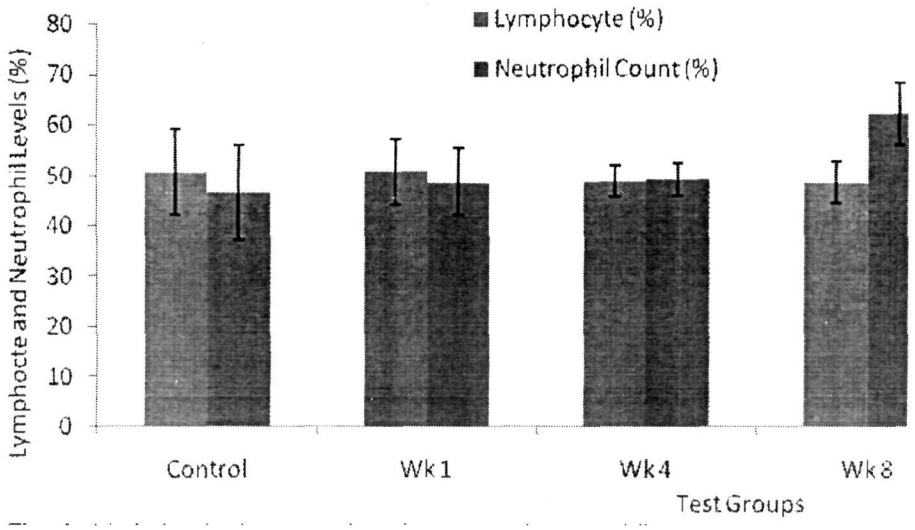

Fig. 4 Variation in the mean lymphocyte and neutrophil count between the control and the test groups

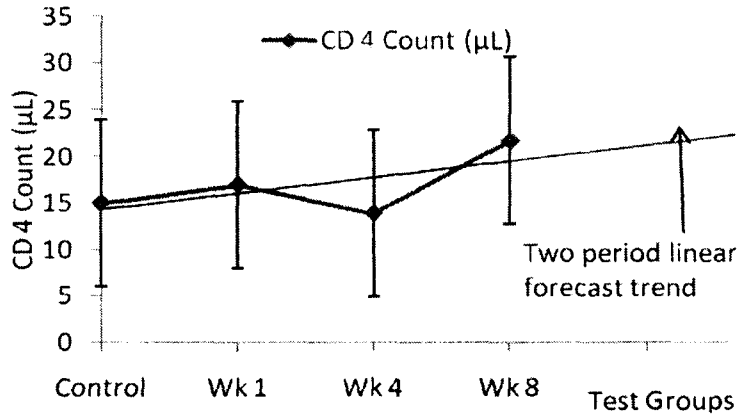

Fig. 5 The variation of the mean CD 4 Count between the control and the test groups

but for the infection observed in week 4.

The CD4 count is one of the subsets of the total lymphocytes count commonly used as the most important hematological test to check for active immunity. It is used to assess the overall health of the immune system in people with HIV (Horn, 2004). This is because it is the cell usually targeted by HIV virus. An increase in the CD4 count means an enhanced immunity, while a decrease implies a fall in immunity. The normal CD4 count lies between 500 - 1500µL for healthy adult human beings (Horn, 2004). This may be quite different in animals.

The results of this study therefore suggest that the increase in leucocytes and neutrophils count could be an indication of the antimicrobial, antifungal and antiviral property of the coconut oil. On the other hand, the increase in CD4 count could be an indication of enhanced immune response property of the coconut oil meal introduced into the animals' feed. In line with David and Oyebila (2002) in their study on bone marrow, the increased mobilization of leucocytes and neutrophils count in the animals fed with the coconut oil meal, could be attributed to the stimulation of the pluripotent haemopoietic stem cell from where leucocytes and neutrophils are produced in the presence of a growth inducer and differentiation inducer, which are protein in nature.

Conclusion

The results of this study have shown that introduction of coconut oil meal in animal feeds significantly increased both the leucocytes and neutrophil levels. Since neutropenia is a direct consequence of reduced neutrophils and leucocytes level in humans, which predisposes to increased risk of diseases and infections because of reduced body immunity, it is suggested that such nutrients as contained in the coconut oil meal be incorporated in human diets. In as far as this food supplement increases the level of neutrophils, leucocytes and CD4 count, the risk of exposure to foreign diseases is most commonly associated with the black race, such as the neutropenia and leucopenia would be drastically reduced or eradicated. It could also be a potential to fight HIV.

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