

## USE OF *STYLOSANTHES HAMATA* AND *SIDA ACUTA* AS SOLE FEEDS FOR RABBITS (*Oryctolagus cuniculus*)

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**ABSTRACT:** A 42-day feeding trial was conducted to determine whether *Stylosanthes hamata* and *Sida acuta* could be used as sole feeds for local weaner rabbits. The experimental diets had three treatments with three replicates each in a Completely Randomized Design. The experimental diets were T1 (100% *Stylosanthes hamata*), T2 (50% *Stylosanthes hamata* 50% *Sida acuta*) and T3 (100% *Sida acuta*). The growth parameters measured/calculated were mean weekly and total feed intake (g), mean weekly and total weight gain (g) and final weight (g). Blood parameters considered included haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and white blood cell (WBC) differential counts (neutrophils, lymphocytes, eosinophils, and monocytes). Additionally, meat colour, juiciness, tenderness and flavour were also noted after animals were sacrificed. Data was analyzed using ANOVA in GenStat (Discovery Edition). There were significant differences ( $P < 0.05$ ) in mean weekly feed intake, total feed intake, mean weekly weight gain, total weight gain and final weight between treatments. T3 animals consumed the highest feed yet T2 animals had the heaviest weight gain at the end of the experiment. Whereas there were no significant differences ( $P > 0.05$ ) between treatments for MCHC and WBC differential counts like neutrophils lymphocytes and eosinophils, significant differences ( $P < 0.05$ ) were observed between treatments for PCV, RBC, MCV and monocytes. The sole *Stylosanthes hamata* feed significantly ( $P < 0.05$ ) improved meat colour and juiciness, while tenderness and flavour, did not record any significant differences ( $P > 0.05$ ) between treatments. The results suggest that *Stylosanthes hamata* and *Sida acuta* may have a potential to enhance rabbit growth as a combined feed. Any negative effect on rabbit health either when fed individually or in combination was inconclusive and *Stylosanthes* in particular as sole feed could improve colour and juiciness of rabbit meat.

**Key words:** Blood indicators, Growth performance, Meat quality, *Sida* spp., *Stylosanthes* spp.

### INTRODUCTION

World production of rabbits (*Oryctolagus cuniculus*) has been estimated by Lebas and Colin (1992) to be of the order of 1200,000 tonnes per annum. Rabbits are prolific breeders, producing large quantities of tasty meat for home consumption and have a faster rate of production than pigs, goats and sheep. If properly managed, a doe can produce more than 15 times her own weight of offspring in a year (Adjare, 1985). Uses to which the rabbit is put by man include; the supply of food (the most extensive of all the uses), the supply of a very high grade wool, as a source of miscellaneous products, assists in laboratory and experimental work, can be used in educational work of varied sorts and could be kept as pet or companion animal.

Animal protein intake in developing countries is still far below the required standards (FAO, 1998). To close up this gap, several strategies have been advocated notably among them is to encourage the developing countries to research and improve the productive potentials of local breeds rather than depend on exotic breeds which are usually not well adapted to the environment and management conditions in those countries (FAO, 1992). Nutrition is one of the factors that could limit productivity (Lukefahr et al., 1983) and nutrients available in forages can be used to replace grain in rabbit diet and therefore reduce concentrate need for rabbit production (Aduku et al., 1986). Good nutrition translates into good hematological characteristics such as erythrocytes, leucocytes and haemoglobin among others which are good indicators of physiological status of animals (Hawkney and Dennet, 1989). Again meat colour of animals fed concentrate is quite white which makes it not very attractive to the consumer, so the diet that the animals consume is very important (Nguyen and Brian, 2008). Most green feed are of quality and high in carotene and xanthophylls, which are important in giving a deep yellow colour to egg and meat quality (Nguyen and Brian, 2008).

ORIGINAL ARTICLE



Although the rabbit is regarded as an herbivorous animal, many rabbit farmers feed their animals with poultry feed (Adjare, 1985). This practice impedes the quick growth of the animals. Domestication has also led to low reproductive performance as well as poor growth rate (Adu et al., 1999). *Stylosanthes* is naturally distributed throughout the tropical and subtropical region in America, Africa, and South Asia (Mannetje, 1984). *Stylosanthes* species may also be used as cover crops, manure and fallow crops, and may be cut and fed fresh or used as hay (Mannetje and Jones, 1990). *Stylosanthes* was introduced into a number of communities in the northern and coastal savanna of Ghana in 1994. It is estimated that about 5,000 ha of natural pastures have been oversown with the legume in almost 300 communities in the Savannah zones since 1994 (Oppong- Anane, 1999). Empirical observation indicates that this forage is readily available and some farmers use it as a sole feed or in combination with *Sida acuta*. This study therefore set out to simulate the farmer scenario of feeding rabbits with *Stylosanthes hamata* as a sole feed or in combination with other forages so as to investigate how this practice impacts on growth, health and the meat quality of rabbits.

## **MATERIALS AND METHODS**

### **Study area**

The experiment was conducted at the livestock farm of the Animal Science Department of the University for Development Studies, Nyankpala campus, Tamale, Ghana. Nyankpala is approximately 16km West of Tamale. It has unimodal rainfall pattern. The area lies on latitude 09°25"N and longitude 00°58"W with an altitude of 183m above sea level. The mean annual rainfall and temperature are 1043mm and 28.3°C respectively. The rainy season is usually between April and October with the dry season from November to March. The mean annual day time humidity is 54% (Kasei, 1990).

### **Experimental animals**

Nine local weaner rabbits about five to six weeks old were used. The mean weights of the animals were 482g, 510g and 550g for T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> (control) respectively.

### **Experimental diets and feeding**

The experimental diets used were *Stylosanthes hamata* and *Sida acuta*. Animals in T<sub>1</sub> were fed 100% *Stylosanthes*. T<sub>2</sub> and T<sub>3</sub> were given 50% *Stylosanthes* 50% *Sida* and 100% *Sida* respectively. Animals were fed 500g of feed daily between 6:00 am and 4:00 pm. Water was provided *ad lib*. Empty polythene was placed under each cage to collect left over feed and faeces. Any leftover feed was discarded the following morning.

### **Housing management**

Experimental animals were managed intensively in a hutch made of nine separated cages. The wood-frame and wire hutch type was used. The flooring, sides and top were all welded mesh to ensure proper sanitation as well as observation and inspection. The dimensions of the hutch were 64cm × 64cm × 64cm (for length, breadth and height respectively).

### **Experimental design**

Completely Randomised Design (CRD) was used for the experiment. The animals were weighed initially and randomly allotted in equal numbers over the three treatments. There were three replicates per treatment.

### **Duration**

The study lasted for six weeks. The adaptation period was seven days with the treatment diets before the start of the experiment.

### **Data collection for growth parameters**

The parameters studied were: weekly feed intake, and live weight gain. Body weights of experimental animals were recorded weekly. Feed was weighed each time before being offered. Leftover feed was collected every morning before feeding and weighed, after sorting out faeces from leftovers.

### **Experimental Procedure for blood parameters**

Samples of blood were collected from each rabbit using the Standard Operating Procedure for Rabbit Immunization and Blood Collection (SOPRIBC, 2006). And each blood sample put into test tubes containing ethylenediaminetetraacetic acid (EDTA). Parameters measured were haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and white blood cell (WBC) differential count (neutrophils, eosinophils, lymphocytes and monocytes).

### **Packed Cell Volume (PCV)**

Heparinised capillary tubes were filled with the blood from the weaner rabbits and sealed using crista seal. The filled capillary tubes were placed in haematocrit centrifuge (CENTROLIT 11) and spun at 10,000 rpm for five



minutes. These ensured maximal packing of the red cells. PCV was subsequently determined by measuring the height of the red cells column using haematocrit reader (Barker and Silverton, 1976).

### Haemoglobin (Hb)

The haemoglobin levels were estimated by adding 20 ul of blood sample to 5 mls of Drabkins solution. This mixture was allowed to rest for 5-10 minutes. Drabkins solution haemolysis the red blood cells, releasing haemoglobin pigment into the solution and then Hb was estimated using spectrophotometer (CECILCE 1011) at 540 nm. This was calculated as,  $Hb = PCV/3$  and expressed in grams per decilitre (g/dl)

### Estimation of Red Blood Cell (RBC)

RBC was calculated as;  $PCV \times 0.13$  and expressed in millions micro per litre ( $10^6 \mu l$ ).

### MCH, MCHC and MCV

The following indices: mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) was calculated according to Seiverd (1964). The MCHC utilizes only the PCV and Hb measurements. Both of these tests are associated with a greater degree of precision than the RBC count. Therefore, of the three indices, MCHC is most accurate (Sirosi, 1995)

### White Blood Cell (WBC) Differential Count

A total white blood cell count is not necessarily indicative of the severity of a disease, since some serious ailments may show a low white cell count. For this reason, a differential white cell count is usually better and that is what was performed in this case. A blood smear was prepared and air dried. It was then stained using Leishman's stain and the slide placed on the microscope stage and examined under  $\times 10$  objective lens was moved out and a drop of oil laced on the slide. The  $\times 10$  objective lens was moved into a position making contact with the drop of oil. It was carefully focused on the selected field using the fine adjustment knob. The numbers of each cell type counted was recorded and then percentages calculated. Suppose you counted 72 neutrophils among 100 cells, then the percentage of neutrophils in the blood is  $72/100 = 72\%$  (Victoria, 2003).

### Estimation of Packed Cell Volume (PCV)

Heparinised capillary tubes were filled with the sampled blood of the weaner rabbits and sealed using cristaseal. The filled capillary tubes were placed in a micro haematocrit centrifuge (CENTROLIT 11) and span at 10000 rpm for 5 minutes. This ensured maximal packing of the red cells. The PCV was subsequently determined by measuring the height of the red cells column using haematocrit reader (Barker and Silverton, 1976).

### Slaughtering

After the experimental period of 42 days, the rabbits were taken to the University for Development Studies Animal Science Department meat processing unit to be slaughtered. Animals were bled and dressed by scalding. Immediately after scalding, evisceration was done to remove the viscera. The carcasses of the individual animals of the various treatments were subjected to sensory analysis after cooking.

### Sensory analysis

Consumption quality was assessed by forming a tasting panel consisting of ten members. They evaluated the intensity of the following characteristics; tenderness, juiciness, colour, and flavour. The carcasses were sliced according to the individual treatment into fifteen pieces of the size of about 2cm and cooked at the same temperature using a regulated electrical stove. During the cooking, the slices were turned over every 10 minutes and the cooking was done for a period of 45 minutes.

After the cooking, the slices were each wrapped in tissue paper based on the individual treatment and placed on a tray and presented to the assessors for the taste evaluation. Bread and water was used as a neutralizer which the assessors took after tasting each slice from each treatment. Assessors scored the attributes of the meat according to Table 1 below.

**Table 1 - Score table used to record the results of the evaluation**

Meat attribute	Score				
	1	2	3	4	5
Meat colour	Very Light	Light	Intermediate	Dark	Very Dark
Juiciness	Very Juicy	Juicy	Intermediate	Dry	Very Dry
Tenderness	Very Tender	Tender	Intermediate	Tough	Very Tough
Flavour	Very Weak	Weak	Intermediate	Strong	Very Strong

### Statistical analysis

The data was analysed using the analysis of variance (ANOVA) in GenStat (Discovery Edition) for growth and blood parameters while the general linear model using diet as factor was used for the sensory analysis.



## RESULTS AND DISCUSSION

The final weights of rabbits in this study for similar ages were comparable to findings by Ansah et al. (2012) who used rabbits in a similar environment that were fed false yam leaves but lower than those of other researchers elsewhere in Africa like Oluremi and Nwosu (2002) in Nigeria and Elamin and Yousif (2011) in Sudan, apparently because of differences in breed, initial weights and unequal duration of experimental period. The overall mean feed intake and average weight gains per rabbit per week was significantly different ( $P < 0.05$ ) between treatments (Table 2). Cook and Gray (2003) and Yarrow and Yarrow (2005) have noted that to reach optimum body size and full antler growth, white-tailed deer require at least 16% protein in the plants they consume, a scenario in which the rabbit may be said to be no exception.

A number of research works have noted that the protein levels of *Stylosanthes hamata* and *Sida acuta* are around 16% or more (Changjun et al., 2004; Skerman et al., 1988; Williams et al., 2012). Changjun et al. (2004) have further observed that metabolisable energy (ME) contents of some *Stylosanthes* spp. are between 1730 and 1815 kcal/kg which though lower than growing rabbits requirement of about 2.5MJ/kg DE (Ibrahim et al., 2009), some reasonable gains in weight was made possibly because there was sufficient but not necessarily adequate energy in the experimental diets, given that Ibrahim et al. (2009) have observed that rabbits fed on diet containing 90% energy requirement with or without supplementation showed high values of total revenue, net revenue, economical efficiency and relative economic efficiency, while recorded the low value of feed cost/kg live body weight. Reasonably reduced energy content of diet may not therefore necessarily be a limiting factor to growth. Rabbits on T2 probably gained the highest weight because the mixture of *Stylosanthes hamata* and *Sida acuta* might have provided higher protein content. Furthermore significant differences ( $P < 0.05$ ) were observed in total weight gain and final weight across all treatments (Table 2). Although T3 rabbits consumed the most feed at the end of the study, T2 animals recorded the highest weight, suggesting some possible positive interaction between the legume (*Stylosanthes*) and forage (*Sida*). Bamikola and Ezenwa (2001) observed a higher growth rate when a mixture of *Stylosanthes* and Guinea grass was used to feed goats in Nigeria. In an experiment with grower goats in Nigeria, the daily live weight gain increased with 30% inclusion level of *Stylosanthes hamata* as compared to the control (100% *Panicum maximum*). An inclusion level above 50% did not however show any significant difference in terms of weight gain (Bamikola, 1999).

**Table 2 - Growth performance of rabbits**

Parameters	Trt 1	Trt 2	Trt 3	S.e.d.	Sig.
Ave. weekly feed intake(g)	1967 <sup>a</sup>	2008 <sup>b</sup>	2116 <sup>c</sup>	5.94	***
Total feed intake(g)	11802 <sup>a</sup>	12048 <sup>b</sup>	12696 <sup>c</sup>	1.53	***
Ave. weekly weight gain(g)	28 <sup>a</sup>	41 <sup>c</sup>	36.6 <sup>b</sup>	2.00	**
Total weight gain(g)	168 <sup>a</sup>	246 <sup>c</sup>	220 <sup>b</sup>	2.45	***
Final weight (g)	650 <sup>a</sup>	756 <sup>c</sup>	670 <sup>b</sup>	4.49	***

S.e.d- standard error of difference, Trt-Treatment, Sig.= significance, different superscripts <sup>abc</sup>= in a row imply significant differences ( $P < 0.001$  or 0.01) between treatments, \*\*\*= significant at  $P < 0.001$  and \*\*= significant at  $P < 0.01$

### Some hematological characteristics

The PCV values (35.3 to 39%) obtained in this study were within the normal range (38 to 45%) as reported by Swenson and Reece (1993) for T1 and T3 but slightly below for only T2 animals. The values (11.8 to 12.7 g/100 ml) for haemoglobin concentration (Hb) obtained in this study again were within the normal range (12.9 to 13.85 g/100 ml) reported by Schalm et al. (1975) for T1 and T3 but slightly below for only T2 animals. The RBC values (4.6 to 5.1 x 10<sup>6</sup>/mm<sup>3</sup> of blood) were slightly lower than the values (6 to 11 x 10<sup>6</sup>/mm<sup>3</sup> of blood) reported by Olomu et al. (2003), but higher than the values (3.10 to 4.67 x 10<sup>6</sup>/mm<sup>3</sup> of blood) reported by Taiwo et al. (2006). There were no significant ( $P > 0.05$ ) differences among the dietary treatments with respect to the erythrocytic index MCH, but were significantly different ( $P < 0.01$ ) for MCV. The MCV and MCH values obtained here were slightly higher to reference values of 60 to 73 fl, 16 to 23pg by Anon (1980). MCHC values (33.0-33.3%) in this study though slightly significant ( $P < 0.05$ ) between the treatments, fell within the normal range of 26 to 34% as reported by Anon (1980). It would thus appear from PCV and Hb values that over time, feeding *Stylosanthes hamata* with *Sida acuta* could probably lead to anemic conditions. This will require further investigation as RBCs counts were not greatly affected (Table 3).

**Table 3 - Effect of diet on hematological characteristics of rabbits**

Parameters	Trt 1	Trt 2	Trt 3	S.e.d	Sig.
PCV (%)	38.000 <sup>b</sup>	35.300 <sup>a</sup>	39.000 <sup>b</sup>	0.678	**
Hb (g/100ml)	12.675	11.775	12.650	0.229	ns
RBC (x 10 <sup>6</sup> µL)	5.000 <sup>b</sup>	4.625 <sup>a</sup>	5.125 <sup>b</sup>	0.096	**
MCV (fl)	76.150 <sup>a</sup>	76.650 <sup>b</sup>	76.500 <sup>b</sup>	0.108	**
MCH (Pg)	25.375	25.550	25.300	0.145	ns
MCHC (%)	33.075 <sup>a</sup>	33.325 <sup>b</sup>	33.025 <sup>a</sup>	0.118	*

S.e.d- standard error of difference, Trt-Treatment, Sig.= significance, <sup>abc</sup>= in a row imply significant differences ( $P < 0.05$ ) between treatments for different superscripts, \*\*\*= significant at  $P < 0.001$  and \*\*= significant at  $P < 0.01$ , ns= non significant





With regard to WBC differential counts, there were no significant differences ( $P>0.05$ ) in the MCH, neutrophils, lymphocytes and eosinophils except for monocytes (Table 4). Not a single differential count was recorded for basophils in all treatments. High WBCs in blood tend to suggest subclinical or clinical conditions. It therefore appeared the treatments in the present study did appear to pose any observable health problems and especially so when no basophils counts could be made at all. Recent data have revealed the role of basophils in the initiation of the T helper cell 2 (Th2)-mediated immune response. Not only do basophils guide the Th1-Th2 balance by providing an early source of crucial Th2-skewing cytokines, interleukin (IL)-4 and thymic stromal lymphopoietin, but recent findings have also illustrated their capacity to function as antigen-presenting cells (Sokol and Medzhitov, 2010). Additionally, basophils, eosinophils, and Th2 lymphocytes are recruited to the site of inflammation during late-phase reactions (LPRs) (Falcone et al., 2000), suggesting the probable inflammatory-free condition of animals during the study.

**Table 4 - Effect of diet on WBC differential counts of rabbits**

WBC differential counts (%)	Trt1	Trt2	Trt3	s.e.d	Sig.
Neutrophils	36.000	36.500	38.000	2.750	ns
Lymphocytes	58.000	60.550	54.150	2.308	ns
Eosinophils	6.000	6.000	5.750	1.643	ns
Monocytes	0.000 <sup>a</sup>	0.500 <sup>b</sup>	0.000 <sup>a</sup>	0.204	*

S.e.d- standard error of difference, Trt-Treatment, Sig.= significance, different superscripts <sup>abc</sup>= in a row imply significant differences ( $P<0.05$ ) between treatments, \* = significant at  $P<0.05$ , ns= non significant.

### Eating quality of rabbit meat

*Stylosanthes hamata* significantly improved colour and juiciness of the meat of the rabbits ( $P<0.05$ ) (Table 5). This improvement was however diluted a little when fed in combination with *Sida acuta* for colour but not for juiciness (Table 5). On the other hand, tenderness and flavour did not show any significant differences ( $P>0.05$ ) between treatments. Priolo and Vasta (2007) hypothesized that tannins are responsible for the differences found in meat colour. Changjun et al. (2004) in their nutritional analysis of some *Stylosanthes* leaf meal found that there were some tannins which may be implicated in this study. Again Guodao et al. (2004) have found that some species of *Stylosanthes* contain  $\beta$ -carotene. Carotene has been noted by Yang et al. (2000) to have effect on the meat colour. Since most forages contain vitamins which improve meat colour as alluded to by Arnold et al. (1993) who stated that supplementing grain-fed cattle with supra-nutritional level of vitamin E (a-tocophery acetate) improved meat colour and lipid stability, it is reasonable to assume vitamins in the forages that were fed may have enhanced the meat colour, too. Post-slaughter handling such as keeping the dead animal for some time before dressing being a contributing factor to the meat colour in this study cannot be overruled because Warris (2001) stated that acidity affects colour and water holding capacity after death. On juiciness, significant differences ( $P<0.05$ ) were recorded between treatments. Factors that influence juiciness include: the animal's age at slaughter, the amount of fat and collagen (connective tissue) contained in particular cuts, and, to a small degree, brining (The Accidental Scientist, 2011). Upon slaughter no reasonable fat was noticed so since a number of research works have reported an increased juiciness and tenderness with an increase in fat content in meat products (Berry and Wergin, 1993; Troy et al., 1999) and given that this fat plays a major role in improving water holding capacity and binding properties, forming rheological and structural properties that trap moisture in the products to improve juiciness (Hughes et al., 1997; Pietrasik and Duda, 2000), the significant differences observed in juiciness may be attributable not to fat but possibly to differences in cuts and finer details in the cooking procedure. No significant differences in tenderness of the meat ( $P>0.05$ ) was observed. The animals used for this study were not fully matured and were also of the same age. Forrest (2009) confirms that maturity is a factor which has the largest effect on meat tenderness. Flavour did not also record any significant differences ( $P>0.05$ ) between treatments, possibly because no spices were added during cooking.

**Table 5 - Effect of diet on eating quality of rabbit meat (5 point category of scale)**

Sensory attribute	Trt1	Trt2	Trt3	S.e.d	Sig.
Colour	1.80 <sup>a</sup>	2.20 <sup>ab</sup>	2.80 <sup>b</sup>	0.373	*
Juiciness	1.50 <sup>a</sup>	1.90 <sup>a</sup>	2.80 <sup>b</sup>	0.375	**
Tenderness	2.00	2.50	2.30	0.427	ns
Flavour	2.80	3.30	3.70	0.383	ns

S.e.d- standard error of difference, P- probability, Trt-Treatment, Sig.= significance, different superscripts imply significant differences ( $P<0.05$ ) between treatments. \*\* = significant at  $P<0.00$  and \* = significant at  $P<0.05$ , ns= non significant.

### CONCLUSION

Feeding local rabbits with *Stylosanthes hamata* in combination with *Sida acuta* can lead to improved weight gains in them but must be done with a good eye on the health of the rabbits. *Stylosanthes hamata* as a sole can also improve the colour and juiciness of the meat of local rabbits. *Sida acuta* as a sole feed could also bring about improved weight gains however the meat colour would be a bit darker. Given that these two forages are readily available in the locality, it is recommended that farmers use 50% *Stylosanthes hamata* and 50% *Sida acuta*, in feeding so as to improve the weight gains of their local rabbits.



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