

Performance, nutrient utilization and blood constituents of goats fed different inclusion levels of sour sop leaf powder in diets containing fluted pumpkin pulp

Abstract

The study was conducted to assess growth performance, nutrient utilization and blood constituents of goats fed diets containing fluted pumpkin pulp and different proportions of sour sop leaf powder. Twenty four West African dwarf female goats of about 7 to 8 months old with an average initial body weight of 7.00 ± 0.15 kg were used for the study. They were randomly allotted to four dietary treatments and each treatment was replicated three times with two goats per replicate in a completely randomized design. The compared diets contained; 6% dried fluted pumpkin pulp without sour sop leaf powder that served as the control group (PPM₁), 6% dried fluted pumpkin pulp with 2% sour sop leaf powder (SLP₂), 6% dried fluted pumpkin pulp with 2.5% sour sop leaf powder (SLO₃) and 6% dried fluted pumpkin pulp with 3% sour sop leaf powder (SLW₄). In all diets examined, results showed that diet PPM₁ was significantly ($P < 0.05$) higher in feed conversion ratio, digestibility of ether extract, gross energy intake, total nitrogen and energy output, creatinine, urea, serum enzymes, total cholesterol and low density lipoprotein. Goats in diet SLO₃ were better significantly ($P < 0.05$) in final, total and daily body weight gain, dry matter, crude protein and crude fibre digestibility, nitrogen retention, digestible and metabolizable energy, packed cell volume, haemoglobin, red and white blood cells, serum total protein, albumin, glucose and high density lipoprotein. However, feed and nitrogen intake as well as triglycerides indicated significant ($P < 0.05$) higher values in diet SLW₄. No significant ($P > 0.05$) difference was found in initial body weight, metabolizability, globulin, eosinophils, monocytes and basophils. It can therefore be concluded that using 6% dried fluted pumpkin pulp with 2.5% or 3% sour sop leaf powder in diets enhanced nutrient utilization for better performance and health status of goats.

Keywords: sour sop leaf, *pumpkin-pulp*, *performance*, *blood*, *goats*.

Introduction

Goats have been identified to play a crucial role in nutritional security for supporting mankind in increasing animal protein availability for human sustainability. Their biological values and domestic demand as choicest milk and meat for consumers have been

ranked higher than sheep and cattle [1] Therefore, the need to increase goat production is a consequence of its capacity to generate income and improve the living standards of rural subsistence-farming communities [2] The degree to which goats survive to marketable age is one of the key indicators for their efficient productivity. However, nutritional constraints remain a concern, because it limits animal digestion, growth rate and weakens animals against parasitic and infectious diseases that bring about high mortality. Tropics experience seasonal dry periods that decreased the quantity and quality of available forages for ruminant livestock. Goats raised in this tropical region are predominantly faced with nutritional stress, due to decrease in the quality of grazing pastures [3] Conventional feeds that supplement poor forages are expensive as a result of high grain price and less diversified animal feeds. One possible way to mitigate this incessant challenge ravaging ruminant livestock production is to explore alternate feed resources that are not consumed by man but can be used as feeds for livestock.

Fluted pumpkin (*Telfairia occidentalis*) belongs to the genus *Cucurbita* and family of *Cucurbitaceae*. The pulp is a by-product of the pumpkin seed which is relatively abundant, most especially in areas where they are produced. The fluted pumpkin pulp is beneficial and economical as feed resource for animals that provide considerable benefits to goat farmers [4] However, inclusion of natural additives to poor quality feeds is currently used by most rural farmers to improve better sustainability of goat farming in Nigeria. They have valuable potentials that are profitable in the aspect of boosting immune system, biodegradable and biocompatible [5]

Soursop (*Annona muricata*) belongs to the family of *Annonaceae*. It is a herb plant that has good natural multiple nutritive values with secondary metabolites that justify its application as phyto-additive [6] It also possesses great potentials as anti-microbial and anti-inflammatory with bioactive components that modulate rumen to regulate micro-flora for better digestion and inhibition of lipids [7] However, there is relatively little information regarding the effect of soursop leaf powder supplementation on feed utilization and performance of goats. Thus, the study was designed to determine the effect of soursop leaf powder on growth, nutrient retention and blood constituents of goats fed diets containing dried fluted pumpkin pulp.

Materials and Methods

Location of study

The study was conducted at the Small Ruminant Unit of the Livestock Teaching and Research Farm of Ambrose Alli University, Ekpoma. The area lies on longitude 6.09°E and

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latitude 6.42⁰N within the humid climatic zone of Nigeria. The average annual rainfall and temperature of the region are about 1556mm and 31⁰C respectively, while relative humidity of the lactation is about 78% averagely.

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Experimental diets

Green matured soursop leaf was obtained from their naturally growing plant within the Teaching and Research Farm environment. They were air dried under a well ventilated shed until the leaves were crispy to touch, while retaining their greenish coloration. Thereafter, they were milled to pass through a 0.15mm sieve to obtain soursop leafy powder and stored in airtight bags at room temperature. Matured harvested fluted pumpkin fruits were cut opened, seeds and pulp were separately removed before pulp was sun dried and crushed into fluted pumpkin pulp meal. However, soursop leaf powder and fluted pumpkin pulp meal with other feeding ingredients were used to prepare the supplementary diets as showed in Table, 1. Succulent elephant grass obtained within the farm was chopped to small lengths of about 4 to 5cm and served as basal diet. The basal and supplementary diets were offered in ratio 60: 40 to the animals respectively. The prepared supplementary diets were composed as follows; PPM₁ (6% dried fluted pumpkin pulp without soursop leaf powder and served as the control group), SLP₂ (6% dried fluted pumpkin pulp with 2% soursop leaf powder), SLO₃ (6% dried fluted pumpkin pulp with 2.5% soursop leaf powder) and SLW₄ (6% dried fluted pumpkin pulp with 3% soursop leaf powder) were the inclusion levels in different treatment diets

Table 1. Gross composition (%DM) of the supplementary diets

Ingredients	Treatment Diets			
	PPM ₁	SLP ₂	SLO ₃	SLW ₄
Wheat offal	47.00	44.00	41.00	38.00
Dried brewers grain	33.00	32.50	32.00	31.50
Rice bran	5.00	8.00	11.00	14.00
Dried fluted pumpkin pulp	6.00	6.00	6.00	6.00
Broken bean /husk	5.50	4.00	4.00	4.00
Soursop leaf powder	-	2.00	2.50	3.00
Bone meal	2.00	2.00	2.00	2.00
Vitamin premix	1.00	1.00	1.00	1.00
Salt	0.50	0.50	0.50	0.50

Total	100.00	100.00	100.00	100.00
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Animal and management;

Twenty four West African dwarf female goats of about 7 to 8 months old with an average initial body weight of 7.00 ± 0.15 kg were used for the study. On their arrival, they were confined for a preliminary period of fourteen days to allow them adapted to the environment and treated against internal and external parasites. After the quarantine period, goats were randomly allocated to the four treatment diets giving six animals per treatments. Thus, each treatment was replicated three times with two goats per replicate in a completely randomized design. They were housed in individual semi-open partitioned pens, equipped with feeders, water troughs and saw dust that was changed twice weekly. Diets that were offered to each goat twice daily (8:00am and 4:00pm) were calculated on the basis of 5% dry matter of their body weight. The ratio of the basal diet of elephant grass to each of the supplementary diet was used to determine the proportion of each diet that was offered to the goat. The amount of diet supplied to each animal was adjusted daily based on the voluntary intake of the animal with an estimated leftover of 15%. They also had free access to drinking water and duration of the experiment was 90 days exclusive of the 14 days of acclimation.

Experimental procedures and measurements

Growth performance

The average daily feed intake was determined by the difference between the amount of feed offered and leftover. Initial body weight of goats were weighed and recorded at the onset of the experiment. Subsequently, weekly weights using the measuretech® hanging scale. Weekly weights were taken prior to morning feeding to estimate change in body weight. Total body weight was obtained by the difference between the final and initial body weight. The average daily weight gain was calculated from weekly weight in individual goats over the entire period of the trial. However, daily feed intake with body weight gain was used to calculate the feed conversion ratio.

Nutrient digestibility, nitrogen retention and energy metabolism

Growth study was preceded by a 14 day metabolic trial to determine apparent nutrient digestion of the diets. Three goats with similar body weight were randomly chosen from each treatment group and housed separately in individual metabolic cages designed for feeding, watering with separate collection of faeces and urine. Feed offered, leftover, voided faeces and urine were collected and recorded daily for the duration of 14 days, which includes 7 days

of total collection after a 7-day of adaption period. Thereafter, a sub-sample of about 10% of the total faecal output was bulked, properly mixed and pooled for each animal before stored in plastic container in the freezer (-20%) for later analysis. Urine from each animal was also collected in a plastic container containing 100 ml of 3.6 mol/L H₂SO₄. The collected urine was strained through a layer of grass wool to remove detached hair fragments and other solid contaminants. The volume was measured and then diluted to 5 L using tap water and a sample of 20 ml was collected, pooled for each animal to prevent loss of nitrogen due to volatilization before stored at -20⁰C for total nitrogen determination. Nutrient Digestibility (ND) of the diet was calculated for dry matter, crude protein, crude fibre, ether extract, ash and nitrogen free extract from each treatment diet using the equation proposed by [8]

$$\text{ND \%} = \frac{\text{Total amount of nutrients in feed} - \text{Total amount of nutrients in faeces}}{\text{Total amount of nutrients in feeds}} \times 100$$

Nitrogen and energy retention were calculated by standard procedures outlined for direct estimation of animal digestion [8]. Nitrogen balance was determined as the differences between nitrogen intake and nitrogen excreted from faeces and urine. The nitrogen retention percentage was estimated from the nitrogen balance expressed as a percentage of nitrogen intake.

Gross energy (GE) of the diets, faeces and urine were measured using an adiabatic bomb calorimeter (C200, IKA Works Inc. Staufen, Germany). The GE intake was calculated as the GE of the feed in dry matter multiplied by dry matter (DM) of the feed intake, while faecal energy (FE) output was determined as the GE of the faeces in DM multiplied by the quantity of the faeces in DM. Urine energy (UE) output was determined as the GE of the urine in DM multiplied by the quantity of the urine in DM. Digestible energy (DE) intake was calculated as the difference between GE intake and FE output divided by GE intake expressed as percentage. Metabolizable energy (ME) was determined by 82% of DE intakes as reported by [9]. The metabolizability (qm) was calculated as ME/GE for each treatment diet [10].

Blood collection and analysis

After the metabolic trial, 10 ml of blood sample was collected from each goat the following day through jugular venipuncture, using disposable heparinised syringes into sterilized evacuated collection tubes before morning feeding. Sub-samples of 5 ml were drawn into labelled sterilized universal bottles containing ethylene diamine tetra-acetic acid (EDTA) as anti-coagulant to determine haematological traits. Packed cell volume (PCV)

was determined with wintrobe's micro-haematocrit while red blood cell (RBC) and white blood cell (WBC) were determined with improved Neubauer haemocytometer while differential counts of the WBC such as lymphocytes, eosinophils, neutrophils, monocytes and basophils were determined by making differential smear using wright stain and percentage. The haemoglobin concentration (Hb) was determined using cyanomethaemoglobin method. The erythrocytic indices, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were computed as described by [11]

The other 5ml sub-samples were transferred into anti-coagulant free plastic tubes and gently inverted a couple of times, kept in an ice box to allow coagulation at room temperature. They were later centrifuged for 15 minutes at 890xg at 4⁰C and maintained for 8 hours in refrigerator at 4⁰C prior to biochemical analysis and separation of supernatant serum. The plasma was also transferred to a storage tube and labelled with the date and animal identification and stored at -20⁰C until analysis.

The biochemical constituents of the serum samples estimated included total protein and albumin that were analyzed by using commercial kits (Biuret method, chemelex, SA Barcelona), but globulin was calculated by subtracting albumin from total protein serum. Urea in serum samples were estimated using the diacetylmonoxime method, creatinine was determined by Jaffe reaction method while blood glucose was obtained by enzymatic colorimetric test. All analyses followed the procedures described by [12] Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), total cholesterol, high density lipoprotein (HDL) and triglyceride concentrations were measured using procedures given by Green Cross MS in Korea as described by [13] The low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were calculated using equations below as presented by [14]

$$LDL = \text{Total cholesterol} - (\text{HDL} + \text{VLDL})$$

$$VLDL = \frac{\text{Triglycerides}}{5}$$

Chemical analysis

Dry matter was determined by drying the fluted pumpkin pulp, soursop leaf, feed, ors, and faecal samples in an air-forced oven at 135⁰C for 2 h [15]; crude ash content was measured by placing the samples into a muffle furnace at 550 ⁰C for 24h. Nitrogen was determined using the Kjeldahl method, using selenium as a catalyst [15] and crude protein was calculated using the formula 6.25 x N. The ether extract was measured by calculating the weight loss of the dry matter on extraction with diethyl ether using the soxhlet

extraction apparatus for 8 h [15] Crude fibre was determined using Tecator Line (FT122 Fibertee TM). The concentration of phytate was determined according to Fredlund *et al.* (1997). Total tannin was determined colorimetrically as described in [15] while total saponin and oxalates were also quantified using colorimetry [15].

Statistical analysis

Data generated from growth, digestibility, nitrogen and energy retention as well as blood constituents were subjected to analysis of variance using the general linear model (GLM) procedure of [16] to determine the effects of the treatment diets on parameters. Where significant difference occurs, means were separated by Duncan's multiple range test [17].

Results

In the feed ingredients examined (Table 2), soursop leaf powder (SL) recorded higher content of crude protein, ether extract and nitrogen free extract but relatively lower in ash values as compared with dried fluted pumpkin pulp (FPP) and elephant grass (EG). However, FPP recorded higher values in dry matter and ash than values obtained in SL and EG, while EG had highest crude fibre content than other feed ingredients. All treatment diets were variable according to types and varying levels of feed ingredients used. Treatment diets SLP₂, SLO₃ and SLW₄ had higher and similar values for dry matter, crude protein, ether extract and nitrogen free extract than PPM₁. On the other hand, crude fibre and ash were relatively higher in diet PPM₁ than diets SLP₂, SLO₃ and SLW₄. Above all, oxalate and phytate showed no remarkable difference among feed ingredients and treatment diets. However, diet PPM₁ had lowest content in tannin and saponin than other treatment diets, SLP₂, SLO₃ and SLW₄.

Table 2: Proximate and phytochemical composition (%DM) of dried fluted pumpkin pulp meal, soursop leaf powder, elephant grass and treatment diets of goats.

	Feed ingredients			Treatment diets			
	FPP	SL	EG	PPM ₁	SLP ₂	SLO ₃	SLW ₄
Dry matter	90.67	87.46	85.04	69.8	75.9	77.8	79.3
Crude protein	3.05	13.98	8.62	10.5	11.2	11.4	11.9
Ether extract	2.03	2.10	1.09	1.78	2.21	2.01	2.46
Crude fibre	19.49	7.53	31.04	33.0	31.9	32.4	30.1
Ash	9.36	5.79	7.41	6.98	5.59	5.19	4.92
Nitrogen free extract	54.74	58.06	35.88	48.1	49.3	48.9	50.1
Phytochemical components							
Tannin	0.68	0.82	0.76	0.87	1.32	1.71	1.56

Saponin	0.79	0.94	0.72	0.49	0.81	0.85	0.89
Oxalate	0.23	0.19	0.58	0.66	0.66	0.53	0.75
Phytate	0.19	0.30	0.51	0.54	0.59	0.67	0.73

FPP = Fluted pumpkin pulp, SL = Soursop leaf, EG = Elephant-grass

There were noticeable significant ($p < 0.05$) difference in mean values for feed intake of goats on the treatment diets (Table 3). Diet SLW₄ appeared higher in value, followed by SLO₃ and SLP₂ compared to the value obtained for PPM₁. However, initial body weight that ranged between 7.09 and 7.20 kg in values for goats did not show any significant differences ($P > 0.05$) among diets. Addition of 2.5% and 3% of soursop leaf powder in diets indicated higher significant values ($P < 0.05$) in final body weight of goats than diet with 2% soursop leaf powder and control diet. Inclusion of test ingredient in diets consequently improved total body weight gain of goats significantly ($P < 0.05$), with highest value recorded in SLO₃ and lowest in those on PPM₁. Daily weight gain was significantly ($P < 0.05$) increased with progressive increase in levels of soursop leaf powder in the experimental diets, but feed conversion ratio showed fairly high values in goats on control diet as compared with diets with soursop leaf powder.

Dry matter digestibility was significantly ($P < 0.05$) higher in values observed for goats on test diets (SLP₂, SLO₃ and SLW₄) than the control diet (PPM₁). Goats placed on diets SLO₃ and SLW₄ had higher crude protein digestibility as compared with those on diets PPM₁ and SLP₂ that showed similar and lower significant ($P < 0.05$) values among treatment diets. Digestibility of crude fiber was found to be significantly ($P < 0.05$) highest in diet SLO₃ followed by diets SLW₄ and SLP₂ compared to diet PPM₁ that recorded the lowest value. The control diet had higher significant ($P < 0.05$) value in ether extract digestibility than values obtained on other test diets. Digestibility of ash varied significantly ($P < 0.05$) in goats on different treatment diets, with test diets being higher in values than value recorded for goats on control group. However, nitrogen free extract values that ranged between 65.47 and 74.52% followed the same pattern of variation as noticed in digestibility of ash.

Table 3: Effect of soursop leaf powder on growth and nutrient digestibility of goats fed diets containing fluted pumpkin pulp meal

Parameters	Diets				SEM ±
	PPM ₁	SLP ₂	SLO ₃	SLW ₄	
Feed intake (g/day)	239.01 ^c	252.64 ^b	254.00 ^b	265.02 ^a	1.02
Initial body weight (kg)	7.20	7.14	7.09	7.18	0.09
Final body weight (kg)	9.14 ^b	9.89 ^b	10.11 ^a	10.01 ^a	0.15

Total weight gain (kg)	1.94 ^c	2.75 ^b	3.02 ^a	2.83 ^b	0.03
Daily weight gain (g/day)	27.71 ^c	39.29 ^b	43.14 ^a	40.43 ^a	0.58
Feed conversion ratio	8.63 ^a	6.43 ^b	5.89 ^c	6.56 ^b	0.07
Nutrient Digestibility (%)					
Dry matter	68.94 ^b	71.82 ^a	73.05 ^a	72.28 ^a	0.57
Crude protein	67.75 ^b	70.93 ^a	71.72 ^a	70.96 ^a	0.64
Crude fiber	60.97 ^c	67.44 ^b	70.03 ^a	69.95 ^b	0.59
Ether extract	63.58 ^a	54.44 ^b	51.43 ^b	50.77 ^b	0.48
Ash	55.22 ^c	60.23 ^b	67.56 ^a	66.02 ^a	0.61
Nitrogen free extract	65.47 ^c	69.14 ^b	74.52 ^a	72.38 ^a	0.79

^{a,b,c} Means in the same row with varying superscript differ significantly ($P < 0.05$).

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The inclusion of soursop leaf powder to diets affected nitrogen retention in goats (Table 4). Nitrogen intake was relatively higher in goats on test diets and significantly ($P < 0.05$) lower in control diet. Nitrogen excreted in faeces and urine (g/day) were significantly ($P < 0.05$) lower in goats that consumed diets with soursop leaf powder as compared with those on control diet. Total nitrogen output was highest significantly ($P < 0.05$) in diet PPM₁, followed by SLP₂ and lowest in diets SLO₃ and SLW₄. Furthermore, nitrogen balance (g/day) and retention (%) were found to be significantly ($P < 0.05$) higher in goats that consumed diets SLO₃ and SLW₄ than those fed on diets SLP₂ and PPM₁.

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Energy utilization was also influenced by different proportion of soursop leaf powder in diets (Table,4). Differences were registered significantly ($P < 0.05$) in gross energy intake in goats among treatment diets. Higher values were recorded for PPM₁, followed by SLP₂ before SLO₃ and SLW₄ that had lower values. However, remarkable difference was also pronounced significantly ($P < 0.05$) for faecal energy output in goats placed on diet PPM₁ than diets SLP₂, SLO₃ and SLW₄. Urinary energy was considerably lower in goats fed diets SLO₃ and SLW₄ as compared with those fed with diets PPM₁ and SLP₂. Total energy output values that ranged between 798.97 and 1196.27 (MJ/g/day/DM) followed the same trends as faecal and urinary energy, although their values were not strictly comparable. Digestible and metabolizable energy intake values appeared higher significantly ($P < 0.05$) in goats on test diets (SLP₂, SLO₃ and SLW₄) than goats placed on control group (PPM₁) that had lower value. Metabolizability indicated no significant difference ($P > 0.05$) between values (0.28-0.49 qm) registered in diets.

Table 4: Nitrogen retention and energy utilization of goats fed diets containing different inclusion levels of soursop leaf powder.

Parameters	Diets				SEM ±
	PPM ₁	SLP ₂	SLO ₃	SLW ₄	
Nitrogen (N) intake (g/day)	10.50 ^b	11.2 ^a	11.4 ^a	11.7 ^a	0.11
Faecal N-output (g/day)	3.00 ^a	2.35 ^b	2.01 ^b	2.24 ^b	0.03
Urinary N-output (g/day)	1.01 ^a	0.94 ^b	0.56 ^b	0.74 ^b	0.02
Total N-output (g/day)	4.01 ^a	3.29 ^b	2.57 ^c	2.98 ^c	0.04
N- balance (g/day)	6.49 ^c	7.91 ^b	8.83 ^a	8.72 ^a	0.06
N-retention of intake (%)	61.81 ^c	70.63 ^b	77.46 ^a	74.53 ^a	0.98
Energy ((MJ/g/day/DM)					
Gross energy	3948.25 ^a	3686.54 ^b	3299.79 ^c	3169.48 ^c	1.20
Faecal energy	979.84 ^a	799.92 ^b	696.42 ^c	701.55 ^{bc}	0.87
Urinary energy	216.43 ^a	168.49 ^b	102.55 ^c	134.22 ^c	1.05
Total energy output	1196.27 ^a	968.41 ^b	798.97 ^c	835.77 ^c	0.92
Digestible energy	1353.29 ^c	1566.03 ^b	1972.38 ^a	1790.91 ^a	1.73
Metabolizable energy	1109.70 ^c	1284.15 ^b	1617.35 ^a	1468.55 ^{ab}	0.99
Metabolizability (qm)	0.28	0.35	0.49	0.46	0.24

^{a,b,c} Means in the same row with varying superscript differ significantly (P < 0.05)

Haematological parameters of goats were significantly (P<0.05) influenced by treatment diets except eosinophils, monocytes, basophils and mean corpuscular volume that were not affected (P>0.05) as pointed out in Table 5. However, packed cell volume (27.01-31.01%), haemoglobin (5.01 -7.02 g/dl) and red blood cell (5.47-6.10x10⁶/ml) values appeared to be higher in goats on test diets SLP₂, SLO₃ and SLW₄ as compared with those on control diet PPM₁. Goats on diets SLP₂, SLO₃ and SLW₄ had higher values of white blood cell than the value recorded in goats on diet PPM₁. Lymphocyte and neutrophil values were highest in diets SLO₃ and SLW₄, followed by SLP₂ and PPM₁ that had the lowest value. Mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration values were found to be improved in goats on diets with soursop leaf than those on diet without soursop leaf powder.

Remarkable significant (P<0.05) differences were observed in all serum biochemical indices of goats except globulin that was unaffected (P>0.05) by the treatment diets. Total protein and albumin values registered for goats on diets with soursop leaf powder were significantly (P<0.05) better than those on control group. Glucose and triglyceride values that ranged from 62.49 to 72.01 g/dl and 20.23 to 22.99 g/dl respectively were highest in animals placed on diets SLO₃ and SLW₄, followed by SLP₂ before PPM₁ that appeared to be lowest.

There were significant ($P < 0.05$) variations in creatinine and urea among treatment diets, these variations were higher in animals on control group than those that were offered test diets.

Table 5: Mean values for haematological, serum biochemical and lipid profile of goats fed experimental diets.

Parameters	Diets				SEM \pm
	PPM ₁	SLP ₂	SLO ₃	SLW ₄	
Haematological indices					
Packed cell volume (%)	27.01 ^b	29.45 ^a	31.01 ^a	30.98 ^a	0.64
Haemoglobin (g/dl)	5.01 ^b	6.79 ^a	7.02 ^a	6.95 ^a	0.05
Red blood cell ($\times 10^6$ /ml)	5.47 ^b	6.04 ^a	6.10 ^a	6.08 ^a	0.07
White blood cell ($\times 10^6$ /ml)	5.98 ^c	7.98 ^b	8.62 ^a	8.43 ^a	0.09
Lymphocytes (%)	43.67 ^c	50.62 ^b	54.96 ^a	52.87 ^{ab}	0.76
Eosinophils (%)	2.19	2.35	2.08	2.31	0.08
Neutrophils (%)	40.01 ^c	45.96 ^b	49.55 ^a	47.02 ^a	0.93
Monocytes (%)	0.25	0.42	0.61	0.58	0.03
Basophils (%)	0.30	0.37	0.41	0.45	0.02
MCV (fl)	34.01	34.58	34.95	34.70	0.67
MCH (pg)	7.99 ^c	9.98 ^{ab}	10.16 ^a	10.07 ^a	0.55
MCHC (%)	30.89 ^b	31.95 ^a	32.07 ^a	32.01 ^a	0.86
Serum biochemical indices					
Total protein (g/dl)	6.52 ^b	6.89 ^{ab}	7.39 ^a	7.06 ^a	0.05
Albumin (g/dl)	2.79 ^b	3.45 ^a	3.65 ^a	3.40 ^a	0.03
Globulin (g/dl)	3.55	3.44	3.74	3.66	0.04
Glucose (mg/dl)	62.49 ^c	68.72 ^b	72.01 ^a	70.11 ^a	0.79
Triglyceride (mg/dl)	20.23 ^b	22.57 ^{ab}	22.90 ^a	22.99 ^a	0.62
Creatinine (mg/dl)	1.07 ^a	0.86 ^b	0.73 ^b	0.70 ^b	0.01
Urea (mg/dl)	12.75 ^a	8.62 ^b	7.85 ^c	7.09 ^c	0.45

^{a,b,c} Means in the same row with varying superscript differ significantly ($P < 0.05$), MCV= Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration.

As indicated in Table 6, are results for serum enzymes and lipid profile of goats fed diets containing soursop leaf powder and fluted pumpkin pulp. Animals placed on diets with test ingredients systemically decreased significantly ($P < 0.05$) in mean values for serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) than those animals on control diet. In this same view, alanine aminotransferase (ALT) and

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aspartate aminotransferase (AST) that ranged from 19.67 to 25.99 and 60.01 to 69.83(U/L) followed the same pattern of variation as observed for SGOT and SGPT.

Total cholesterol values recorded for goats in this study were significantly ($P < 0.05$) varied by treatment effect, goats on diets with 2.50% (SLO₃) and 3.00% (SLW₄) soursop leaf powder inclusion were significantly ($P < 0.05$) lower than goats on diets without soursop leaf powder (PPM₁) and 2% inclusion of soursop leaf powder (SLP₂). However, high density lipoprotein (HDL) was increased significantly ($P < 0.05$) across treatment diets as soursop leafy powder inclusion levels were increasing progressively in test diets. On the other hand, low density lipoprotein (LDL) values decreased significantly ($P < 0.05$) as addition of soursop leafy powder was increasing in the treatment diets of goats. It is worthy to note that, very low density lipoprotein (VLDL) values were not significantly ($P > 0.05$) influenced by treatment diets in this study.

Table 6: Effect of soursop leaf powder inclusion on serum metabolites and lipid profiles of goats fed diets containing fluted pumpkin pulp.

Parameters	Diets				SEM ±
	PPM ₁	SLP ₂	SLO ₃	SLW ₄	
Blood serum enzymes					
SGOT (U/L)	45.04 ^a	32.83 ^b	32.01 ^b	31.03 ^b	0.87
SGPT (U/L)	20.21 ^a	16.32 ^b	15.95 ^b	15.28 ^b	0.39
ALT (U/L)	25.99 ^a	20.85 ^b	19.93 ^b	19.67 ^b	0.46
AST (U/L)	69.83 ^a	62.53 ^b	60.47 ^c	60.01 ^c	0.74
Lipid profile					
Total cholesterol (mg/dl)	70.03 ^a	65.27 ^b	62.13 ^c	61.99 ^c	0.96
HDL (mg/dl)	30.96 ^c	33.57 ^b	35.72 ^a	36.01 ^a	0.85
LDL (mg/dl)	35.02 ^a	27.21 ^b	21.58 ^c	21.32 ^c	0.45
VLDL (mg/dl)	4.05	4.51	4.58	4.60	0.07

^{a,b,c} Means in the same row with varying superscript differ significantly ($P < 0.05$). SGOT = Serum glutamate oxaloacetate transaminase, SGPT = Serum glutamate pyruvate transaminase, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, HDL = High density lipoprotein, LDL = Low density lipoprotein, VLDL = Very low density lipoprotein.

Discussion

Proximate composition and anti-nutritional factors of fluted pumpkin pulp, soursop leaf powder and elephant grass were comparable with range values reported in literature by [4]; [7]; [18] respectively. The phytochemical levels in these plants species were considered low and safe with regards to phytochemical poisoning critical values of 7.3 – 9.0mg/g noted by [19]. Variation observed in nutrient content of treatment diets could probably be due to the reflection of different feed components that supplied nutrient to diets. However, it was

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interesting to know that crude protein values recorded in diets were above 8% CP minimum value required for goat maintenance [20]

The test ingredient seem beneficial as it was able to reduce the level of anti nutritional factors and possibly increase the ability of the animals to properly modify and improve feed efficiency for better nutrient supply, absorption and utilization by animals. This resulted in higher significant final, total and average daily weight gain in animals on the test diets. This inference was in consistency with the earlier report by [18] that efficient feed utilization by animals supply adequate balance of nutrients requirement at the site of metabolism for heavy weight gain. Daily weight gain values recorded in the work was comparable with the mean value (38.64g/day) reported by [21]. The obvious variation in daily feed intake at similar growth stage of the animals could be related to the type as well as physical nature of diet components, length of time spent on diet and acceptability with other unknown contributing factors of the feeds. It is worthy to mention that feed conversion ratio (FCR) that measures feed intake per unit weight gain was lowest in diet SLOP₃, indicating better feed efficiency. However, the higher weight gain and FCR recorded in diets with test ingredient attest to the superiority of the diets.

In accordance with the previous findings, digestibility in goats is governed by many factors that include nature and quality of feeds, level of feed intake, salivary secretion, manner of rumen fermentation and flow rate in gastro-intestine [18]. The improvement obtained in dry matter digestibility might be channelled to unlimited nutrient from greater proportion of forage grass that was compensated with appreciable percentage of supplement diet. This nutrient availability could have possibility stimulated the activity of cellulolytic rumen flora for better feed ingested and digestibility. The higher crude protein digestibility in test diets showed that phyto-genic bioactive components in the test ingredient did not cause harmful effect on activities of rumen bacteria but improved digestibility. This is in harmony with previous study of [21] who observed that secondary components in diets suppress rumen degradation to enhance appreciable quantity of protein availability in post-rumen digestion. Likewise fibre digestion in test diets that could be linked to modulation effect that appeared to favour proper functioning of rumen ecology for high fermentable cell wall fractions and digestibility of fibre. This was earlier observed by [20], who stated that easy digestible features in feeds increase activity of rumen degrading microbes that break fibre feed constituents to provide energy for themselves and host animals. Interestingly, it was apparent that the addition of soursop leaf powder to diets was inversely proportional to the digestibility of ether extract. The reason for this inconsistency could presumably depend on the bioactive

ingredients in the diets that could have disrupted the activity of enzymes reaction in the rumen by forming an indigestible complex for oil degrading components. Notwithstanding, there were considerable increase in digestibility of ash and nitrogen free extract in goats on test diets. This observation further affirmed the positive interaction effect of the feed components that were found to enhance nutrient availability for effective digestion. The low digestion in almost all nutrients in the control diet could be traced to an inhibition of digestive enzymes activity by anti-nutritional factors in fluted pumpkin pulp and their interactions with fibre which adversely reduced cell wall carbohydrate as demonstrated in goats on diet with no soursop leaf powder.

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The discrepancy noticed in values of nitrogen intake could mainly be attributed to the difference in contributory effects of the dietary components that tends to increase the protein content of test diets. However, the high numerical values of faecal, urinary and total nitrogen excreted in control group could be as a result of rapid breakdown of dietary protein to ammonia which increased nitrogenous excretion [8]. It is worth pointing out that all diets in the study had positive nitrogen balance, explaining treatment diets were tolerated with provision of adequate protein requirement for the animals. The higher nitrogen retention noticed in diets with 2.5 and 3% soursop leaf powder inclusion, could be connected to the presence of optimum anti-microbial effects that delay rumen protein degradation by suppressing bacteria population and made denatured protein absorbed and utilized in the intestine by animals for maintenance and production purposes [22]. Nitrogen retention values obtained in this work were within the reference values reported by [23].

The observed increment in gross energy (GE) intake in control diet could perhaps be linked with ingestion of the feed components that had measureable effect on energy concentration. This possibly explained the excessive amount of GE intake by the animal to meet up with energy requirement for their body physiological activities. The higher faecal, urinary and total energy output noted in control diet, further buttress the imbalanced level of energy catabolism that was associated with poor energy utilization in the diet. The improved digestible energy values in animal on test diets showed that the phyto-genic components in the feeds were activity to make nutrient available for efficient feed utilization and reduced faecal energy. Metabolizable energy (ME) is often used as sensitive yardsticks for determination of overall energy intake of feed and measures metabolic sum of the process of digestion, absorption and utilization of feed in energy unit [24]. Thus, the efficiency with which higher ME was retained and utilized for growth in animals on test diets was influenced by low energy loss as heat. This low heat increment was a function of nutrient adequacy in feeds that

gave better ME which was expressed in higher weight gain. However, this depends on interaction of two principle factors; the nature of the chemical compounds in which ME was contained and purpose for which these compound were used by the animals. The similar values of metabolizability (qm) has laid credence to the work reported by [25] who noted that qm of complete feed is relatively constant and equivalent to about 0.96 MJ/g/day DM.

Blood examination is a scientific way of screening and assessing the nutritional and health status of an animal. Thus, haematology provides opportunity to clinically investigate the presence of several metabolic needs and other constituents in the body of animals [26]. Packed cell volume (PCV) is an indicator of blood volume or blood dilution that may result from toxic substance, while haemoglobin (Hb) and red blood cell (RBC) measure the ability to withstand some levels of respiratory stress and oxygen carrying capacity. The positive comparative values of these indices showed that animals were in normal healthy condition and not anaemic. Mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration values that explain the bone marrow capacity for blood cells production and diagnosis of anaemia were correlated with PCV, Hb and RBC obtained in this study. This further explains the quality of the balanced feeds that had no adverse effect on blood and health status. White blood cell (WBC) improves immune system, antibodies production and disease fighting ability of the animal. The immune response of animals in this study were not negatively affected by test feeds as showed in their values that tallies with average value ($7.68 \times 10^3/m^3$) for goats as reported by [26]. The results of neutrophils and lymphocytes for white blood differential counts imply that goats on these diets had the tendency of not being susceptible to microbial infection or disease condition. While low values of monocytes, eosinophils and basophils were indications that their immune system were not challenged with toxic substance present in the test feeds. However, their values showed good resistance to diseases and did not portend any danger, since they fell within the normal range values of 10-50% neutrophils, 40-75% lymphocytes, 1-5% monocytes, 1-8% eosinophils and 0-3% basophils [11].

The higher serum total protein and albumin concentration obtained in animals on diets with soursop leaf powder could be traceable to low content of secondary compounds and availability of better nutrient utilization that were able to supply adequate digestible amount of protein needed to make nitrogen available for rumen microbes. Consequently, this increased protein absorption in the intestine that responsible for maintaining normal serum protein levels in animals. However, total protein and albumin values obtained in this study, were in line with average range values of 7.02 and 3.18 g/dl respectively, earlier noted

by[23]This indicates that anti nutritional factors in test diets could not reduce nutrient absorption but proposed intact health hepatic cellular functions. The constant globulin values observed, could probably be due to the goats adaptation to stress that made them disease free and cause an excessive production of antibodies through gamma globulin production[12]It might be logical to say that the favourable role of test feeds gave way to increased soluble carbohydrate for metabolic efficiency in the stomach which modulated nutritional metabolism. This might probably be translated to the improvement in serum glucose and triglyceride that was linked to increment in body weight gain of the animals. The low creatinine levels in serum, suggests no muscle or tissue wastage and animals did not survive at the expense of their body reserves. This is proportional to the low urea levels with positive nitrogen balance observed in animals on test diets, reflecting the superiority of the dietary protein that was efficiently utilized and did not cause excessive protein catabolism that is associated with protein deficiency. [27] noted in literature that urea test measure the amount of nitrogen in the serum and high serum urea level is an indication of kidney dysfunction because protein intake and kidney functioning are affected by quantity of blood urea nitrogen.

Serum enzymes are found in practically every tissue of animal body, including red blood cell are highly concentrated in cardiac muscle and liver intermediate in skeletal muscle and kidney but much lower concentration in other tissues [28] Their measurements are helpful for diagnosis and following cases of myocardial malfunction, hepatocellular disease and skeletal muscle disorders. They are excellent markers of hepatic liver damage caused by exposure to toxic substances [22]The reference values of SGOT (14.0 to 123 U/L), SGPT (15 to 44 U/L), ALT (10.3 to 53.3 U/L) and AST (43 to 230 U/L) reported by Adebayo *et al* (2019); Chen *et al.* (2015) for goats were within the recorded values obtained in this study. These normal physiological serum enzymes levels imply that the phyto-genic bioactive components in the test diets had no debilitating effect on the liver of the animals. Kim *et al.* (2012), asserted that clinical significant risk of serum enzymes activities above normal range is an aberration and indication that animals may suffer from liver diseases. Moreover, the clear low values of serum enzymes, creatinine and cholesterol in conjunction with urea observed in this study could be conventionally used as tools to show that the test ingredients did not interfere with renal and hepatic functions, explaining absence of damage in liver and myocardial.

The residual effect of tannin and saponin in the test diets could be responsible for scavenging effect of free radical in the blood that suppressed serum as expressed in low cholesterol. Okoruwa (2021), observed that cholesterol level of 180mg/dl and below may not

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result in arteriosclerosis, hence the values registered in this work suggest that a safe concentration for good animal health. The increased levels of highdensity lipoprotein cholesterol noted in animals on test diets further attest the good effect of the feeds that could prevent peripheral artery disease and arteriosclerosis. Ugochukwu *et al.* (2003) found that low and very low density lipoprotein are considered as bad cholesterol because their high levels can cause build up of cholesterol in arteries. However, the reduction of their concentration levels in the test diets could be attributed to high dietary fibre utilization and presence of secondary metabolites which could possibly depress hepatic activities of lipoprotein and cholesterol enzymes.

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Conclusion

The study discovered that feeding dried flutedpumpkin pulp with different proportions of soursop leaf powder as dietary supplement to goats can serve as potential alternative feed that will provide more practical approach in their feeding regime. Thus, it is expected that this alternative feedcan go a long way in making animal protein available at affordable price to an average Nigerian and also reduce competition between man and ruminant livestock for feeds, most especially during the dry season.

It can therefore be concluded that, efficacy of processing fluted pumpkin pulp and soursop leaf powder in the study diets reduced anti nutritional factors and provided phytochemical components. This increases nutrient utilization as demonstrated in greater feed ingestion capacity with nutrient digestibility that consequently improved health status and body tissue build-upas seen in growth performance without any negative effect in goats. However, the response in terms of goats improvement were more pronounced in diets that contained 6% dried fluted pumpkin pulp with 2.5% or 3% soursop leaf powder (SLO₃ and SLW₄)

- Authors' contribution: This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.
- Competing interest: No competing interest exists between us.

References

1. Gursoy, O. Economics and profitability of sheep and goat production in Turkey under new support regimes and market conditions. *Small Ruminant Research*.2006;62: 181 –191.

Comment [I14]: Keep uniformity while referencing, follow journal's guideline. Somewhere used punctuation marks comma/ full stop, different hyphens, with or without space, volume/article etc. Keep uniform punctuation marks.

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2. Lawal-Adebowale, O. Dynamics of ruminant livestock management in the context of Nigerian agricultural system. *Livestock Production*, 2012;4: 1 – 20
3. Okoruwa M I and Ikhimioya, I. Influence of browse-tree leaves supplementation on digestibility, rumen fermentation, and performance of goats fed mixed grass hay. *Livestock Research for Rural Development*. Volume 2020;32,(6) Article # 93,<http://www.lrrd.org.lrrd32/06/odion200416.html>.
4. Adebayo, O R Farombi, A G and Oyekanmi, A M. Proximate, mineral and anti-nutrient evaluation of pumpkin pulp (*Cucurbita pepo*). *Journal of Applied Chemistry*,2013;4(5); 25-28
5. Li, Y, Zhang, G. Xu, H. Zhou, S, Dou, X, Lin, C, Zhang, X, Zhao, H and Zhang, Y. Effects of replacing *alfalfa* hay with Moringa leaves and peduncles on intake, digestibility and rumen fermentation in dairy cows. *Livestock Science*, 2019; 220: 211 – 216
6. Adanma, C I and Ugochi C O. Quality evaluation of tea brewed from blends of soursop (*Annona muricata*) and moringa (*Moringa oleifera*) leaves. *European Journal of Nutrition & Food Safety* 2019;10 (1); 1-15
7. Ogbonna, H.P, Ogbonna, P.C. and Ogujiofor, I.B. Proximate composition, vitamin, mineral and biologically active compounds levels in leaves of *Mangifera indica* (Mango) *Parsea Americana* (Avocado pea) and *Annona muricata* (Soursop). *Journal of Applied Science and Environmental Management*,2019; 23 (1): 65 - 74
8. McDonald, P. R.A., Edward, J.F.D. Greenhaigh and G.A. Morgan. *Animal Nutrition* 6th. Ed. Pearson Education Limited Edinburgh. Great Britain.2002;Pp 544.
9. NRC (National Research Council). *Nutrient requirements of beef cattle*. 7th Revised edition. National Academy Press. Washington DC. 1996;23pp
10. AFRC. Agricultural and Food Research Council. *Energy and Protein ruminants*. Wallingford Commonwealth Agricultural Bureaux International. 1993;Pp 159
11. Jain, N. C. *Essentials of veterinary haematology*, Lea and Febiger, Pennsylvania, USA, 1993; pp. 7.
12. Chen, G J., Song, D. S., Wang, B.X. Zhang, Z. F., Peng Z. L. Gou, C.H Zhong, J. C. and Wang, V. Effects of forage concentration ratio on growth performance, ruminal fermentation and blood metabolites in housing feeding Yaks. *Asian Australasian Journal of Animal Science*,2015;28 (12): 1736--1741
13. Kim, S. H., Alam, M. J., Gu .M. J., Park K. W. Jeon C. O. Ha J. K, Cho K. K and Lee S.S. Effect of total mixed ration with fermented feed on ruminal *in vitro* fermentation,

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- growth performance and blood characteristics of hanwoo steers. *Asian-Austrian Journal of Animal Science*, 2012; 22 : 213 - 223
14. Fredlund K, Asp N. N., Larsson, M, Marklinder, L and Sandberg, A.S. Phytate reduction in whole grains of wheat, rye, barley and oats after hydrothermal treatment. *Journal of Cereal Science*, 1997;25 (1): 83 -91.
 15. AOAC International Official Methods of Analysis .Washington D C. Association of official analytical chemist, 18th Edition (Kjelrick, editor) Arlington.2005; pp 1230.
 16. MINITAB Minitab Statistical software, Release 10.2. Minitab Inc. State College PA. USA.2000
 17. Steel, R.G.D. and Torrie, J.H. Principle and procedure of statistics. A Biometrical Approach 3rd Edition. MacGraw Hill Book Co. New York.1990.
 18. Osakwe, I.I. and Udeogu, R.N. Feed Intake and nutrient digestibility of West African Dwarf goats fed *Pennisetum purpureum* supplemented with *Gmelina arborea*. *Animal Research International*, 2007;4 (3) : 724 – 727.
 19. Akomolafe, S.F. and Ajayi, O.B. A comparative study on antioxidant properties, proximate and mineral composition of the peel and pulp of ripe *Annona muricata* (L) fruit. *International Food Research Journal*, 2015; 22(6); 2381-2388.
 20. Okoruwa M I and Ikhimioya, I. Influence of browse-tree leaves supplementation on digestibility, rumen fermentation, and performance of goats fed mixed grass hay. *Livestock Research for Rural Development*. 2020. *Volume 32,(6) Article # 93*, <http://www.lrrd.org.lrrd32/06/odion200416.html>..
 21. Ayandiran, S. K Odeyinka, S. M. and Odedire, J.A. Growth performance and nutrient digestibility of West African Dwarf (WAD) goats fed bread waste and *Moringa oleifera* leaf. *International Journal of Animal Science*, 2019;3 (2): 1047.
 22. Okoruwa M.I. Impact of herb oils on digestion, lipid profile and methane emissions in goats fed mixed maize/rice straw-hay as alternative basal diets. *International Journal of Research and Innovation in Applied Science*; 2021; 6 (2):5-12
 23. Okoruwa M I and Edoror O.M. Influence of *Garcinia kola* seed meal in diets of goats ; effects on rumen fermentation, nutrient utilization , blood metabolites and faecal flora. *Australian Journal of Basic and Applied Science*, 2019;13 (18):40-47.
 24. Okoruwa M I. Energy utilization for body weight gain of lamb-rams fed differently processed breadfruit (*Artocarpus altilis*) meal in total mixed rations. *Nigerian Journal of Animal Production*.2016. Vol. 43, No.2 pp. 147-154

25. Ahamefule. F.O. and Udo. M. D. Performance of West African dwarf goats fed raw or processed pigeon pea (*Cajanus cajan*) seedmeal based diets. Nigeria Journal of Animal Production, 2009; 37 (2) :227-236..
26. Okoruwa, M. I., Bamigboye, F. O. and Ikhimioya, I. Dietary Albizialebbbeck leaf and millet sievate effects on digestibility, rumen environment and haematological indices of goats. *Livestock Research for Rural Development*,2022. Vol.35 (9), Article No 79.http://www.lrrd.org./lrrd35/09/3579_odio.html.
27. Almeida O C, Pires A V, Susin I, Gentile R S, Mendes C O, Queiroz M A A., Ferreira E M and Eastridge M I.Milk fatty acid profile and arterial blood milk fat precursors concentration of dairy goats fed increasing doses of soybean oil. *Small Ruminant Research*, 2013;114; 152 – 160.
28. Ugochukwu, N.H., Babady, N. E. Cobourne, M. And Gasset S.R. The effect of Gongronemalatifolium extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. *Journal of Biological Sciences*, 2003;20 (1) : 1-5