



Intestinal Histomorphometry, Relative Organs Weight and Apparent Nutrient Digestibility of Rabbits Fed Varied Replacement Levels of Soybeans Meal with *Ipeomea Asirifolia* Leaf Meal

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Abstract: Cost of soybeans meal contribute high percentage of total cost of feed in rabbit's production in developing countries apart from the maize, therefore experiment was carried out to investigate the effect of replacing soybeans meal with *Ipomoea asarifolia* leaf meal (IALM) as a plant protein for rabbit production and gut responses, apparent nutrient digestibility were therefore investigated. Twenty-four weaned, 6-8 weeks old rabbits, were randomly allotted to four dietary treatments in which IALM was used to replaced soybean meal (SBM) at 0% (T1, control), 10% (T2), 20% (T3), and 30% (T4), with six (6) rabbits per treatment in a completely randomized design experiment. Each rabbit served as a replicate. Feed and water were offer *ad libitum* while other standard management practices were observed. Relative organs and gut weight and length were determined using sensitive digital scale and tape rule respectively, while apparent nutrient digestibility, gut histomorphometry were evaluated following standard procedures. Data were analysed using ANOVA at $\alpha_{0.05}$. Control rabbits had the highest value for villus height (77.68 μ m), villus width (13.99 μ m) and crypt depth (38.03 μ m) while the largest relative gut weight was caecum with the value ranges from 5.33% (treatment 3, 20%IALM) to 7.51% (treatment 4, 30%IALM). The longest relative gut length (19.16) was obtained in treatment T4 (30%IALM). Other relative organ weights were not affected by dietary treatment except abdominal fat with highest values under T3 and T4 and nutrients were highly digested under control diet than other treatments. It was concluded that soybeans can be replaced with IALM up to 30% without having negative effect on gut response, organs weight and nutrients digestibility.

Keywords: *Ipomoea asarifolia*, Gut, Gut Weight, Gut length.

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INTRODUCTION

In order to bridge the gap of animal protein malnutrition in developing countries around the

world, relatively cheap, high feed conversion, high prolificacy, and short gestation period and easy to manage animal like rabbit must be considered. One

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of the globally accepted ingredients used in the rabbit production is soybeans. High cost of this ingredient has forced the price of rabbit up beyond expected despite global production of soybean [1] of 366 Mt in 2016. Although Nigeria production level was not capture as at the time but according to FAO [2], United States has the highest rate of soybean production (117.2 tons), followed by Brazil (96.3 tons) and Argentina (58.8 tons) and was not sufficient enough to take care of animals and man. Therefore, there is need for alternative to this ingredient for animal consumption especially rabbit been a pseudoruminant.

Various studies [3, 4] have been conducted to determine the feeding quality, value and composition of many plants in the production of rabbits (*Orytolagus cuniculus*). According to Abdu *et al.* [5], rabbit can survive on about 90-80% forage as feed sources. The author opined that rabbit can survive in the wild by eating a variety of plants. This makes the feeding of rabbit easier than any other domesticated animals. Examples of such plants include but not limited to the following: *Tridax procumbens*, Milk weed, *Aspilia africana*, *Leucaena leucocephala*, *Tithonia diversifolia*, *Acacia nilotica*, *Vernonia Amygdalina*, *Calycopteris floribunda*, *Brassiopsis mitis* and *Ipeomea asarifolia* etc. *Ipeomea asarifolia* is common in the derive savannah of Nigeria. The origin of *Ipeomea asarifolia* has been hypothesized to originate in Southern India and those early European visitors of the region spread it around the world because of its medicinal uses. It has also been stated that *Ipeomea asarifolia* is native to tropical America. It occurs almost throughout the tropics, including, Nigeria [6].

Ekenyem and Madubuike [7] gave description of *Ipeomea asarifolia* (morning glory) to belong to the family convolvulaceae, perennial herbaceous plant, preponderant in South Eastern Nigerian and rapidly multiplying by seed and stolon. The authors reported that the *Ipeomea asarifolia* leaf meal contain high crude protein level and metabolisable energy, good mineral profile, and no-cost. Also Ekenyem and Madubuike [7] established *Ipeomea asarifolia* as a potential cheap feed ingredient for optimum and sustainable poultry production.

According to Ekenyem and Madubuike [7] *Ipeomea asarifolia* leaf meals was reported to have 32.00% crude protein, 2,760.00 metabolizable energy (Kcal/Kg) and 16.90% crude fibre, while Shittu *et al.* [8] reported 28.40%, 3,236.15 (Kcal.kg) and 7.15% for crude protein, metabolisable energy and crude fibre, respectively for *Ipeomea asarifolia* leaf meal. Also growth performance was reported [8] to be in favour of *Ipeomea asarifolia* leaf meal based

diets when compare with the control rabbit without *Ipeomea asarifolia* leaf meal but soybeans meal. Therefore, based on the aforementioned qualities of *Ipeomea asarifolia* leaf meal, the gut, organs response and nutrient digestibility of rabbit fed this unique protein-rich-plant need to be examined.

MATERIALS AND METHODS

Experimental site

The research was carried out at the Rabbitry Unit, Teaching and Research Farm, Ladoko Akintola University Ogbomosho, Oyo State, Nigeria. The study was conducted from January to March, 2017.

Preparation of *Ipeomea asarifolia* leaf meal and formulation of experimental Diets

Ipeomea asarifolia leaves were harvested around the school premises, Ogbomosho, air-dried under uncompleted roofed-house to reduce moisture until it's crispy to touch. The leaves thereafter crushed with hammer mill to form *Ipeomea asarifolia* leaf meal (IALM). The IALM was incorporated in the experimental diets to have four (4) experimental diets (table 1). Diet 1 tagged T1 serve as the control diet and contains no IALM while Diet 2, Diet 3, Diet 4 were made to contain IALM at 10%, 20% and 30% respectively in replacement of soybean meal in the control diet.

Experimental animals and management

Prior to the arrival of the animals, the rabbit hutches were cleaned and disinfected, feeding and drinking troughs and collection trays were washed and cleaned. Twenty-four (24) male weaned rabbits of mixed breeds were used for the experiment. The rabbits were between 6-8 weeks of age and weighed between 400g and 500g. The rabbits were randomly divided into four groups of six rabbits per treatment and each rabbit serve as a replicate under Completely Randomized Design Experiment. There was an adjustment period of two weeks in which the rabbits were dewormed, given vitamins and antibiotics before the beginning of the experiment. During the acclimatization period, the animals were fed on control (Diet T1). The rabbits were housed individually in a cage measuring 47×57×48cm and provided with drinking and feeding facilities made of earthenware pots re-enforced with cement to prevent feed wastage by tipping. The rabbits were fed twice per day, at 8.00hr and 16.00hr (100-120g each). Water and feed were provided *ad libitum*. The initial weight of rabbits were determined and recorded. The rabbit weights and feed consumed were also weighed and recorded weekly. The experiment lasted for 8 weeks. Daily management involved includes cleaning of cages, surrounding floor, feeding troughs and drinking troughs. Fresh

feed and water were served every day. Known quantities of feed were fed to the rabbits twice daily, feed intake was determined by subtracting the weight of the feed refused from feed served. The Proximate analysis of the diets, leaf and feces were determined according to the AOAC [9] for Crude protein, Crude fibre, Ether extract (fat) and Ash while the result was used to calculate the apparent nutrient digestibility. Gut morphometric characteristics was done using measuring tape and digital sensitive scale and the results were calculated on relative basis.

Gut histomorphometry analysis

Gut histomorphometry was handled as described by Onderci *et al.* [10]. The samples were placed in a 10% buffered neutral formaldehyde solution (pH 7.2 - 7.4) and were gradually dehydrated with increasing concentrations of ethyl

alcohol (50 - 100%). The dehydrated specimens were embedded in paraffin, sectioned at 5µm and stained with hematoxylin and eosin [11]. The sections were analyzed under a light microscope (AmScope Biological Microscope B120C-E5 with USB camera) and the height and width of the villus were measured using a computer assisted image analysis of the same microscope. A total of 15 intact well oriented crypt villus units were selected randomly for each sample. The mean values attributed to individual bird were used in the statistical analysis. Villus height was measured from the tip of the villus to the crypt-villus junction, whereas crypt depth was defined as the depth of the invagination between adjacent villi [12]. The villus width was defined as the distance from the outside epithelial edge along a line passing through the vertical midpoint of the villus.

Table-1: Gross Composition of Experimental Diets (g/100g)

Ingredients	T1 (0%IALM)	T2 (10%IALM)	T3 (20%IALM)	T4 (30%IALM)
Maize	19.08	19.08	19.08	19.08
PKC	15.27	15.27	15.27	15.27
Soya meal	9.54	8.59	7.63	6.68
I. asarifolia Leaf Meal	0.00	0.95	1.91	2.86
Wheat Offal	41.98	41.98	41.98	41.98
Maize bran	7.63	7.63	7.63	7.63
Bone meal	3.82	3.82	3.82	3.82
Limestone	1.91	1.91	1.91	1.91
Premix	0.38	0.38	0.38	0.38
Salt	0.38	0.38	0.38	0.38
Total	100.00	100.00	100.00	100.00
Calculated Nutrients				
Crude Protein (%)	16.65	16.46	16.28	16.09
Crude Fiber (%)	7.32	7.33	7.34	7.33
Energy (Kcal of ME/kg)	2220.69	2225.78	2230.93	2155.02

IALM = *Ipomoea asarifolia* leaf meal

RESULTS AND DISCUSSIONS

Except muscular thickness and villus height crypt depth ratio, all other parameters measured were significantly affected and control animal had the highest value respectively for villus height (77.68µm), villus width (13.99 µm) and crypt depth (38.03µm). The values for other treatment were similar (Table 2). This result established the effect of *Ipomoea asarifolia* leaf meal on gut histomorphometry. Although decrease in villus height suggests decrease in the surface area capable of lowering absorptive capacity but the performance of rabbit buck presented earlier disproved the assertion. Shittu, *et al.*, [8] has earlier established the improved final weight gain of rabbit fed *Ipomoea asarifolia* leaf meal. It therefore shows that the minimum heights of villi were achieved in the course of the gut growth and that the nutrient absorption does not dependent on the height of villi alone but

also includes some other integral component of gut. This is in line with the report of Hofacre *et al.* [13] and Pelicano *et al.* [14] that absorption is totally dependent on the mechanisms that occur in the intestinal mucosa. It established that the nutritional quality of *Ipomoea asarifolia* leaf meal can compare favourably to soybeans. Variation in the mucosa histomorphometry was not depressed to affect the nutrient intake. The *Ipomoea asarifolia* leaf meal does improve the crypt which is tubular organ found in-between villus in the intestine which produces enterokinase that is the precursor to pepsinogen that is responsible for the production of pepsin that help to digest protein [15]. This is contrary to the effect of feed restriction as reported by Oliveira *et al.* [16], that feed restriction resulted in several metabolic changes that lead to lower body weight, immunodepression and modified function of the digestive system especially the liver and small

intestine. The changes can affect the enzyme activity in brush border, mucosa cell mass, protein content and mucosa integrity [16]. Shortening of gut small intestine histomorphometry of rabbit in this experiment did not cause atrophy (gradual loss of muscle) that can result into nutrient malabsorption in the rabbit.

Table 3 shows the relative gut weight of rabbits fed varied replacement level of soybeans meal with *Ipomoea asarifolia* leaf meal. All the parameters measured were significantly ($P < 0.005$) affected by the dietary treatments. The largest percentage of gut was caecum with the value ranges from 5.33% (treatment 3, 20% IALM) to 7.51% (treatment 4, 30% IALM). Small intestine recorded the second largest gut relative weight when all the gut sections were compared and the least gut weight percentage was rabbits' relative oesophageal weight. Caecum is the largest of all the gut section weighted. This is in accordance with the report of Jenkins [17], that rabbit's caecum is proportionally the largest of any mammal. It is twice the length of the abdominal cavity and 40–60% of the total volume of the gastrointestinal tract. Higher caecum relative percentage recorded in this research was in accordance with report of Abdel-khalek *et al.* [18]. The author reported largest digestive compartment to be caecum in their research that examined functional, anatomical and histological development of caecum in rabbits. This might be connected to the volume of feed intake by the rabbit and microbial population/fermentation that normally occur in the caecum. Also Abdel-khalek *et al.* [18] have earlier linked the increase to feed intake, fermentation and activation of microbial metabolism that capable of stimulating the development of the caecum and colon weight changes. Development of rabbit hindgut follows the small intestine and reaches maturity at 8 weeks [19].

Table 4 shows the relative gut length of rabbits fed *Ipomoea asarifolia* leaf meal (IALM). Except caecum, other parameters measured were significantly affected by the dietary treatments. Oesophageal length on treatment 1, 2, and 3 were statistically similar and higher compare to treatment 4 (30% IALM replacement). Also 20% IALM (T3) had numerically higher value (0.87%) of oesophagus compare to other treatments with were statistically similar. Longer relative small intestinal length was recorded under treatment 4 (30% IALM). Similarity recorded in the caecum relative length may be an indication of normal microbial fermentation taking place in the caecum and the organ were not subjected to extra work as a result of dietary variations. According to Orayaga *et al.* [20], this implies that the diets did not place extra load on the GIT components to necessitate extra development of

the organs to cope, as in the case when there was high fibre than necessary in monogastric diets. Caecum is a very important organ of digestion in rabbit because rabbits are pseudoruminant and by that there will be similarity in nutrient obtainable from the caecum activities. The rabbits in all the treatments were able to reingest the caecum by-products as required. According to Davies and Davies [21], caecal fermentation and the feces, allowing reingestion and absorption of bacteria and their by-products in the small intestine. Given that the system is geared for rapid elimination of digester component that is capable of been acted upon by the caecum microbes. Oesophagus only serves as passage for feed and contributed no or little to the digestive process other than that. Esophagus serves as a transport duct from the oral cavity to the stomach and that structure and function of the rabbit esophagus differs little from that of other non-ruminant species, and has little or no effect on digestion (Davies and Davies, [21] and Ruckebusch *et al.*, [22].

Table 5 shows the relative organ weights of rabbit fed *Ipomoea asarifolia* Leaf Meal. All the organs measured were not affected by the dietary variations except abdominal fat. The abdominal fat increase with increase replacement levels of soybeans meal with *Ipomoea asarifolia* Leaf Meal. This might be as a result of high energy concentration in the IALM [8], the authors reported higher metabolisable energy of 3,236.15 (Metabolisable Energy (Kcal/kg) compared to the reported value of 2,760.00 (Metabolisable Energy (Kcal/kg) according to Ekenyem and Madubuike [7]. Fat deposit might be a good sign of excess energy in the diet that the animal was trying to preserve in their system. This is in line with report of Liu *et al.* [23], that fat is largest energy reserve in mammals. Dietary variations generally have no significant effect on the weights of internal organs in rabbits, including liver relative weight, which is contrary to the report of Attia *et al.* [24] when rabbits were fed manna oligosaccharides and zinc-bacitracin continuously or intermittently, but in line with the report of Tumova *et al.* [25], during restricted and *ad libitum* feeding of broiler rabbits. Similarity in the value of the other organs weight may also be an indication of diet free from anti nutritional factors and similar digestive activity of the organs. Berardini *et al.* [26] implicated anti-nutritional factors as been responsible for disproportionate enlargement of organs such as gall and liver, indicating improper health conditions. Abnormal blood circulation occasioned by dietary factors would cause variation in the size of the heart. Non-significant difference among the treatment groups for heart (percent live weight) indicated a normal blood circulation among all the dietary groups [20].

Also Olabanji *et al.*, [27] had earlier linked the significantly ($p < 0.05$) higher liver weight of rabbits to possibility of liver overload. Pálsson [28] long ago opine that vital organs such as brain, lungs, kidneys, heart, esophagus, abomasum and small intestine are proportionally more developed at the birth time and, as a consequence, grow up proportionally less in the postnatal life.

Apparent nutrients digestibility of rabbits fed *Ipomoea asarifolia* leaf meal varied significantly ($p < 0.005$, Table 6) across the dietary variations and except nitrogen free extract, control diet had the highest crude protein, crude fibre, ash, ether extract and dry matter digestibility compared to other treatments with varied replacement levels of soybeans meal with *Ipomoea asarifolia* leaf meal. The high nutrient digestibility observed in control diet may be as a result of processing of soybeans meal grain which may include drying, roasting and heavy industrial grinding. Also this might be as a result of high fibre content of forage compare to nutrient concentrated conventional ingredient of soybeans. It is recognized that soybean meal is often used as feed material, as it has high contents of amino acid profiles and energy in livestock and poultry rations [29]. Lower nutrient (although this did not affect growth according to Shittu *et al.*, [8] digestibility observed in this study might be as a result of the ant nutritional factors in *Ipomoea asarifolia* leaf

which may be natural defensive mechanism for the plant. This can make the feed to form complex with the enzymes in the gastrointestinal tracts. It is known that feed is digested by enzymes and acid in the stomach, and soluble constituents are absorbed mainly via the epithelial cells in the small intestine [29]. According to the same authors indigestible compounds, such as non-starch polysaccharides, proteins, and resistant starches subjected to Maillard reactions, as well as some fiber bound proteins and tannins, where together with endogenous secretions fermented by the gut microflora. Rabbit with large caecum also have capacity of hind gut fermentation. The products of that fermentation might have helped the rabbit to obtain the required nutrients. The end products of the fermentation in hindgut are short-chain fatty acids (SCFA), are equally important energy source for the microbiome [29, 30] and microbial protein is a good protein source for the rabbits.

CONCLUSION

Ipomoea asarifolia leaf meal did not affect gut enterocytes function, thereby support the nutrient digestion of the feed and organs relative weight were not overload as a result of different replacement levels of soybeans meal with *Ipomoea asarifolia* leaf meal. This has established the quality of this leaf in the production of rabbits.

Table-2: Gut histomorphometry Characteristic of Rabbits fed *Ipomoea asarifolia* Leaf Meal

Parameters (μm)	T1 (0%IALM)	T2(10%IALM)	T3(20%IALM)	T4 (30%IALM)	SEM
Villus height	77.68 ^a	30.59 ^{bc}	41.41 ^b	33.88 ^{bc}	2.99
Villus width	13.99 ^a	9.20 ^b	10.71 ^b	11.96 ^{ab}	0.71
Crypt Depth	38.03 ^a	15.01 ^b	19.77 ^b	16.39 ^b	1.89
Muscular Thickness	8.64	7.92	7.82	7.31	0.49
VH:CD	2.04	2.04	2.09	2.06	0.04
Sub muscular Mucosa	12.12 ^a	2.86 ^b	2.24 ^b	3.90 ^b	0.81

IALM = *Ipomoea asarifolia* leaf meal, VH = villus Height, CD = crypt Depth

Table-3: Relative Gut Weight of Rabbits fed *Ipomoea asarifolia* Leaf Meal

Parameters	T1 (0%IALM)	T2(10%IALM)	T3(20%IALM)	T4 (30%IALM)	SEM
Oesophagus	0.13 ^b	0.20 ^a	0.12 ^b	0.12 ^b	0.01
Small intestine	3.07 ^a	2.86 ^a	2.34 ^b	3.04 ^a	0.08
Large intestine	2.27 ^b	1.95 ^b	1.79 ^b	2.67 ^a	0.08
Caecum	7.20 ^a	7.01 ^a	5.33 ^b	7.51 ^a	0.21
Stomach	2.73 ^b	2.59 ^b	2.51 ^b	3.29 ^a	0.08

IALM = *Ipomoea asarifolia* leaf meal

Table-4: Relative Gut Length of Rabbits fed *Ipomoea asarifolia* Leaf Meal

Parameters	T1 (0%IALM)	T2(10%IALM)	T3(20%IALM)	T4 (30%IALM)	SEM
Oesophagus	0.83 ^a	0.79 ^a	0.87 ^a	0.62 ^b	0.04
Small intestine	18.27 ^a	17.79 ^a	16.11 ^b	19.16 ^a	0.31
Caecum	2.63	2.40	2.46	2.76	0.06

IALM = *Ipomoea asarifolia* leaf meal

Table-5: Relative Organs Weight of Rabbits fed *Ipomoea asarifolia* Leaf Meal

Parameters	T1 (0%IALM)	T2(10%IALM)	T3(20%IALM)	T4 (30%IALM)	SEM
Lung	0.67	0.58	0.48	0.56	0.07
Kidney	0.80	0.78	0.72	0.86	0.02
Abdominal fat	1.00 ^b	1.36 ^b	2.55 ^a	2.55 ^a	0.18
Heart	0.33	0.26	0.29	0.31	0.01
Spleen	0.10	0.09	0.06	0.06	0.01
Liver	2.53	2.72	2.15	2.61	0.05

IALM = *Ipomoea asarifolia* leaf meal

Table-6: Apparent Nutrient Digestibility of Rabbits fed *Ipomoea asarifolia* Leaf Meal

Parameters	T1 (0%IALM)	T2(10%IALM)	T3(20%IALM)	T4 (30%IALM)	SEM
Crude protein	89.01 ^a	77.3 ^b	65.37 ^c	78.68 ^b	2.12
Crude Fibre	87.46 ^a	77.73 ^c	68.63 ^c	79.91 ^b	1.78
Ash	82.17 ^a	67.86 ^c	74.07 ^b	76.17 ^{ab}	0.18
Ether Extract	83.51 ^a	73.16 ^b	70.91 ^b	79.81 ^{ab}	0.01
Dry matter	83.44 ^a	75.65 ^{bc}	70.14 ^c	80.11 ^{ab}	1.51
Nitrogen Free Extract	79.31 ^{ab}	75.12 ^{cb}	71.86 ^c	82.41 ^a	2.05

IALM = *Ipomoea asarifolia* leaf meal

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