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The Influence of Different Levels of Dietary Energy on Complement Levels `in *Trypanosoma congolense* infected West African Dwarf goats .

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Abstract: Thirty six healthy WAD goats, divided into three groups A, B and C of twelve each with eight infected and four controls were placed respectively on low -2426.74kcal/kg, medium -2548.57kca/kg and high-2670.40kcal/kg levels of dietary energy but isonitrogenous feed and subsequently infected with a virulent strain of *Trypanosoma congolense*. The influence different levels of dietary energy on serum complement C3,total and alternative haemolytic complement parameters were evaluated up to 5 weeks post infection (p.i). The respective decline in levels of these complement system parameters were significant ($P<0.05$) and more pronounced in group A than B and C. There were highly significant decline ($P<0.001$) in complement system values of infected goats in groups A, B and C when compared with pre-infection values (C3, THC and AHC) while infected groups A, B and C were significantly different ($P<0.05$) to one another in values which worsened with decreasing levels of dietary energy and this indicated that dietary energy ameliorates and influences the hypo-complementaemia observed during the course of experimental trypanosomosis in goats.
Keywords: WAD goats, Dietary energy, Complement levels, Trypanosoma congolense.

INTRODUCTION

The complement system comprises a group of serum proteins and cell membrane receptors that function primarily to fight infection. Clinically, measurement of complement pathway activity and individual component levels is of value in cases of immunodeficiency and inflammatory conditions that involve complement activation[1].

Historically, the term complement (C) was used to refer to a heat-labile serum component that was able to lyse bacteria and its activity is destroyed (inactivated) by heating serum at 56 degrees C for 30 minutes. However, complement is now known to contribute to host defenses in other ways as well. Complement can opsonize bacteria for enhanced phagocytosis; it can recruit and activate various cells including polymorphonuclear cells (PMNs) and macrophages; it can participate in regulation of antibody responses and it can aid in the clearance of immune complexes and apoptotic cells [2]. Complement can also have detrimental effects on the host; it contributes to inflammation and tissue damage and it can trigger anaphylaxis. Complement comprises over 20 different serum proteins that are produced by a variety of cells including, hepatocytes, macrophages and gut epithelial cells. Some complement proteins bind to immunoglobulins or to membrane components of cells. Others are proenzymes that, when activated, cleave one or more other complement proteins. Upon cleavage some of the complement proteins yield fragments that activate cells, increase vascular permeability or opsonize bacteria. Trypanosomal infections results in a profound hypocomplementamia in humans [3], cattle [4-5] and sheep [6]. *Trypanosoma congolense* and *T.brucei.brucei* organisms that are not coated with variant surface glycoprotein (VSG) are lysed by human serum via the alternative complement pathways (ACP) [7]. Blood stream forms of *T.b.gambiense* also activate the ACP of human serum directly [8]. The serum complement concentration C3,total and alternative haemolytic complement had been studied in goats[9] in cattle[10] but there was no information on the influence of different levels of dietary energy on these complement system, therefore, the influence of different levels of dietary energy on complement C3, total and alternative haemolytic complement were studied .

MATERIALS AND METHODS

Experimental Site: The experiment was carried out at the large animal ward II, Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Oyo state, Nigeria.

Experimental Animals: A total of thirty six (36) adult West African Dwarf (WAD) goats were used, they were purchased at a local market in Ibadan, and were acclimatized for four weeks at the experimental site. They were treated against worm infestation, haemoprotozoan diseases such as babesiosis and trypanosomosis as clinically indicated *Pestes des petit ruminantum* (PPR) vaccine was administered to all goats. Antibiotics, antihelmintics and acaricide dips were also administered as clinically indicated.

Grouping of Animals: The goats were randomly divided into three experimental groups (A, B and C) based on weight and sex and each group contained twelve goats.. The animals were on these rations for 4 weeks before experimental infections were carried out. This was to create the desired differences in the nutritional status of the animals before experimental infection. In each group, eight goats were infected with trypanosomes while the remaining four served as non-infected controls.

Feeding of experimental animals: Animals in group A, B, and C were placed on low, medium, and high plane rations respectively based on different dietary energy but isonitrogenous (the crude protein levels were the same). The proximate compositions of the feeds were determined [11]. Gross energy values of the feeds were estimated with Gallenkamp ballistic bomb calorimeter. Daily feed offered was based maintenance ration at 4.0% of their body weights [12]. The feeds were given in two split doses at 008h and 016h daily and water was provided ad libitum.(Table 1 and 2).

Infection with Trypanosomes: The *Trypanosoma congolense* parasites used in this experiment was obtained from the National Institute for Trapanosomosis Research (NITR), Vom, Nigeria. *Trypanosoma congolense* (Binchi Bassa Strain) was obtained and subjected to six passages in albino mice, prior to use. The infected animals were inoculated intraperitoneally with equal number of *Trypanosoma congolense* at the rate of $1.0\times10^{6}mL^{-1}$ in sterile normal saline. This experiment lasted a period of 5 weeks .

Parasitaemia: was determined by haemocytometry as described by Herbert and Lumsden [13].

Serum complement: Five milliliters (5ml) of blood sample was collected in anticoagulant free plastic tube and allowed to clot at room temperature within 3 hours of collection. The serum samples were separated and stored at -20^{0C} for complement system analysis. Testing of haemolytic complement and its components was done as described by Kumshe [9]. The procedure involves preparation of erythrocytes, sensitization of erythrocytes complex formation, standardization of erythrocyte complex formation cells, testing of haemolytic antibody titre, haemolytic complement determination and interpretation of results. Determination of serum complement C3 was determined by radio immunization assay (RIA) using Mancini test as described [14], total and alternative haemolytic complement was determined as described by Phimister and Whaley [15].

The formula use to determine the concentration of the complement in test serum of the complement.

$$\% \text{ complement} = \frac{\text{Mean OD of test sample- Mean OD of negative}}{\text{Mean OD of positive control-Mean OD of negative}} \times 100$$

Statistical Analysis and Experimental Design: The data obtained from serum C3, total and alternative haemolytic complements were observed at pre and post infection phase and data were statistically compared with the respective control and infected groups by a one –way analysis of variance (ANOVA). Duncan's multiple range tests were applied to compare the significance of differences of groups. ($P < 0.05$ and $P<0.001$ were considered significant and very highly significant difference).

RESULTS:

Parasitaemia: It was first detected at week 1 post infection (p. i.) in all the groups with log parasitaemia of 4.0 ± 0.13, 3.6 ± 0.05 and 3.2 ± 0.02 in all infected animals on low (A), medium (B) and high (C) levels of dietary energy respectively. The lowest parasitaemia level was found in the goats placed on a high (C1) level of diet, while the highest level was observed in goats on the low (A1) level of dietary energy as revealed by examination of the buffy coat. The parasitaemia persisted with the peak parasitaemia occurring on week 5 post infection (Table 3).

The group mean serum C3 component concentration values of infected animals decreased by 55.18% (from pre-infective values of 139.75 ± 0.01 to 62.63 ± 10.64), 47.38% (from pre-infective values of 138.25 ± 0.41 to 72.75 ± 1.91) and 44.60% (from pre-infective value of 138.75 ± 0.49 to 76.88 ± 0.49) in groups A, B and C respectively. When the C3 complement component concentrations of infected animals in groups A, B and C were statistically paired with their respective corresponding pre infective values they had $P<0.001$,$P<0.001$ and $P<0.001$. Statistical pairs of infected A/B, A/C and B/C had $P<0.05$,$P<0.05$ and

P>0.05 respectively. (Table 4). The group mean total haemolytic complement (THC) of infected animals declined by 69.57% (from pre-infective values of 57.50±0.42 to 17.50±0.42), 58.33% (from pre-infective values of 60.00±0.60 to 25.00±0.60) and 54.04% (from 58.75±0.31 to 27.00±0.60) in groups A ,B and C respectively. When the THC concentrations of infected animals in groups A,B and C were statistically paired with their respective corresponding pre infective values, they had P<0.001,P<0.001 and P<0.001. Statistical pairs of infected A/B, A/C and B/C had P<0.05,P<0.05 and P>0.05 respectively. (Table 4). The group mean alternative haemolytic complement (AHC) of infected animals decreased by 55.99%(from pre-infective values of 19.88±0.28 to 8.75±0.31), 30.86 %(from pre-infective values of 20.25±0.56 to 11.00±0.27) and 26.74%(from pre-infective values of 21.50±0.42 to 13.00±0.27) in groups A,B and C respectively . When the AHC concentrations of infected animals in groups A,B and C were statistically paired with their respective corresponding pre infective values, they had P<0.001,P<0.001 and P<0.001. Statistical pairs of infected A/B, A/C and B/C had P<0.05,P<0.05 and P>0.05 respectively.(Table 4).

Table -1: Composition of rations offered to WAD Goats

Feed ingredients	**Percentage of ration**		
	A	B	C
Panicuum maximum	20.00	15.00	10.00
Air dried cassava	50.00	55.00	60.00
GNC	10.00	10.00	10.00
PKC	4.00	4.00	4.00
Fish meal 65%	4.25	4.25	4.25
Wheat offals	10.00	10.00	10.00
Oyster shell	1.00	1.00	1.00
Vitamin premix	0.50	0.50	0.50
Salt	0.25	0.25	0.25
Total	100.00	100.00	100.00

Table- 2 :Proximate Analysis of ration feeds offered to the WAD Goats

Components	RATION		
	A	B	C
Dry matter %	72.95	75.25	77.57
Crude protein %	13.17	13.26	13.35
Ether extract %	24.64	26.92	29.20
Crude fibre %	16.82	16.82	16.81
Nitrogen free extract %	62.40	54.50	45.80
Total Ash %	3.60	11.60	15.20
Calculated Gross Energy(kcal/kg)	2426.74	2548.57	2670.40

Table-3: Influence of different levels of dietary energy on Parasitaemia (log ^{10}) Trypanosomes ml^{-1} blood in *T.congolense* infected WAD goats.

Week(post infection)	Low plane(A)	Medium plane(B)	High plane(C)
Week 1	4.0 ± 0.13^{a}	3.6 ± 0.05^{b}	3.2 ± 0.02^{c}
Week 2	2.9 ± 0.02^{a}	3.0 ± 0.04^{b}	2.7 ± 0.05^{c}
Week 3	6.8 ± 0.02^{a}	6.2 ± 0.03^{b}	6.0 ± 0.03^{c}
Week 4	8.0 ± 0.05^{a}	7.0 ± 0.02^{b}	6.8 ± 0.03^{c}
Week 5	8.5 ± 0.03^{a}	8.1 ± 0.03^{b}	7.6 ± 0.02^{c}

Table-4: Influence of different levels of dietary energy on C3, Total haemolytic complement (THC) Alternative haemolytic complement (AHC %) in *T.congolense* infected WAD goats.

Complement	Groups	Pre-infection Value	Post infection Values	% decrease
C3(%)	A	139.75± 0.001	62.63± 1.64	55.18
	B	138.25± 1.44	72.75± 0.91	47.38
	C	138.75±0.49	76.88± 0.04	44.59
THC	A	57.50 ±0.42	17.50± 0.42	69.57
	B	60.00 ±0.60	25.00±0.60	58.33
	C	58.75 ± 0.31	27.00 ± 0.60	54.04
AHC	A	19.88± 0.28	8.75± 0.31	55.99
	B	20.25± 0.56	14.00± 0.27	30.86
	C	21.25± 0.42	15.75± 0.31	26.74

DISCUSSIONS

The present study showed that dietary energy had a marked influence on parasitaemia and complement levels during infections. The trypanosomal infections in these studies were characterised by an undulating parasitaemia and the host goats did not reveal parasites in their peripheral blood until the 7th day post infection. Following the establishment of infection, infected animals fed different levels of dietary energy experienced greater retardation of complements than their control groups.

The group mean serum C3,total and alternative haemolytic complement in all infected goats in group A, B, and C decreased as the infection progressed .This may be due to consumption of this component and it is an important candidates for improve generalized defense mechanism ,control of parasitaemia and immunosuppression as described in trypanosome infected animals [16] and the decline in mean serum C3, may be due to failure of mononuclear phagocytic system and liver to synthesize this complement component and since liver is one of the vital organs that is clinically affected during trypanosomosis [17]. The fall in these complements agrees and in consistent with the finding of *T.congolense* infected cattle [18-19], sheep [6], *T. congolense* infected camel [20], *T.congolense* infected mice [21]. The depletion in serum C3 and total concentration is more severe and significantly lower in WAD goats on low levels of dietary energy than medium and high levels of dietary energy. This further confirms the influence of dietary energy in the susceptibility of WAD goats to *T.congolense* infection. This suggested that different degrees of hypocomplementaemia were observed in all infected goats and may due to different levels of dietary energy and the mechanism by which this influences serum C3 component was not known. The amelioration of hypocomplementamia observed in this study may be due to the influence of improved nutrition vis-à-vis increased dietary energy in groups B and C of infected animals.

There was a significant decrease in total haemolytic complement (THC) in all infected and re infected groups. The fall in THC agrees and corroborates the observation of *T.evansi* infected goats [20]. *T.brucei* infected Saneen goats [22]. *T.vivax* infected cattle [18] in both WAD and Born white (BW) goats infected with *T. congolense*[9-10] in cattle infected with *T. congolense*. The depletion in serum THC concentration is more severe and significantly lower in WAD goats on low levels of dietary energy than medium and high levels of dietary energy. This further confirms the influence of dietary energy in the susceptibility of WAD goats to *T.congolense* infection. A significant decrease was observed in alternative haemolytic complement (AHC) in all the infected groups . The fall in AHC has been observed and reported by [6] of *T.congolense* infected sheep[23]. *T.brucei brucei* infected WAD short horn cattle [22], *T.vivax* infected cattle [18] in both WAD and Born white (BW) goats infected with *T. congolense* in cattle infected with *T. congolense*. The depletion in serum AHC concentration is more severe and significantly lower in WAD goats on low levels of dietary energy than medium and high levels of dietary energy. This further confirms the influence of dietary energy in the susceptibility of WAD goats to *T.congolense* infection. The decline in mean C3, THC and AHT agrees with finding of Kumshe in both WAD and Born white (BW) goats infected with *T. congolense* and Talabi, in cattle infected with *T. congolense.*

The exact mechanism by which dietary energy influence complement system is not known but Faye *et al.*, [24] reported that the high energy demands of trypanosome infection may lead to severe energy shortage and this might be reflected in the changes from energy and to protein metabolism, since the complement system are made up serum protein ,catabolism of serum protein due to shortage of dietary energy might be responsible for the differences observed in this study and hence the different levels of dietary energy influence the activities of these complement system of *T.congolense* infected goats in term of depletion by trypanosomes and the rate of synthesis by mononuclear phagocytic system and liver. In conclusion, *Trypanosoma congolense* infection in goats causes major changes in parasitaemia and serum complement system as a result of modification and influence of dietary energy. This is an indication that dietary energy influences the susceptibility of WAD goats to experimental *T.congolense* infection .

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