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RUMEN METABOLITES AND THERMO-PHYSIOLOGICAL RESPONSE OF WEST AFRICAN DWARF SHEEP AS INFLUENCED BY FICUS FOLIAGE WITH DIFFERENTLY PROCESSED BREADFRUIT MEALS.

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ABSTRACT: *This experiment was conducted to assess the rumen metabolites and thermo-physiological response of West African Dwarf (WAD) sheep fed ficus foliage with differently processed breadfruit meals. Twenty (20) healthy West African Dwarf sheep with average weight of 6.00±0.55kg and aged approximately 7months were randomly allotted to four (4) dietary treatments with five (5) animals per treatment in a completely randomized design. The diets compared were A (50% unpeeled raw breadfruit meal + 20% ficus foliage + 30% concentrate diet), B (50% peeled raw breadfruit meal +20% ficus foliage + 30% concentrate diet), C (50% unpeeled soaked breadfruit meal +20% ficus foliage + 30% concentrate diet) and D (50% unpeeled boiled breadfruit meal +20% ficus foliage +30% concentrate diet). The results obtained showed that the rumen temperature (41.68 ^{0}C), rumen ammonia nitrogen (NH_3-N) concentration (70.23mg/100ml), total volatile fatty acids (72.03mmol/liter), acetic acid (46.65%), respiratory rate (16.23 breaths/mintues), pulse rate (80.07 beats/min) and urea (14.92 mg/dl) were significantly ($P<0.05$) best with animals on diet A than those on diets B, C and D. Animals on diet B had the highest significant ($P<0.05$) values in terms of rumen pH (6.60), propionic acid (25.02%), butyric acid (9.62%), total protein (8.08g/dl), glucose (64.01mg/dl), sodium (136.03mmol), potassium concentration (5.44mmol/l) and phosphorus (7.34mg/dl). Significant ($P>0.05$) difference did not exist between animals on treatment diets with regards to rectal temperature and serum calcium levels. It was therefore concluded that 50% peeled raw breadfruit meal + 20% ficus foliage + 30% concentrate diet improved rumen metabolites and thermo-physiological response of West African dwarf sheep.*

KEYWORDS: *Breadfruit, ficus foliage, rumen, thermo-physiology response and sheep.*

INTRODUCTION

Ruminants are important livestock sector in virtually every country in the world, due to their contribution as animal protein to the diet of an average human populace. However, one of the major problems facing the holders of small ruminant in Nigeria is how to adequately feed their livestock most especially during the off- season. Arigbede *et al* (2005) noted that inadequate feed supply in both quantity and quality is responsible for low ruminant animal productivity in the tropics. Though forages are abundant in the tropics, seasonal changes in their nutritive values have been the major problem in ruminant animal production. Nevertheless, the use of conventional feed resources in ruminant nutrition are undesirable and further compounded the problem as they become insufficient and put ruminants into direct competition for feeds with human population. Consequently, this become a great interest and concern to the researchers, hence there is need to find alternative ways of improving ruminant

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feeds. Breadfruit *(Artocarpus altilis)* meal and ficus *(Ficus thionning*) foliage are less-known feeds that are rich in energy and protein for ruminants. They are generally cheap and available with good nutritional potentials, if properly harness as feedstuffs for ruminant animals (Ajayi *et al*., 2005; Oladunjoye et al, 2010).

Notwithstanding, what makes ruminant animals unique in feeding status is their rumen, which is the largest muscular organ in the fore-stomach. The rumen is characterized as the primary site for microbial fermentation of ingested feeds. The management of microbial population in the rumen is achieved by feeds and pH control. The rumen temperature and pH are critical phenomenons that depend on the fermentation of ingested feeds in the rumen. However, thermoregulatory indices of animals that are often measured as rectal temperature, heart rate and respiratory rate have been found to be of value in exterminating the health status of livestock. It was reported in literature (Sanusi *et al*., 2011) that rectal temperature increases, when the physiological mechanism of animal fails to negate the excessive heat load, such exposure of animal to heat stress evokes a series of drastic changes in the biological functions like a decrease in feed intake, disturbances in protein and energy balances, enzymatic reactions and blood metabolism. Thus, they are important index in assessing the body's ability to respond to haematological and serum biochemical upset of animals (Silanikove, 2000). Moreover, thermo-physiological response of animals may give some insight as to the potentials of a test diet to meet the metabolic needs of animals. Hence, the present study was undertaken to assess the rumen metabolites and thermo-physiological response of West African dwarf sheep as influenced by ficus foliage with differently processed breadfruit meals.

MATERIALS AND METHODS

Experimental Site:
The study was carried out at the Sheep and Goat Unit of the Teaching and Research Farm, Ambrose Alli University, Ekpoma, Nigeria (6^0 42^0N, $6^0$09'E). The temperature ranged from 26^0C to 36^0C with mean annual rainfall of about 1556mm.

Experimental Diet's Preparation:
Ficus foliage was freshly harvested within the Teaching and Research Farm, allowed to wilt over night before they were individually chopped into thin pieces of about 5cm and used. Breadfruits were harvested from the same tree in a farm plantation around Ekpoma in Edo – State, Nigeria. The breadfruits were differently processed into meals in the following ways:

URBM = Slicing - Sun dry - Milling
PRBM = Peeling Slicing - Sun dry - Milling
USBM = Slicing - soaking - Sun dry - Milling
UBBM = Slicing - Boiling - Sun dry - Milling

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URBM = Unpeeled Raw Breadfruit Meal
PRBM = Peeled Raw Breadfruit Meal
USBM = Unpeeled Soaked Breadfruit Meal
UBBM = Unpeeled Boiled Breadfruit Meal

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The concentrate diet that was used in combination with the differently processed breadfruit meal and ficus foliage is indicated in Table 1.

Table 1. Gross composition (%DM basis) of the concentrate

Ingredient	Composition
Brewer's dry grain	50.00
Wheat offal	35.00
Rice bran	10.00
Vitamin	1.50
Bone meal	2.00
Limestone	1.00
Salt	0.50
Total	100

The prepared breadfruit meals were used in combination with ficus foliage and the formulated concentrate diet as the experimental diets in a ratio of 50:20: 30 respectively. However, the four experimental diets prepared were: A (50% URBM + 20% ficus foliage + 30% concentrate diet), B (50% PRBM + 20% ficus foliage + 30% concentrate diet), C (50% USBM +20% ficus foliage + 30% concentrate diet) and D (50% UBBM +20% ficus foliage +30% concentrate diet). The experimental diets were offered at the rate of 5% (dry matter basis) of their body weight.

Experimental Animals and Management

Twenty (20) West African dwarf male Sheep approximately 7 months old with average weight of 6.00±0.55kg that were used for the study lasted for 84 days with 14-day of adjustment period. The experimental animals were sourced from villages and markets within Ekpoma, Edo State. The animals were randomly allotted to the four (4) treatment diets (A, B, C and D) with five (5) replicates per treatment group in a completely randomized design.

Prior to the experiment, pens were cleaned and disinfected before animals were housed in individual pens. Each animal was treated against ecto and endo-parasites. They were also vaccinated with peste des potits ruminant vaccine (PPRV) during the 14-days adjustment period. The health of the animals was properly monitored and immediately adequate treatments were given to the unhealthy animals. Animals were offered their treatment diets once daily at about 08.00am and any feed remaining in the troughs was removed before fresh feed was given in the following morning. Clean water and salt licks were provided for all the animals without restriction throughout the period of the experiment.

ISSN 2053-5805(Print), ISSN 2053-5805(Print)

Rumen and Physiological Studies:
Rumen parameters were assessed on every two weeks till the termination of the experiment. Rumen fluid samples were obtained once daily (morning) for six consecutive time for 12 weeks from four (4) animals per treatment group. The rumen fluid was collected using suction stomach tube from sheep that were previously fed with experimental diets before they were offered morning feed. The pH of the rumen fluid readings were recorded within two minutes of collection through the use of a digital pH meter which had been stabilized in distilled water with specific pH recommendation. The temperature of the rumen fluid samples were also determined through insertion of thermometer into the fluid samples for 2–3 minutes and readings were recorded, before the rumen fluid samples were stored at -20^0C for analysis of rumen ammonia nitrogen concentration (NH_3-N) and determination of volatile fatty acid concentration and its fractions.

Rectal temperature, respiratory rate and pulse rate of the animals were measured early in the morning, afternoon and evening for every day throughout the experimental period.
Rectal temperature was taken on each animal using a digital thermometer. The sensory tip was disinfected and inserted into the rectum at the display of L ^{0}C by the thermometer (which indicated that the thermometer was set for temperature reading). This was removed after the sound of the alarm signal. The displayed body temperature was then recorded. Respiratory rate was determined by counting the number of abdominal movements per minute, while pulse rate was determined for each animal by placing the fingertips on the femoral arteries of the hind limb for one minute.

At the end of the experiment, animals were starved overnight and blood samples were collected in the morning from four (4) animals per treatment group. The blood samples (5ml) were collected by jugular venipuncture from each animal into labeled sterilized universal bottles without anti-coagulant which were used for serum separation for the determination of serum biochemical indices and plasma electrolytes.

Laboratory Analysis
Proximate composition of differently processed breadfruit meals, ficus foliage and concentrate diet were analsyed using the procedures of AOAC (1990). Rumen ammonia nitrogen concentration was measured using the method of Mebrahtu and Tenaye (1997). Total volatile fatty acids production was determined by steam distillation process using markham micro-distillation apparatus as reported by Yusuf *et al*. (2013). Individual volatile fatty acids production was determined using gas chromatography (Mebrahtu and Tenaye, 1997). Serum total protein, glucose, urea, sodium, potassium, calcium and phosphorus were determined using the standard clinical chemistry according to the procedures of Al-Eissa and Saad (2001).

Statistical Analysis
Data collected from rumen and physiological parameters were subjected to analysis of variance (ANOVA) and significant differences between means were separated using Duncan Multiple Range Test (SAS, 1997).

ISSN 2053-5805(Print), ISSN 2053-5805(Print)

RESULTS

Chemical composition of the differently processed breadfruit meals, ficus foliage and the concentrate diet are shown in Table 2.

Table 2. Proximate composition (% DM basis) of differently processed breadfruit, ficus foliage and concentrate diet.

Component	URBM	PRBM	USBM	UBBM	Ficus Foliage	Concentrate
Dry Matter	86.02	86.00	85.98	85.99	62.78	89.06
Crude Protein	4.60	4.87	4.85	4.53	22.88	20.01
Crude Fibre	5.01	5.33	5.29	5.20	25.74	13.00
Ash	3.82	3.95	2.72	3.09	11.31	7.98
Ether Extract	2.41	2.91	2.11	2.56	3.30	1.09
Nitrogen Free Extract	84.16	82.94	85.03	83.92	36.77	57.92

URBM = Unpeeled raw breadfruit meal, PRBM = Peeled raw breadfruit meal, USBM = Unpeeled soaked breadfruit meal, UBBM = Unpeeled boiled breadfruit meal.

The dry matter values were quite high and varied from 62.78 to 89.06%. The crude protein values ranged from 4.53% to 22.88% with ficus foliage recorded the highest and UBBM the lowest. Crude fiber and ash values were ranged from 5.01 to 25.74% and 2.72 to 31.06% respectively. Ether extract and nitrogen free extract values varied from 1.09 to 3.30% and 36.77 to 85.03% respectively.

Table 3: Rumen parameters of West African Dwarf sheep fed experimental diets.

Parameters	Diets				
	A	B	C	D	SEM ±
Rumen pH	5.92^{b}	6.60^{a}	6.17^{a}	5.99^{b}	0.21
Temperature (^{0}C)	41.68^{a}	39.08^{b}	38.96^{b}	40.82^{a}	0.09
NH_3-N (mg/100l)	70.23^{a}	68.96^{b}	67.51^{c}	63.84^{c}	0.38
TVFAs (Mmole/litre)	72.03^{a}	66.95^{b}	65.84^{b}	71.93^{a}	0.42
Molar (%)					
Acetic acid	46.65^{a}	45.32^{a}	42.03^{b}	45.66^{a}	0.83
Propionic acid	16.07^{b}	25.02^{a}	23.56^{a}	18.34^{b}	0.50
Butyric acid	6.03^{b}	9.62^{a}	8.72^{a}	6.98^{b}	0.45

$^{a, b, c}$ means within the same row with different superscripts differ significantly (P<0.05).
SEM = Standard error of mean.
NH_3–N = Ammonia nitrogen, TVFAs = Total volatile fatty acids

Presented in Table 3 are the rumen parameters of West Africa Dwarf Sheep fed experimental diets. Significant differences (P<0.05) were observed among treatments for all the parameters assessed. The apparent pH of the rumen fluid values were 5.92, 6.60, 6.17 and 5.99 for diets A, B, C and D respectively. Diets A(5.92) and D(5.99) were significant similar (P>0.05) between treatment groups but significantly (P<0.05) lower than diets B(6.60) and C(6.17). The apparent rumen temperature levels observed in this study did not follow the same pattern of variation as observed in rumen pH levels. The values ranged from 38.96 to 41.68^{0}C with animals on diets A and D significantly higher (P<0.05) than those on diets B and C.

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Apparent rumen ammonia nitrogen (NH_3-N) concentration levels in sheep were varied significantly (P<0.05) with animals on diet A (70.23mg/100ml) being the highest, followed by diets B (68.96mg/100ml) and C (67.51mg/100ml) before diet D (63.84mg/100ml) which was the least. The rumen total volatile fatty acid values that ranged between 65.84 and 72.03mmol/liter among treatments were significantly (P<0.05) higher in sheep on diets A and D compared with diets B and C. The molar proportion of acetic acid values observed almost followed the same trend as obtained in total volatile fatty acids. The values were higher in diets A (46.65%), B(40.32%) and D (45.66%) significantly (P<0.05) and lower in diet C(42.03%). The propionic and butyric molar proportions of the total volatile fatty acids were significantly (P<0.05) better in diets B(25.02 & 9.62%) and C(20.56 & 8.72%) compared with diets A(16.07 & 6.03%) and D(18.34 & 6.98%).

Table 4: Thermo-physiological parameters of West African Dwarf sheep fed ficus foliage with differently processed breadfruit meals.

	Diets				
Parameters	A	B	C	D	SEM±
Thermoregulatory					
Rectal temperature (^{0}C)	39.02	38.91	38.97	38.60	0.86
Respiratory rate (breaths/min)	16.23^a	15.06^b	15.13^b	14.98^b	0.96
Pulse rate (beats/min)	80.07^a	75.40^c	76.03^c	78.09^b	0.84
Blood metabolites					
Total protein (g/dl)	6.72^b	8.08^a	7.96^a	6.46^b	0.59
Glucose (mg/dl)	53.97^b	64.01^a	60.31^a	59.01^b	0.73
Urea (mg/dl)	14.92^a	7.83^c	9.82^b	9.66^b	0.42
Sodium (Mmole)	112.20^c	136.03^a	122.34^b	116.09^c	0.35
Potassium (Mmole)	4.96^a	5.44^a	5.21^a	2.93^b	0.68
Calcium (mg/dl)	5.93	5.39	5.29	5.12	0.19
Phosphorus (mg/dl)	4.56^b	7.34^a	6.62^a	4.93^b	0.05

$^{a, b, c}$ means within the same row with different superscripts differ significantly (P<0.05).
SEM = Standard error of mean.

Table 4 shows the physiological parameters of West African dwarf sheep fed experimental diets. Rectal temperature values observed among treatment diets were ranged between 38.60 and 39.02^{0}C. Though no significant difference (P>0.05) was observed among treatment diets, animals on diet A were higher in terms of numerical value than diets B, C and D. The observed results obtained for respiratory rate was significantly (P<0.05) better for animals on diet A (16.23 breaths/min) compared with diets B (15.06 breaths/min), C (15.13 breaths/min) and D (14.98 breaths/min). However, pulse rate values that ranged between 75.40 and 80.07 beats/min did not follow the same variation pattern as observed in respiratory rate. Significant differences (P<0.05) were observed in pulse rate with animals on diet A (80.07 beats/min) being the highest followed by diet D (78.09 beats/min) before diets C (76.03 beats/min) and B (75.40 beats/min).

Serum biochemical parameters of West African Dwarf sheep fed experimental diets is also shown in Table 4. Total protein and glucose values recorded were significantly (P<0.05) influenced by treatment groups. Treatment diets B (8.08g/dl & 64.01mg/dl) and C (7.96g/dl & 60.31mg/dl) were higher compared with diets A (6.72g/dl & 53.97mg/dl) and D (6.46g/dl & 59.01mg/dl). Serum urea levels was significantly highest in sheep on diet A (14.92mg/dl)

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followed by diets C (9.82mg/dl) and D (9.66mg/dl) before diet B (7.83mg/dl). Sodium values were highest in animals on diet B (136.03Mmol) than those on diets A (112.20 Mmole), C (122.34Mmol) and D (116.09Mmole) .Potassium values of 4.61Mmole, 5.44Mmole, 5.21Mmole and 2.93Mmole were obtained for treatment diets A, B ,C and D respectively. Animals on diets B and C were significantly ($P<0.05$) higher than animals on diets A and D. The mean values obtained for phosphors were significantly ($P<0.05$) highest in animals on diets B (7.34 mg/dl) and C (6.62 mg/dl) followed by diet A (5.93 mg/dl) and D (5.12 mg/dl) was the least. No significant ($P>0.05$) variation was found in calcium across the treatment diets with mean values ranged from 8.21 to 8.61mg/dl.

DISCUSSION

The observed ranged values of 4.53–4.87% crude protein values of the differently processed breadfruit meals were below the 6–8% crude protein values recommended by NRC (1985). Gratemby (2002) indicated that 10–12% crude protein values are recommended as moderate levels for ruminant production. Therefore, the 22.88% and 20.01% crude protein values in ficus foliage and concentrate diet were added to provide adequate nitrogen requirement for rumen micro-organisms to maximally digest the components of the dietary fiber leading to the production of volatile fatty acids. The crude fiber, ash and ether extract contents in the study were highest in the ficus foliage than breadfruit meals and the concentrate diet. However, the differently processed breadfruit meals were better in nitrogen fee extract than the ficus foliage and concentrate diet.

The higher apparent rumen pH observed in animals on diets B and C could probably due to less fermentable of feed components that were consumed by the animals. The implication of these higher rumen fluid pH values observed was that ciliate protozoa population might established well in such rumen environment and encourage fauna and faunation in the rumen. Ranjhnan (2001) reported that diets which are very high in protein also give alkaline reaction which brings about high pH in the rumen. The decreased rumen pH values observed in animals on diets A and D could probably due to faster fermentation by cellulolytic or amylolytic bacteria activities leading to lactic acid production as earlier reported by Jyoti *et al* (2000). This high concentration of lactic acid and low pH could lead to low protozoa population or total disappearance in the rumen and encourage flora and defaunation with rapid absorption off undissociated volatile fatty acids. However, the rumen pH values observed in this study were compared favourable with the findings of Yusuf *et al.* (2013). Whose values (5.5 – 7.5) were within the recommended standard rumen pH values. The relatively constant temperature of the rumen has been reported (Ranjhnan, 2001) to be a factor of great importance to the stability of microbial fermentation in the rumen. The slight difference noticed in values of apparent rumen temperature between treatment diets might be as a result of difference in evolution of heat in fermentation of feeds. This is in agreement with the report of Dung *et al* (2011) who found that the trends at which temperature rises in the rumen following ingestion of feed is due to the evolution of heat in the fermentation process which has been used as a measure of fermentation rate in the rumen. However, the higher rumen temperature observed in animals on diets A and D collaborated with the relatively acidic rumen environment which do not favour protozoa, hence protozoa do not thrive well or survive in rumen temperature much above 40^0C (Jyoti *et al*., 2000). The rumen ammonia nitrogen (NH_3-N) concentration levels were highest in animals on diet A. This could be a reflection of the extent of crude protein degradability and nitrogen uptake by the

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rumen microbes in preference to amino acid in the animals. Cabrita *et al.* (2006) stated that one of the most intriguing problems in rumen ecology is the extent to which NH_3-N serves as nitrogenous materials for synthesis of microbial cells. The minimum NH_3-N level of 2–5mg/100ml rumen fluid has been suggested to maximize rumen microbial synthesis, 15mg/100ml to maximize fibre digestion and 20mg/100ml to maximize intake (Yashim *et al.*, 2014). Nonetheless, the rumen NH_3-N levels obtained in this study were within the normal ranged of 0 to 130mg/100ml reported by Yusuf *et al.* (2013). Volatile fatty acids contribute about 70% of the energy requirement for ruminants; hence they are classified as one of the universal end-product of anaerobic microbial fermentation of carbohydrates in the rumen. However, the disparity observed in total volatile fatty acids values were linked with the combination of ficus foliage and differently processed breadfruit meals in the experimental diets, which increased the microbial fermentation, production and accumulation of total volatile fatty acids at low pH in diets A and D. Okoruwa (2015) reported that, if volatile fatty acids production rate exceeds the clearance rate, they will accumulate in the rumen, this may lower the rumen pH and cause metabolic disturbance known as rumen acidosis. However, there was no case of rumen acidosis in this study, meaning that the rumen pH values (5.92-6.60) obtained in this study was still within the normal range for the sheep as earlier reported by Yusuf *et al* (2013). The variation observed in acetic acid molar proportion might be due to the differences in processing methods of the breadfruit meals. Widiawati and Thaliab (2009) reported that feeds resulting in increase of acetate production will promote an increase of methane production, which represent a net loss of feed energy as well as inefficiency in feed utilization.

Notwithstanding, the higher proportion of propionic and butyric acids recorded in diets B and C revealed the better rumen fermentation of the diets by the microbial activity and good nutrient utilization to yield energy, thus propionic acid has been classified as the major precursors of glycogenic fatty acid in ruminants (Vasta *et al.*,2009). The slight difference observed in animals' response to changes in rectal temperature in terms of diets could probably due to the environmental temperature and evolution of heat in the fermentation process. This is in consistent with the earlier reports by Hicks *et al* (2001) who reported that the rumen temperature is an effective of body temperature and may be used to predict diseases and heat stress. The rectal temperature values obtained in this finding were within the normal ranged of values (38.40 to 39.30^{0}C) earlier reported by Sanusi *et al* (2011) for West African dwarf goats and sheep. The respiratory rate values observed were slightly higher than the stipulated ranged of values (12 to 20 breaths/min) reported by Takuji and Kazuo (2004) for sheep and goats. The observed higher respiratory rate in this study was a clearly indication of environmental temperature response in sheep in order to maintain their homeostasis. Variation in pulse rate has been reported to reflect the rate at which the heart pumps blood through the body (Adetunji *et al.*, 2002). However, the higher values found in rectal temperature and pulse rate in animals on diet A was an indication of greater stress at that period. This is an agreement within the reports of other researchers (Badru *et al.*, 2009) that rectal temperature and pulse rates are both used to determine the health status and adaptability of domestic animals to stressful condition.

Blood provides a valuable medium for clinical investigation and assessment of nutritional status of animals. Serum total protein and glucose values observed in this study were influenced by ficus foliage and differently processed breadfruit meals. The higher serum total protein and glucose values obtained in animals on diets B and C could reflected to the

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nutritional adequacy and safety of the test diets which agrees with the report of Aruwayo *et al* (2011) who found that total protein and glucose syntheses in animals are related to the amount of protein and energy present in the diets. However, urea levels were higher in diet A than other diets. This increment in urea levels might perhaps be ascribed to the effect of some endogenous anti-quality component which could reduced protein utilization and subsequently degraded in urea. Notwithstanding, the higher serum total protein obtained in animals on diet B were indirectly proportional to the lower serum urea levels of the same diet. This is in consonance with the report of the Omoikhoje *et al* (2003) that urea is an indirect measure of protein utilization in animals. Sodium concentration values observed in this study fell within the normal mean values (113.02 to 139.17mmol/l) for healthy sheep as reported by Taiwo and Ogunsanmi (2003). The low sodium levels observed in animals on diet A could be as a result of varied dietary intake of salt and loss of sodium and chlorine ion in the diet. The potassium concentration levels observed were slight lower than the ranged values (3.9 to 5.3mmol/l) reported for sheep and goats (Daramola *et al*., 2005). The low potassium levels recorded in animals on diet D in this finding explains the tendency of infection. The observed better calcium levels in the experimental animals generally could probably be an account of higher intake of available calcium content in the diets that were utilized by the animals. This also applicable to phosphorous levels in diets B and C that were better utilized by the sheep.

CONCLUSION

Based on the results obtained in this study, it was therefore concluded that feeding ficus foliage with differently processed breadfruit meals to West African Dwarf (WAD) sheep have the potential of meeting the nutritional needs, in terms of basal component of feeds. The response in terms of improving rumen metabolites and thermo-physiological response by WAD sheep indicated that 50% peeled raw breadfruit meal + 20% ficus foliage + 30% concentrate diet can serve as a suitable feeds for WAD sheep.

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