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Original Paper

Influence of soil texture and compost on the early growth and nutrient uptake of *Moringa oleifera* Lam

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Soil is the main reservoir of water and nutrients, and thus controls the availability of most essential plant nutrients for crop growth and establishment. Therefore, a study was conducted at Ladoke Akintola University of Technology, Ogbomoso, Nigeria to investigate the effects of soil texture and compost on early growth of *Moringa oleifera* (*M. oleifera*). The experiment was a split plot laid out in a randomized complete block design with three replications. The main treatment comprises of three soil texture; sand, loamy sand, and clay while the sub-plot treatment was compost at four rates of 0, 2.5, 5 and 10 t/ha per 10 kg of soil and NPK 15 : 15 : 15 at the rate of 90 kg N/ha. Data on plant height, number of leaves, stem diameter were measured at 2 week interval for 10 weeks. Results showed that Moringa plant produced in loamy sand was superior in plant height, number of leaves and stem girth irrespective of compost applied. At 10 weeks after sowing, fresh shoot weights/pot was 73.3, 31.7, 30.3 g respectively for loamy sand, clay and sand. *M. oleifera* N uptake in loamy sand was significantly (P <0.05) greater by 57 and 50%, respectively, than sand and clay. P uptake was significantly higher at 5 ton per ha than the control and other treatments. The study concluded that, combination of loamy sand and 5 ton per ha of compost was suitable for the early growth of *M. oleifera*.

Keywords: soil texture, compost, *Moringa oleifera*, growth, nutrient uptake

1 Introduction

Moringa oleifera has gained a lot of popularity due to recent discoveries of its usefulness to mankind thus resulting in rapid growth in interest for the plant. Consequent upon this, considerable research has been conducted on the extraction of its seed oil for use in agro forestry systems, water purification property, medicinal and nutritional benefits (Fuglie, 2001). Moringa is rich in health promoting phytochemicals such as carotenoids, phenolic, various vitamins and minerals (Foidl et al., 2001; Becker and Siddhuraju, 2003; Bennett et al., 2003). It leaves are highly nutritious, one serving of the plant contains 125% calcium, 61% magnesium, 41% potassium, 71% iron, 272% vitamin A and 22% vitamin C daily value, 5–10% crude protein and it does not easily turn rancid (Fuglie, 2001). It has more beta carotene, protein, vitamin, calcium, potassium and iron than carrots, peas, oranges, milk, bananas and spinach respectively (Palada and Chang, 2003).

Soil texture can influence growth and yield of crop, as it could have tremendous effect on retention and uptake of water and nutrient by the plant. Generally, most soils in Nigeria have organic carbon content falling below one percent, low phosphorus and slightly acidic medium (pH below 6.5) leading to low plant productivity (Esu, 1991). Furthermore, the rising cost of inorganic fertilizers coupled with their inability to condition the soil has directed attention to organic manures in recent times (Agyenim-Baoteng et al., 2006; Oyedeji et al., 2014). The use of organic manure as fertilizer releases many important nutrients into the soil and also nourishes soil organisms, which in turn slowly and steadily make minerals available to plants (Erin, 2007). Organic materials serve not only as sources of plant nutrients but also as soil conditioners by improving soil physical properties, as evidenced by increased water infiltration, water holding capacity, aeration and permeability, soil aggregation and rooting depth, and by decreased soil crusting, bulk

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density and erosion (Franzluebbers, 2002; Zebarth et al., 1999).

Organic wastes of acceptable quality usually, when returned to agricultural soils on regular basis contribute greatly to the overall maintenance of soil fertility and productivity, and reduce the need for mineral fertilizer (Parr and Colacicco, 1987). Compost is an organic residue that has decomposed and recycled as a fertilizer and for soil amendment. It is a potential source of nutrients and is also useful in soil amelioration especially for communal farmers who cannot afford fertilizers. However, getting the maximum value of the compost requires applying it at the proper rates and frequency in conjunction to a particular soil. Research on the establishment and growth of *Moringa oleifera* seedlings is the realization that production can be adversely affected by soil types and nutrient status of the soil or media. This having received much attention depends on the availability of materials used in composting which varies from different locations. Considering the nutritive value of *M. oleifera* as well as the variability of soils, the present study was carried out with the objective of determining the effects of soil texture and compost rates on the early growth and biomass yield of *M. oleifera*.

2 Material and methods

2.1 Study site, soil sampling, and laboratory analyses

The study was carried out at the screen house of the Department of Agronomy, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

Soil samples used for the experiment were collected from three different locations. Surface soils sample (0–15 cm depth) taken from the three sites were air-dried and pass through 2 mm sieve and then stored for the pot experiment. Sub-samples of the composite soil samples were analyzed for selected physical and chemical properties (Table 1). The compost used for the study was also analysed for some chemical properties as shown in Table 2. The routine analysis of the soil for the experiment was carried out at IITA (International Institute for Tropical Agriculture) Ibadan. Particle size analysis was done by hydrometer method (Gee and Or, 2002). Soil pH was determined with the pH meter using glass electrode in a soil: water ratio of 1 : 1. Organic carbon was determined by the Walkley-Black procedure described by (Nelson and Sommers, 1986). Exchangeable cations were determined according to the procedure described by (Tel and Roa, 1982). Available phosphorus

Table 1 Physico-chemical properties of soils at different location (0–15 cm) used in filing the pot for sowing *Moringa oleifera* seeds in a screen house

Soil properties	Soil texture		
	Sand	Loamy sand	Clay
Physical properties (g/kg)			
Sand	930	790	370
Silt	10	90	160
Clay	60	120	470
Chemical properties			
pH (H_20) (1 : 1)	5.70	7.8	7.5
N (g/kg)	0.16	2.75	0.28
OM (g/kg)	0.49	7.48	0.76
Avail P (mg/kg)	3.10	3.49	2.26
Exchangeable bases (cmol/kg)			
Ca	0.54	0.82	3.97
Mg	4.13	1.38	2.82
K	0.60	0.32	0.20
Na	0.08	0.08	0.09
Micro-elements (mg/kg)			
Mn	29.12	7.03	26.11
Fe	296.12	61.90	26.11
Cu	4.05	2.33	0.52
Zn	48.18	34.70	4.46

was determined using Bray II method (Bray and Kurtz, 1945) while Total nitrogen was determined by Macro-Kjeldahl method (Bremner, 1965).

Table 2 Chemical composition of compost used for the screen house experiment

Parameter	Values
pH	8.1
OC (%)	61.8
TN (%)	1.73
C : N ratio	36 : 1
P (%)	2.52
K (%)	1.48
Ca (%)	3.74
Mg (%)	0.16
Mn (mg/kg)	350.0
Fe (mg/kg)	315.0
Cu (mg/kg)	25.4
Zn (mg/kg)	42.5

2.2 Screen house experiment

The experiment was a split plot laid out in a Randomized Complete Block Design with three replications giving a total of 45 experimental units. The main treatments consisted of three soil types; sand, loamy sand, and clay and four rates of compost (prepared from sawdust and poultry manure mix) at 0, 1.5, 5.0,10 ton/ha represented by C0, C1, C2, C3 respectively, and NPK 15 : 15 : 15 fertilizer (F4) at recommended rate of 90 N kg/ha. The compost was applied per 10 kg of soil weighed into a 10 litres capacity plastic pots perforated at the bottom for drainage outlets. Moringa seeds were sourced from Department of Agronomy, University of Ibadan, Nigeria. The pots were watered to field capacity and left for two weeks before sowing the seed while the recommended rate for NPK 15 : 15 : 15 (90 kg N/ha) equivalent to 0.03 g was applied at sowing. Three seeds of *Moringa oleifera* were sown per pot and later thinned to one vigorous plant 2 weeks after sowing. Data on seedling height, stem girth, number of leaves were collected at 2 week interval for 10 weeks. Fresh and dry shoot (g) and root (g) weights were determined at harvesting (10 weeks after sowing) using weighing balance. Proximate analysis of total dry matter was used to determine the percentage of Nitrogen, Phosphorus and Potassium which was used to calculate the nutrient uptake of *Moringa oleifera* seedlings.

Data were subjected to analysis of variance (ANOVA) using the SAS package 2002.Treatment means were separated using the Fisher's Least Significant Differences at 5% level of probability.

3 Results and disscussion

The physical and chemical status of the soils used for the experiment is shown in (Table 1).

Analysis of soil texture revealed that soils from the three locations 1, 2 and 3 were clay, sand and loamy sand, respectively. Soil pH was acidic in coarse textured soil than medium and fine textured soil which tends towards neutral. Soil organic matter, Total N and available P were in the order of loamy sand > clay > sand which is a reflection of the nutrient potential of the soil. The chemical properties of the compost used for the study is present in Table 2.

The effects of soil texture and compost on Moringa plant height are represented in Figure 1. Moringa plant height was not affected by soil texture at 2 weeks after sowing (WAS). However, at 4, 6, 8, and 10 WAS loamy sand recorded significantly higher plant height than sand and clay soils. At early growth stage of Moringa, fertilizer treatment did not significantly influenced plant up to 4 WAS. Thereafter, fertilizer application significantly influenced the heights at 6, 8, and 10 WAS. At 6 WAS, treatments were in the order of C2 > F > C1 > C3 which were significantly higher height than the control (C0). At 8 WAS, the order was C2 > C1 > C3 > F4.Whereas at 10 WAS, C2 recorded significantly higher height than all the treatments, while the lower plant height was observed in C0, but there was no difference between F4 and C0.

Soil texture had significant effect on stem girth of Moringa throughout the growing stage except at 2 WAS (Figure 2). Stem girth from loamy sand was consistently significantly higher than sand and clay starting from 4 WAS to 10 WAS. The higher stem girth values obtained from loamy sand could be attributed to higher nutrients status of loamy sand compared with other textural classes as reflected in Table 1. At 4WAS fertilizer treatments revealed that C2 significantly produced higher stem girth than F4 and C0 while C1 and C3 are not different from C2 and C0. At 6 WAS, the order was C2 > C3 > C1 > F4 which were significantly higher than C0. At 8 WAS, and 10 WAS, C3, C1, F4 significantly contributed to the improvement of the stem. The lowest stem girth was found in C0.

Soil texture significantly affected number of leaves of Moringa at 4 WAS up to 10 WAS excluding 2 WAS (Figure 3). At 4 WAS loamy sand had higher number of leaves when compared to clay, while number of leaves in sand was not different from loamy sand and clay. However, at 6 WAS, number of leaves was in the order of loamy sand > clay > sand. Also, at 8 and 10 WAS, number of

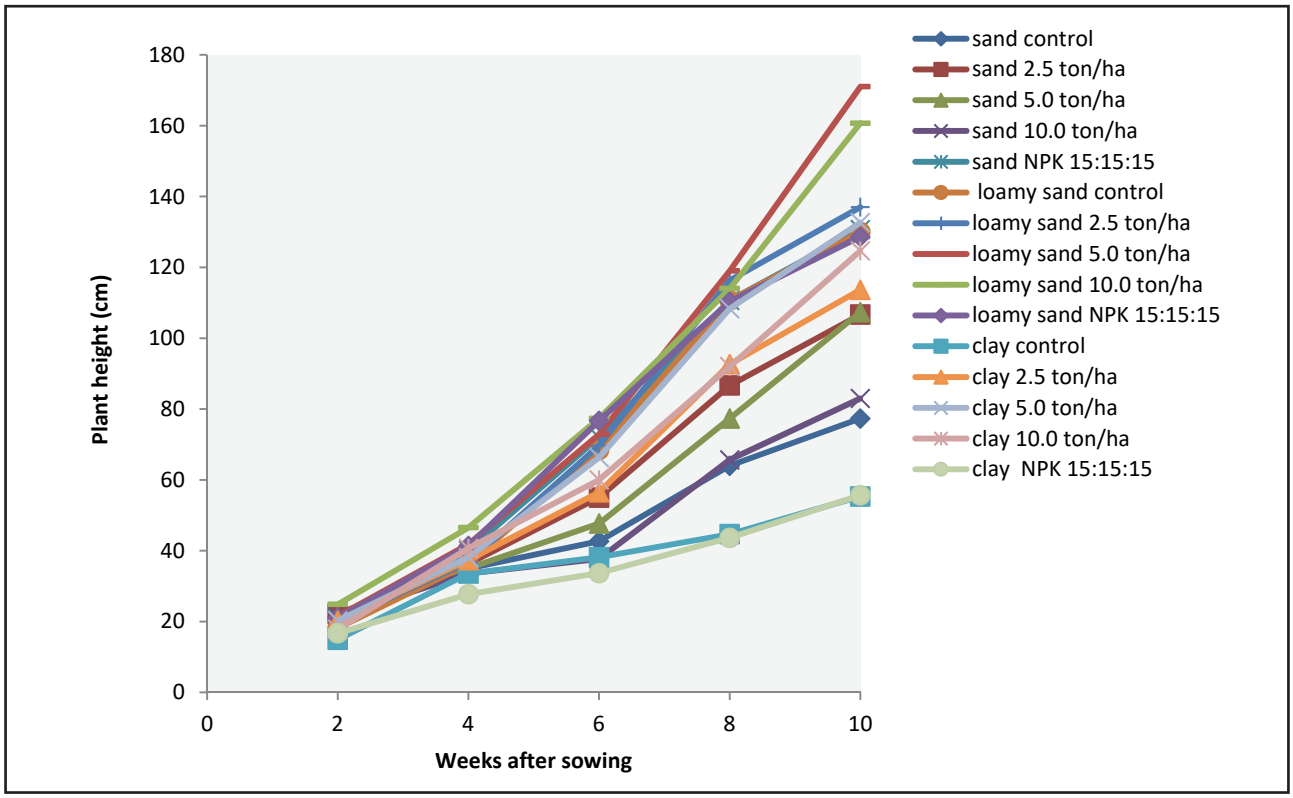

Figure 1 The interaction effects of soil texture by compost effects on *M. oleifera* plant height

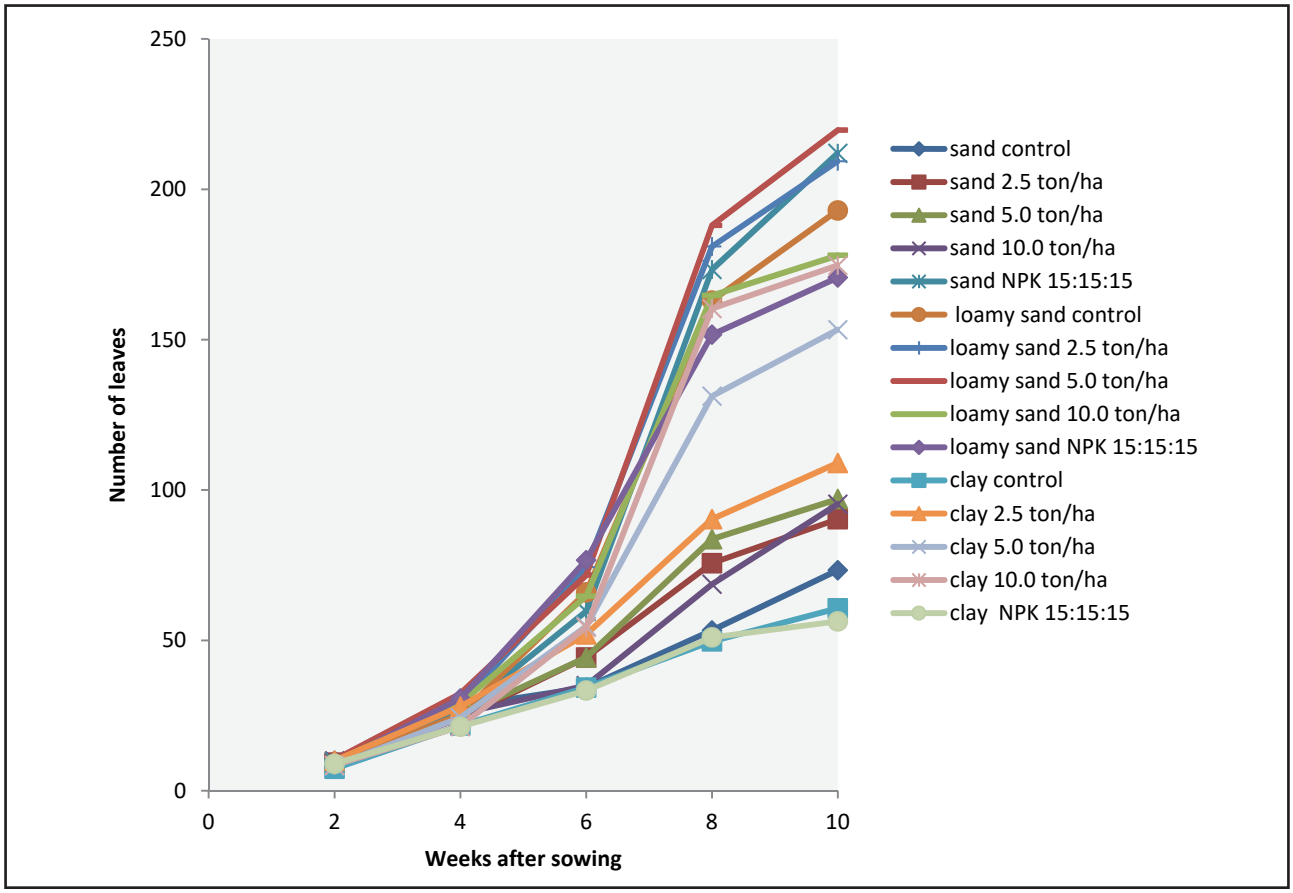

Figure 2 The interaction effects of soil texture by compost effects on *M. oleifera* number of leaves

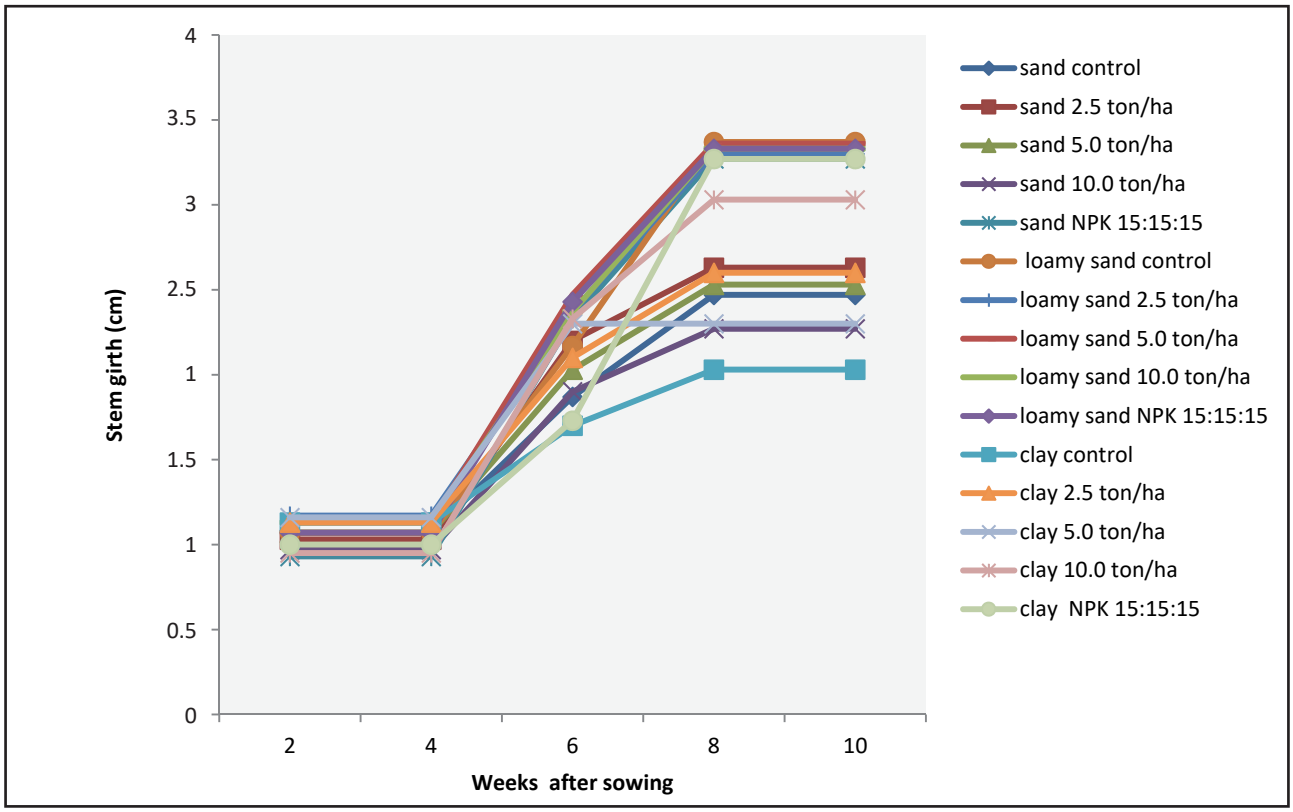

Figure 3 The interaction effects of soil texture by compost effects on *M. oleifera* seedlings stem girth

leaves recorded were significantly higher in loamy sand than sand and clay. Fertilizer treatment had no significant effect on number of leaves at 2 and 4 WAS. Conversely, at 6 to 10 WAS, there were significant effects of fertilizer treatment on number of leaves. At 6 WAS, C2, C1 and F4 had significant higher number of leaves than C0 while C3 was not different from the control and other treatments. At 8 WAS, number of leaves was in the order of C2 > C3 > F4 > C1 which were significantly higher than C0. Furthermore at 10 WAS, treatment C2, C3 and F4 were higher in number of leaves than C0.

The effects of soil texture and compost rates on fresh and dry weights of Shoot and Root of *M. oleifera* seedlings is presented in Table 3. Soil texture significantly increased fresh and dry shoot weight of Moringa. Loamy sand produced significantly higher fresh and dry shoot compared with sand and clay. Compost applied at the rate of C2, C3 and NPK fertilizer increased Moringa fresh shoot weight than C0 while C2 was not different from C0 and other treatments.

The influence of soil texture and compost on Moringa is presented in Table 3. Nutrient uptake of *M. oleifera* seedlings was significantly influenced by soil texture. Loamy sand significantly increased N, P, and K uptake by 43, 51 and 61%, respectively than sand, while there was no significant difference between sand and clay.

Compost and NPK 15 : 15 : 15 had significant influence on uptake of N and K. All compost treatments and NPK significantly increased N and P uptake when compare with the control. However, P uptake was significantly greater on C2 (5 ton/ha) than C0, C1, and F4, while C3 did not differ from C2.

The pre-planting soil analysis revealed that major nutrients of the soil (clay and sand) except for loamy sand (Table 1) was below the critical level where the minimum reported for growth was to be 0.6–1.0 gk/g for N, 3–7 mg/kg for P and 0.21–0.3 cmol/kg for K. Also, the low levels of N, available P, and organic carbon observed in the soils used for the experiment corroborate with the findings of (Aduayi et al., 2002); they reported that most of Nigerians soil is deficient in the major nutrients. Therefore, there is need for sustainable amendment to increase soil productivity in order to enhance the optimum growth and nutrient concentration of the crop.

All growth parameters (plant height, stem girth, and number of leaves) had significant performance in loamy sand than sand and clay. The inherent nutrient status of loamy sand could be attributed to the higher performance than all other textural classes. Also, loamy sand producing higher growth and biomass is an indication of sharing the properties of sand and clay which provided a good drainage and nutrient retention. The increasing effects of loamy sand on *M. oleifera*

Table 3 Effects of soil texture and compost rates on yield component of *Moringa oleifera* seedlings at 10 weeks after sowing

Treatment	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight
	g/plant			
Soil Texture (ST)				
Sand	30.31	5.82	7.49	1.01
Loamy sand	73.27	10.74	9.46	0.99
Clay	31.67	5.93	9.61	1.07
$LSD_{(0.05)}$	15.65	4.43	2.77	0.14
Compost (C)				
Control	37.00	6.53	8.39	1.12
2.5 ton/ha(C1)	45.08	6.20	7.96	1.01
5 ton/ha (C2)	50.28	8.28	9.41	0.99
10 ton/ha (C3)	46.16	8.50	8.30	0.92
NPK 15 : 15 : 15 (F4)	46.91	7.97	10.20	1.08
$LSD_{(0.05)}$	8.23	3.61	1.55	0.23
$LSD_{(0.05)}$ ST * F	19.97	Ns	3.63	Ns

LSD = Least significant difference; ST*F = Interaction of soil texture by composts

early growth in this study is in line with the findings of (Oshunsanya et al., 2015) that loamy sand supplied more nutrients for Moringa development which other textural class could not met. He also observed that Moringa plant height was significantly higher in a loose texture than a well a packed fine texture throughout the study period.

Fresh shoot and root weight followed the same trend as other growth parameters. Loamy sand producing the highest fresh shoot and root weight could be explained to a large extent, the soil potential nutrient capacity to sustain crops which is in the order of loamy sand > sand > clay. This confirms the report of Imoro et al. (2012) that increased nutrient status favoured the growth performance of *M. oleifera* seedlings. Furthermore, Swaider et al. (1992) had similar report that the best soil for growing vegetables is one that is well drained, fairly deep and has a relatively high amount of organic matter (3 to 5%). Nutrient uptake of Moringa seedlings consistently follow the same trend with growth and fresh shoot and root weight of Moringa. Loamy sand aided uptake of N, P and K of Moringa seedlings compared to sand and clay. This could be explained from the perspective that nutrient status of the soil could influence nutrient concentration of *M. oleifera* seedlings.

In terms of compost applied with NPK 15 : 15 : 15, *M. oleifera* seedlings growth parameters was consistently superior in compost applied at the rate of 5 ton per ha compared with the control, NPK and other treatments. The result revealed that 5 ton per ha gave the optimum plant growth beyond which application at higher rate than this retarded growth and might be due to toxicity effect of the compost. Furthermore, the higher response to compost application of 5 ton per/ha could be ascribed to the ameliorative effect of compost on the soil which increased Moringa growth. Asante et al. (2012) reported higher stem height values on compost treated plot and they attributed this in part to the fact that compost treated plot contained an appreciable amount of N which is responsible for promoting vegetative growth. The

Table 4 Influence of soil texture on nutrient uptake of *Moringa oleifera* seedlings

Treatments	N	P	K
	(%)		
Soil Texture (ST)			
Sand	8.78	1.69	2.42
Loamy sand	20.46	3.44	6.18
Clay	10.14	2.45	3.05
$LSD_{(0.05)}$	2.56	1.38	0.97
Compost (C) control	8.29	1.94	2.58
2.5 ton/ha compost	13.49	1.30	4.10
5 ton/ha compost	12.91	4.23	3.93
10 ton/ha compost	15.63	2.81	4.93
NPK 15 : 15 : 15	15.33	2.36	3.88
$LSD_{(0.05)}$	3.30	1.78	1.25
ST*F	5.71	NS	NS

result is in line with Palm et al. (2012) who reported that maintaining soil organic matter through use of organic materials has potential to increase crop yields.

The better production of fresh shoot and root weight with application rate of compost at 5 ton/ha suggested that it was appropriate for the vegetative growth of *M. oleifera* seedlings. Application of 5 ton per ha that was optimum for *M. oleifera* seedlings early growth beyond which retardation sets in contradicts the findings of Pahla et al., 2013; they reported that Moringa growth increased with increase application of manure. The increasing of compost on Moringa development in this study also corroborates the observation of Akanbi et al. (2005) that compost releases considerable soil organic matter, available P and exchangeable cations when applied to soil.

The increase in N, K uptake of *M. oleifera* seedlings from compost applied was comparable to NPK fertilizer, but lesser than the control. However, 5 ton per ha increased P uptake than the control, NPK 15 : 15 : 15 and other treatments. The result revealed that N and K uptake of Moringa could be increased by any application rate of compost which is comparable to NPK 15 : 15 : 15 while P uptake is best with 5 ton per ha. Consequently, application of compost contains beneficial microbes that can promote more effective root growth which aided the uptake of nutrients. This can be corroborated by the report of (Murwira and Mugwira, 2012) that application of manure promotes sustainability of soil fertility through the recycling of nutrients.

Also, the observation agreed with Adebayo et al. (2011) that application of different organic amendment significantly influenced the accumulation of N, P, and K in Moringa seedlings. Chukwuka and Omotayo (2009) also supported that, there was improvement in chemical properties of soil and nutrient uptake in plants due to application of organic amendments.

4 Conclusions

Loamy sand with moderate macro and micro nutrients had a remarkable performance in terms of plant height, number of leaves, stem girth and biomass yield. This implies that Moringa could thrive very well in a medium textured soil than either very loosed or a compacted fine textured soil due to fragile nature of the roots which may find it difficult to penetrate. Also, for optimum growth of the seedlings application rate of compost should not exceed 5 ton per ha beyond which may pose a threat to seedling growth. The results also demonstrated that nutrient concentration could be increased with the application of compost specifically P uptake which was optimum at 5 ton per ha. Therefore, loamy sand in combination with 5 ton per ha is recommended for optimum early growth of *Moringa oleifera*.

References

ADEBAYO, A.G. et al. (2011) Assessment of organic amendments on vegetative development and nutrient uptake of *Moringa oleifera* Lam in the nursery. *Asian J. Plant Sci.*, vol. 10, pp. 74–79. doi: http://dx.doi.org/10.3923/ajps.2011.74

ADUAYI, E.A. et al. (2002) *Fertilizer use and management practices for crops in Nigeria.* Abuja: Federal Ministry of Agriculture and Rural Development.

AGYENIM-BAOTENG, S. et al. (2006) Poultry manure Effect on growth and yield of maize. *W. Afri. J. Appli. Ecol.*, no. 9, pp. 61–70.

AKANBI, W.B. et al. (2005) Suitability of composted maize straw and mineral nitrogen fertilizer for tomato production. *J. Veg. Sci.*, vol. 11, no. 1, pp. 57–65.

AMANUALLAH, M.M et al. (2010) Prospects and potential of poultry manure. *Asian Journal of Plant Science*, vol. 9, pp. 172–182.

ASANTE, W. J. et al. (2012) Initial growth response of *Moringa oleifera* seedlings to different soil amendments. *African Journal of Agricultural Research*, vol. 7, no. 45, pp. 6082–6086.

BECKER, K. and SIDDHURAJU, P. (2003) Antioxidant properties of various solvent extracts of Total Phenolic Constituents from Three Different Agro Climatic Origins of Drumstick Tree (*Moringa oleifera*). *Agric. Food Chem.*, vol. 51, no. 8, pp. 2144–2155.

BENNETT, R. N. et al. (2003) Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees *Moringa oleifera* L. (horseradish tree) and *Moringa stenopetala* L. J. *Agric. Food Chem.*, no. 51, pp. 3546–3553.

BRAY, R. H. and KURTZ, I. T. (1945) Determination of total and available forms of phosphorus in soils. *Soil Science*, no. 59, pp. 45–49.

BREMNER, J. N. and MULVARY, C.S. (1965) Total nitrogen. In: SPARKS (Ed.). *Methods of Soil Analysis*. Wisconsin: American Society of Agronomy, pp. 599–622.

CHUKWUKA, K.S. and OMOTAYO, O.E. (2009) Soil fertility restoration potentials of tithonia green manure and water hyacinth compost on a nutrient depleted soil in Southwestern Nigeria. *Res. J. Soil Biol.*, no. 1, pp. 20–30.

DOERR, B. and CAMERON, L. (2005) *Moringa Leaf Powder. Echo Technical Note.*

ERIN H. (2007) "*Organic Farming" Microsoft Student 2008* (DVD). WA: Microsoft Corporation.

ESU, Z.E. (1991) Detailed Soil Survey of NIHORT Farm at Bunkure, Kano State, Nigeria. Zaria: Institute for Agricultural Research, Ahmadu Bello University.

FOIDL, N. et al. (2001) The Potential of *Moringa oleifera* for Agricultural and Industrial uses. In: FUGLIE (Ed.). *The Miracle Tree/The Multiple Attributes of Moringa CTA*, pp. 45–76.

FRANZLUEBBERS, A.J. (2002) Water infiltration and soil structure related to organic matter and its stratification with depth. *Soil Till. Res.*, vol. 66, pp. 197–205.

FUGLIE, L.J. (2001) *The Miracle Tree, Moringa oleifera: Natural Nutrition for the Tropics. Training Manual.* Dakar: Church World Service.

GEE, G.W. and OR, D. (2002) Particle size analysis. In: DANE AND TOPP (Eds.) *Methods of Soil Analysis, Methods of Soil Analysis.* Wisconsin: American Society of Agronomy, pp. 255–293.

IMORO, A.W.M. et al. (2012) Preliminary study on effects of two different sources of organic manure on the growth performance of *Moringa oleifera* seedlings. *J. Bio. Agric. Health Care*, vol. 2, no. 10, pp. 147–158.

MURWIRA, H.K. and MUGWIRA, L.M. (1997) *Use of cattle manure to improve soil fertility in Zimbabwe.* Zimbabwe: Department of Research and Specialist Services, Chemistry and Soil Research Institute.

NELSON, D.W. and SOMMERS, L.E. (1996) Total carbon, organic carbon and organic matter. In: SPARKS (Ed.). *Methods of Soil Analysis.* Wisconsin: American Society of Agronomy, pp. 961–1010.

OSHUNSANYA, S.O. et al. (2015) Growth and mineral composition of *Moringa oleifera* as affected by soil texture under water stress conditions. *Journal of Applied research*, vol. 7, pp. 151–160.

OYEDEJI, S. et al. (2014) Effects of NPK and poultry manure on growth, yield, and proximate composition of three *Amaranths*. *J. Bot.*, Article ID 828750.

PAHLA, I. et al. (2013) Effects of soil type and manure level on the establishment and growth of *Moringa oleifera*. *International Journal of Agriculture and Forestry*, vol. 3, no. 6, pp. 226–230.

PALADA, M.C. and CHANG, L.C. (2003) *Suggested Cultural practices For Moringa AVRDC international cooperators. Guide.* [Online]. Retrieved 2018-11-28 from http://www.avrdc.org/Lc/indigenous/moringa.pdf

PALM, A.C. et al. (2001) Organic input for soil fertility management in tropical agroecosystems: Application of organic resource database. *Agriculture, Ecosystems and Environment*, vol. 83, pp. 27–42.

PARR, J. F. and COLACICCO, D. (1987) Energy in Plant Nutrition and Pest Control In: *Energy in World Agriculture*. London: Elsevier Science Publishers, pp. 81–129.

SAS institute, 2002. SAS/STAT User's Guide. In: Version 8.2. SAS Institute Cary, NC.

SWAIDER, M.J. et al. (1992) *Producing Vegetables.* 4th ed., Vero Media Platform.

TEL, D. and RAO, F. (1982) *Automated and semi-automated methods for soil and plant analysis.* Ibadan: IITA, pp. 201– 270.

ZEBARTH, B. J. et al. (1999) Influence of organic waste amendments on selected soil physical and chemical properties. *Can. J. Soil Sci.*, vol. 79, pp. 501–504.

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Original Paper

The morphological changes of *oviduct*al mucose in oestral cycle of sows

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The aim of this work was to describe microscopic and submicroscopic changes in *uterine tube* of 40 sows in the estral cycle. Samples of the *uterine tube* were obtained for histological studies by vivisection from three sections of *uterine tube*. Samples were fixed for light microscopy (LM) in formaldehyde and in glutaraldehyde paraformaldehyde for scanning (SEM) and transmission (TEM) electron microscopy. They were subsequently processed in the usual manner in the LM and electron microscopic studies laboratories. We did not detect progressive changes in the length of the *uterine tube*. Unlike the sows' weight (2.57 ±1.12 g or 2.26 ±0.96 g), the length of the *uterine tube* was virtually unchanged depending on the stage of the cycle (30.2 ±2.51 cm in FF or 30.1 ±2.39 cm in LF). The largest relative volume of the epithelial layer was at the follicular stage of the cycle along the entire *uterine tube*. The difference varied from 4.99% – *isthmus* to 13.62% *infundibulum* between each part. Significant changes were seen between the ciliary and secretory cells during the estral cycle in the various parts of the *uterine tube*. Ciliary cells dominated throughout the cycle in infindibulum and *ampulla*, whereas secretory cells in *isthmus*. Their changes and differentiations are the manifestations of hormonal changes that direct the estral cycle. Submicroscopic changes of cells in the estral cycle have also been described.

Keywords: sows, *oviduct – uterine tube*, histology

1 Introduction

The *uterine tube* is a tubular organ that ensures transitory survival of gametes and embryos. Series of precisely initiated processes requiring full completion take place in the *uterine tube* (Besenfelder et al., 2012). Oviduct plays a key role in sperm fertilization, development of a zygote, capacitation and transport of a zygote to the uterus (Lauschova 2003, Sangha et al. 2003, Tienthai et al., 2009, Sharma et al., 2013).

The *uterine tube* is composed of the *infundibulum*, the *ampulla* and the *isthmus*, connecting *uterine tube* to the uterus (Hafez, 1987; Sharma et al., 2013). The *oviduct* wall is made up of muscle layers arranged lengthwise and circularly and of the *mucous membrane*. The mucouse is organized into a number of folds (Kenngott and Sinowatz, 2007), consisting of ciliary and non ciliary secretory cells and lamina propriae mucosae layer (Uhrín,1992).

These structures provide the conditions for a transport, a survival, a capacitation of sperm and fertilization of an ovulated oocyte (Koelle et al., 2010). The secretory cells play an important role in the developmental changes taking place in the *uterine tube* (Prichard et al., 1992). The ciliary cells fulfill the transport role in transporting the immobile oocyte through the *uterine tube*.

The importance of the *uterine tube epithelium* and its secretions has been described in various studies during the estrus, or menstrual cycle in mammals, including humans, monkeys, cows, sheep and pigs (Verhage et al., 1979; Brenner, 1969; Bjorkman and Fredricsson, 1961; Hadek, 1955). The fastest growing part of the *uterine tube* is the *mucous membrane*, which plays an essential role in the physiologyIn general, the *infundibular*y and *ampullar*y regions have more ciliary cells than the isthmic region.

The ciliary cells are predominant in the *uterine tube* region with a prevalence of the folds. The quantity of the secretory cells gradually increases towards the istmus (Abe, 1994; Senger, 2003). In the period of ovulation the quanity of higher ciliary cells is increased. Steinhauer et al. (2004) describes a slightly coloured ciliary cells with

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localized apical nuclei differing from basofil secretory cells with apical projections. Cell height and percentage of ciliary cells were significantly higher than in the anestrus. High levels of P4 during the middle phase of the luteal phase is associated with differentiation and dedifferentiation of cells, as well as with regression of cells that are visible on the lining of the folds in the *uterine tube*. The percentage and amount of the ciliary cells were significantly lower than during the late follicular phase.

Shirley and Reeder (1996) describe large amounts of the secretory cells in rats. The ciliary cells are smaller with a shorter cilium in an *ampulla*ry region during the estrus and metestrus. In the course of the estrus the secretion of the secretory cells accumulates in the apical end of the cells. This causes the cell protrusion to the lumen of the *uterine tube* at the time of diestrus (Shirley and Reeder, 1996).

The longest part of the *uterine tube*, the *ampulla*, is the extended tubular area where the process of fertilization is completed (Bosch and Wright, 2005), and its lining is made up of primary and secondary folders (Abe, 1994) and tertiary folders are created as well in the 9 month old sows during estrus (Šťastný and Lacková,1987). Bullón et al. (1980) describes epitelium cells in the *oviduct*, which he named as "basal", "storage", or as "indifferent" cells. Those are localized in the basal layer of the *epithelium*, are small, round, or oval in shape and having the heterochromatic nuclei. The cells are specified as non-differentiated and can be transformed into a secretory or ciliary cells.

Between the ciliary cells are the plug cells that contain the apical granules and create the secretions of the fallopian tube. Progesterone increases the number of plug cells, whereas estrogen increases their height and secretory activity (Shirish and Chakravarti, 2011; Lacková and Šťastný, 1987). The secretory cells of the *epithelium* contain a considerable amount of intra-cytoplasmic granules, which are considered to be released by the mechanism of the exocytosis (Abe,1994). These granules are distinctly smaller in the cells of the *ampulla* during the luteal phase. These granules were present in a smaller quantities also in the cytoplasm of the *isthmus* cells during the luteal phase of the cycle (Abe and Hoshi, 2007). The structure of the fallopian tube is primarily affected by the influence of hormonal activity as evidenced by changes in the mucous of the fallopian tube during the estral cycle (Hackett and Hafs,1969; Shirley and Reeder, 1996; Abughrien and Dore, 2000; Ulbrich et al., 2010, Lewis and Berardinelli, 2001; Steinhauer et al., 2004). It has also been found that the process of ciliation and deciliation of the *oviduct epithelium* depends also on steroid hormones (Verhage et al., 1979). Shirley and Reeder (1996) describe changes in the number of secretory and ciliary cells in rats during the estral cycle.

2 Material and methods

2.1 Animals

Tissue samples were taken from the fallopian tube of 40 sows (crossbreds Slovak Large White and Landrace breeds). Animals (primipara) with a small litter were excluded from further breeding. Sows were divided into 2 groups according to the stage of the estral cycle (the follicular phase and the luteal phase, with each group consisting of 20 animals). Age of the pigs at the time of slaughter was 368–382 days. The body weight of sows was in the range of 123–141 kg. The ovarian tube changes in the estral cycle were assessed according to the individual cycle control and according to the post mortem image on the ovaries. The follicular stage (FS) of the estral cycle was limited by the 20th day of the estral cycle (beginning) and the first day of reluctance to mate (end). The luteal stage (LS) of the estral cycle was limited by the third day from the reluctance to mate (start) and the 17th day of the estral cycle (end). All the animals have been slaughtered in a slaughterhouse in the usual way and immediately after bleeding their reproductive organs were remove.

2.2 Material

The samples from *oviduct* were taken from *infundibulum*, *ampulla* and *isthmus* for light (LM), transmission (TEM) and scanning electron microscopy (SEM). Samples for LM were fixed in 10% formol (Merck Millipore), dehydrated by a sequence of alcohols and sealed in paraffin 8–10 µm thick slices were made from blocks, which were coloured by haemalaun eosine (Merck Millipore) and by greens trichrome (Merck Millipore). For histochemical proof of glycogen and PAS – positive substances we have used samples fixed in Gendres solution (Vacek, 1974) with PAS reaction (Schiff´s reaction periodic acid-Schiff, Merck Millipore). Sections were evaluated on LM (Olympus Provis AX) with Image ProPlus (Spectra Services Inc, NY) program designed for assessment of individual morphological structures and MS Excel 2000. Samples from the same parts and regions of *oviduct* were taken for electronmicroscopic studies (TEM, SEM). Samples were fixed in 4% solution of glutaraldehyde paraformaldehyde (pH 7.4 Merck Millipore) with 0.08 M – cacodylate buffer (pH 6.9–7.1). For post fixation for TEM we used 1% osmiumoxid (Merck Millipore) with phosphate buffer (Milloning, 1962), samples were rinsed by Milloning´s phosphate buffer and sucrose. They were dehydrated by ascending sequence of ethanols, rinsed by propylene oxide (Merck Millipore) and deluged in the compound Durcupan ACM (A Fluka A. G., Buchs.

Switzerland-Registered Trademark). Semi-thick (1 μm) and ultra-thin slices were made on ultramicrotome (LKB 8800 III). Semi-thick slices were coloured by Toluidine blue (Merck Millipore) and assessed on (Olympus Provis AX). Samples for SEM were rinsed and dehydrated after fixation (3 hours) in ascending sequence of acetones and desiccated with CO_2 (CPD Polaron, England). Dried samples on fixtures were then vacuum-coated with 20 nm thick layer of gold (All Chemie LTD, US). Ultra-thin slices were contrasted with lead citrate (Reynolds, 1963) and uranyl acetate (SPI Supplies and Structure Probe, Inc). Electronograms were made on TEM (TESLA BS 500) and SEM (TESLA BS 301). Morphometric methods were used for objectification of results (Weibel et al., 1966; Mráz and Polónyi,1988).

3 Results and disscussion

Macroscopically *uterine tube* is quite simple organ, in the cyclic gilts is 16.0 cm to 27.0 cm long with weight from 0.86 g to 2.33 g (Šťastný and Lacková, 1987). In sows in the estrusl cycle its length is virtually unchanged (30.2 cm or 30.1 cm, Table 1) in contrast to the weight (2.57g or 2.26 g, Table 1). In the gilts (Šťastný and Lacková, 1987) as well as in the case of heifers, Šťastná et al. (2013) found that the length of the *uterine tube* changed with the estrus cycle change. Significant gradual shortening has passed from estrus to diestrus (P <0.01). The *uterine tube* is divided into three parts: *infundibulum*, *ampulla* and *isthmus* (Menezo and Guerin, 1997; Ellington, 1991).

Table 1 Weight and length of the *uterine tube* (n = 40)

	FF	LF
Weight (g)	2.57 ±1.12	2.26 ±0.96
Length (cm)	30.2 ±2.51	30.1 ±2.39

FF – follicular phase; LF – luteal phase

Sperm and oocyte enter the *uterine tube* from opposite ends. In order to meet, they use the *oviduct*al countercurrent system for transfer. Its structure is also adapted for this purpose. The *oviduct* consists of three parts (*infundibulum*, *ampulla* and *isthmus*) that have a longitudinal and circular muscular layer and a *mucous membrane* (Besenfelder et al., 2012; Menezo and Guerin, 1997; Ellington, 1991). These parts are characterized by polymorphic folds and ridges of varying sizes. The folds are longitudinally different, forming secondary – to – tertiary folds, forming irregular net-like structures forming different troughs, vesicles and crypts (Yaniz et al., 2000; Kenngott and Sinowatz, 2007). Sharma et al. (2015) describes characteristic pattern of variations in the *ampulla*ry segment of the *uterine tube* during follicular and luteal phases of estrous cycle in goat. The *tunica mucosa* of *ampulla* was characterized by presence of longitudinal mucosal folds throughout the length with extensive secondary and tertiary branches in follicular phase, whereas the luteal phase was characterized by primary and secondary branching patterns. Similar differences can also be seen when comparing the *ampulla* mucosa and the *isthmus* of sows in the same phase of the estrus cycle (Figure 1).

The epithelial layer fold volume varied depending on the ongoing phase of the estrus cycle of cycling heifers (Šťastná et al., 2013). The size of the epithelial cells is minimal in the proestrus (Hackett and Hafs, 1969 presented as well by the relative volume of the epithelial layer in the follicular phase of sows (Figure 2), which is dominant in all parts of the *oviduct* (80.96%, 74.64%, 71.55%, respectively.). Also according to Uhrin (1992) in cows after the follicular phase the volume of the mucosa gradually decreases in the diestral stage of the cycle. Natarajan et al. (2003) believes that increasing level of estrogen in the follicular phase is responsible for increasing the height of the mucosa that

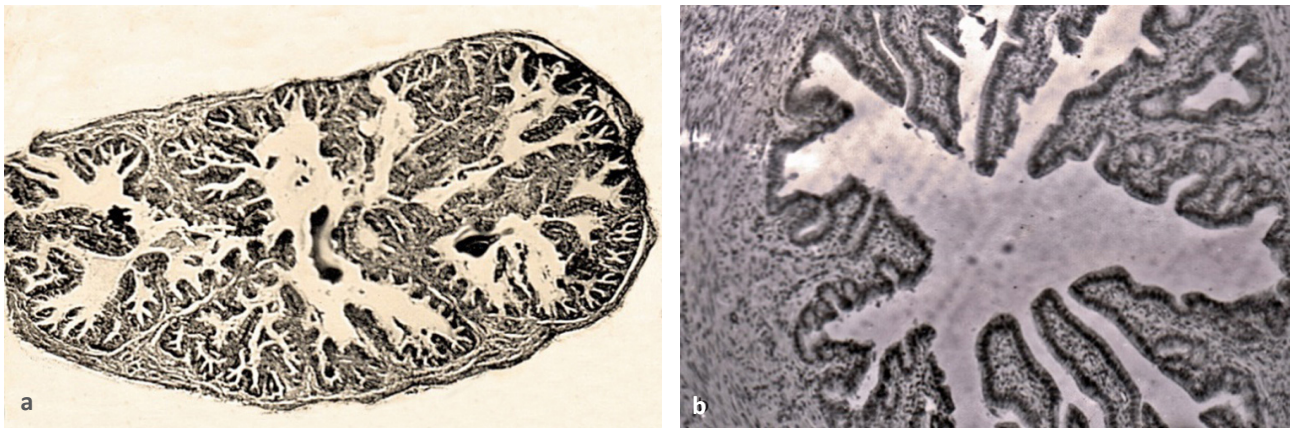

Figure 1 Pronounced primary and secondary folds leading to the centre of the duct can be seen on the transverse section of the *ampulla* of the *oviduct* in the follicular phase of estrus cycle (a) and transverse section of *isthmus* (b) has mainly primary folds in the follicular phase, HE, mag. ×330

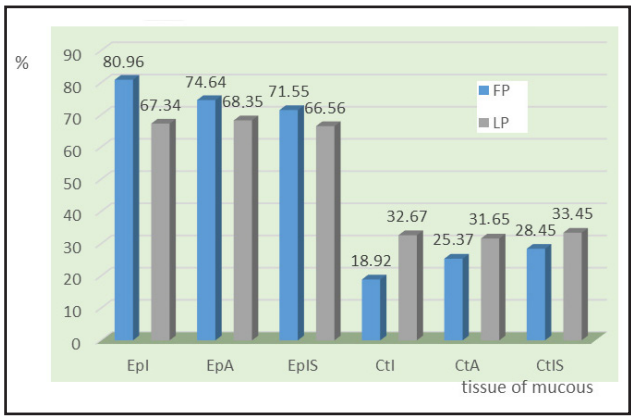

Figure 2 Epitelial and conectical tissue percentage in the different parts of the *uterine tube*
FP – follicular phase; LP – luteal phase; EpI – epitel of the *infundibulum*; CtI – connective tissue of the *infundibulum*; EpA – epitel of the *ampulla*; CtA – connective tissue of the *ampulla*; EpIS – epitel of the *isthmus*; CtIS – connective tissue of the *isthmus*

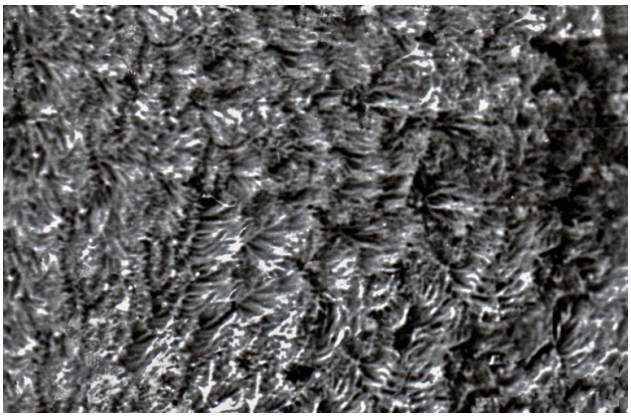

Figure 3 Scanning electron micrograph of infundibullary region of *oviduct* during follicular phase revealing a richly ciliated *epithelium* with uniformed cilia length, coated in gold, mag. ×6,320

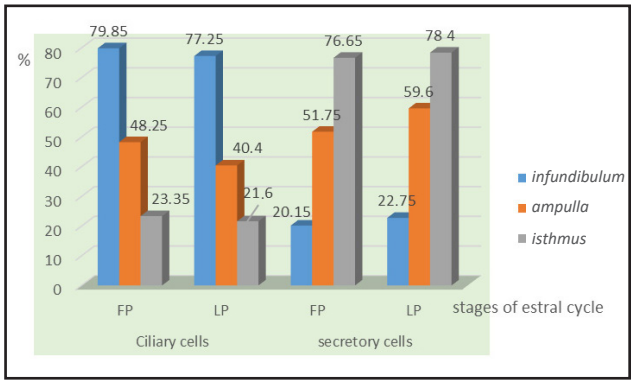

Figure 4 Ciliary and secretory cells precentage in different parts of the *uterine tube*
FP – follicular phase; LP – luteal phase

decreases during the luteal phase. The epithelial layer is composed of ciliary and non-ciliary secretory cells. Their representation varies in the individual parts of the *oviduct* depending on the stage of the estrus cycle. *Infundibulum* and *ampulla* usually have more ciliary cells than *isthmus*. The ciliary cells are slim, cylindrical in shape, attached to the basal membrane and covered by dense and relatively long cilia. They may be lighter and darker with condensed chromatin in the nucleus (Uhrin, 1992).The *infundibular* ciliary cells virtually overlapped the secretory cells in the follicular phase (Figure 3), which formed only 20.15% in this part of the *oviduct* (Figure 4). This ratio varies mainly in *ampulla* (P <0.01) and *isthmus* in the luteal phase of the estral cycle when the predominance of secretory cells is evident (Figure 5). In contrast, the ratio of secretory

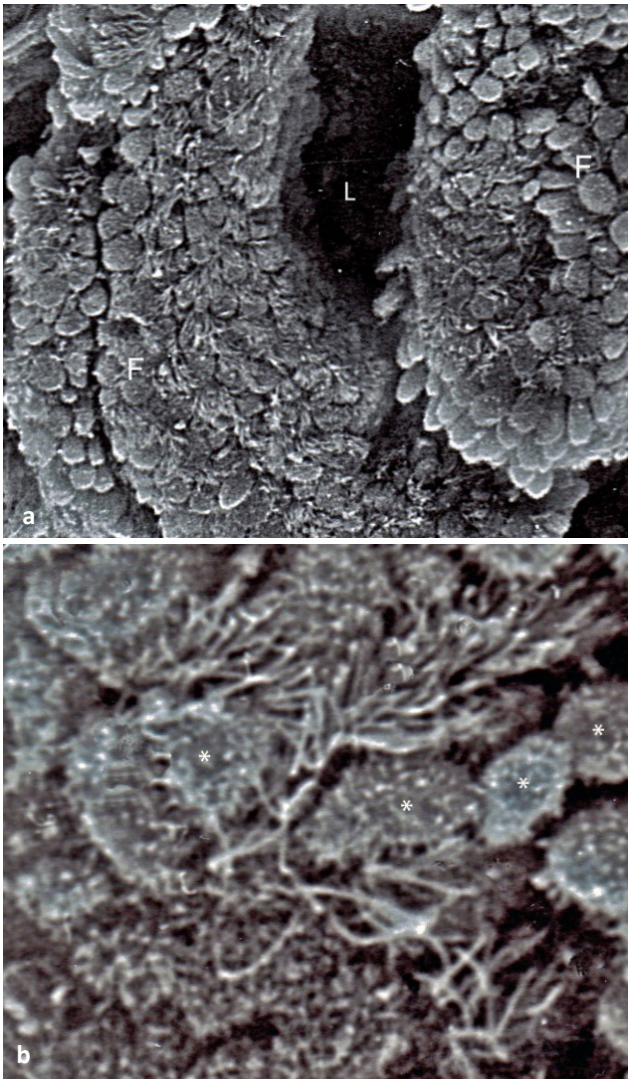

Figure 5 Scanning electron micrograph of *ampullary* region of *oviduct* during luteal phase of estral cycle revealing a richly evident secretory cells on mucous lines (a), coated in gold, mag. ×5,000, enlargement (b) mag. ×16,200
L – lumen, F – folds, * – secretory cells

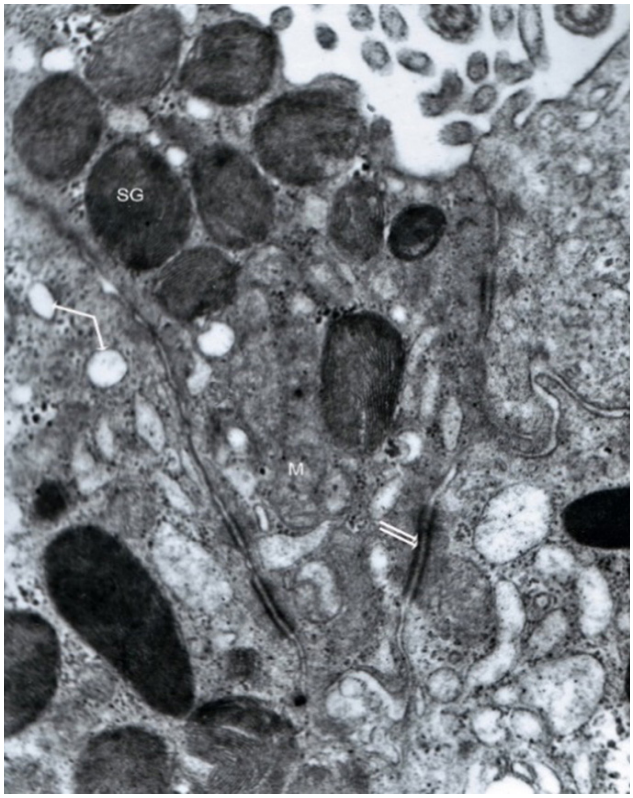

Figure 6 Transmission electron micrograph of *ampulla*ry region of *oviduct* during follicular phase showing quantum of the secretory granules in the apically ends of the secretory cells
SG – secretory granules, arrows – lipidic drops, double arrows – desmosome), mag. ×14,000

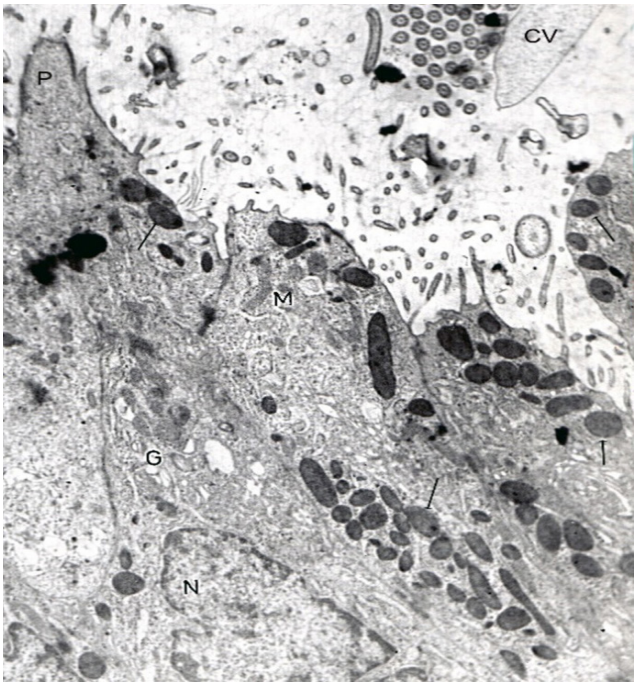

Figure 7 Transmission electron micrograph of *ampulla*ry region of *oviduct* during luteal phase showing apical protrusions (P) of secretory cells with the secretory granules (arows)
N – nucleus, M – mitochondria, G – Golgi, mag. ×19,920

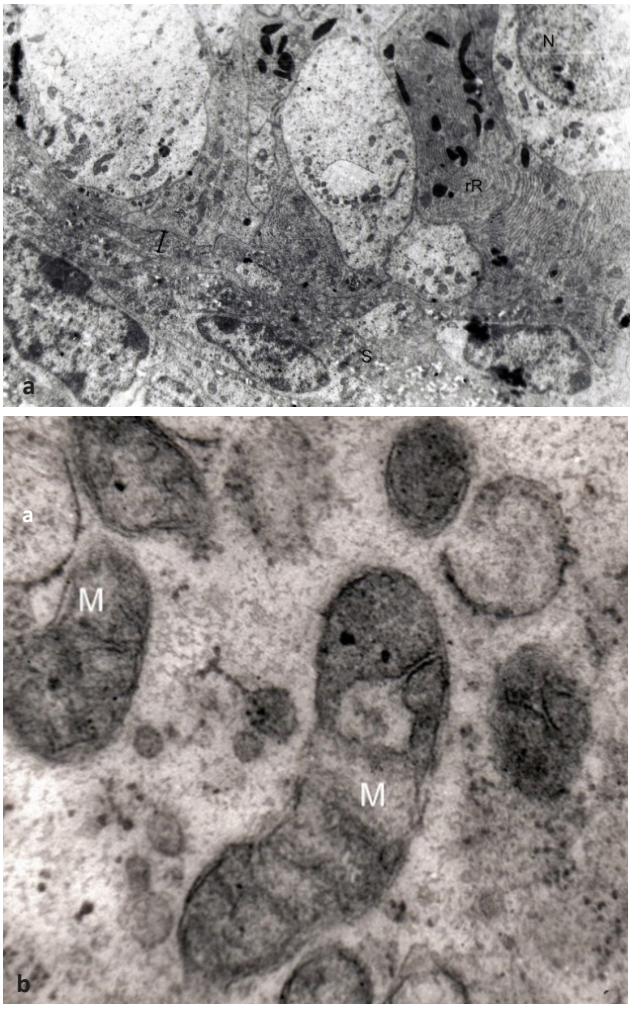

Figure 8 Transmission electron micrograph of *ampulla*ry region of *oviduct* during luteal phase showing the basal cells with the presence of numerous ribosomes, a large rough endoplasmic reticulum (a) and well-developed mitochondria (b), mag. ×9800 (a), ×76,000 (b)
N – nucleus, R – rough endoplasmic reticulum, S – submucous, arrows – basal membrane, M – oval mitochondria

and ciliary cells according to Abe et al. (1999) in the goat *oviduct* is not different in the luteal and follicular phases, but reduction in cell size, especially ciliary cells in the luteal phase of the cycle has been found. Similar findings are reported by Abe and Oikawa (1992) in sows and by Abe and Oikawa (1993) in cows.These cells are characterized by a decreased number of secretory granules, the presence of numerous ribosomes, a large and rough endoplasmic reticulum and a well-developed mitochondria (Figure 8a, b). These changes can also be seen in basal cells.The most characteristic property of secretory cells are the secretory granules. Their amount and size vary depending on the stage of the cycle (Abe et al., 1999). It is found in the follicular phase in the *ampulla* and less in the *infundibula* and the least in the

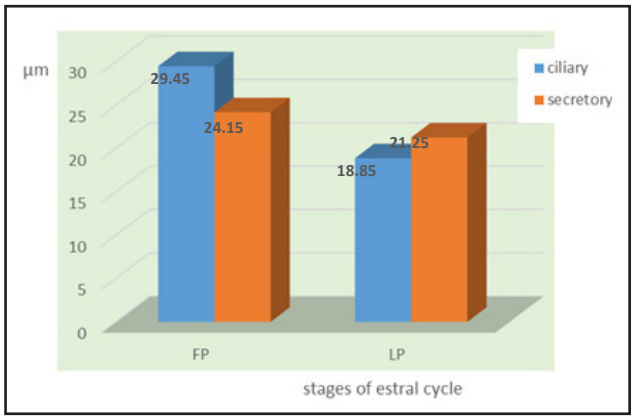

Figure 9 The heigth of the epitelial cells of the *uterine tube* mucosa
FP – follicular phase; LP – uteal phase

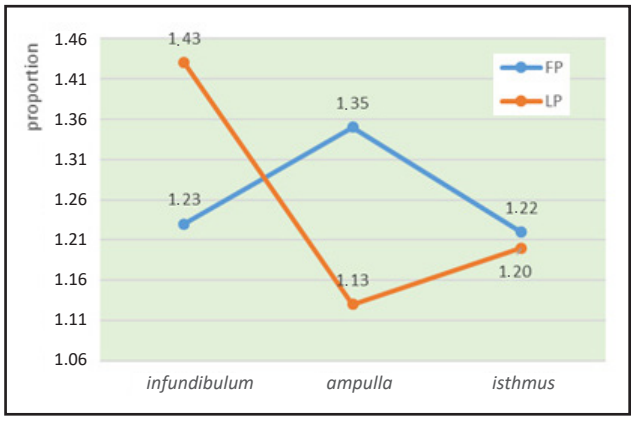

Figure 10 Nucleo-cytoplasmatic proportion of cells of the *uterine tube* mucous (nucleus = 1)
FP – follicular phase, LP – luteal phase

isthmus. This also assumes a different secretory activity of cells (Abe et al., 1999). These changes in secretory cells should be the result of the steroid hormones action on epithelial cells and the different reaction of different sections of the *oviduct* to these hormones (Abe et al., 1999). A number of intracytoplasmic granules and lipid droplets (Figure 6) are seen in the secretory cells of the *epithelium*, that are released from the cell by the mechanism of exocytosis (Figure 7). Secrete granules are located primarily in apical cell sections as densely oval forms. They occur the most in the later follicular phase of the cycle (Figure 6), followed by their intense excretion and the decrease in the luteal phase (Uhrin, 1992). Density structures that are similar in size and shape to the cell core have often been extruded from the epithelial layer of the *oviduct*. These granules significantly decreased in *ampulla*ry cells in the luteal phase. Many granules were observed throughout the ovarian cycle in the cytoplasm of isthm cells, with the exception of the luteal phase, where granules were reduced (Abe and Hoshi, 2007). The epithelial cells are higher in the estrus and in the time of incoming ovulation and are reaching the peak when compared to diestrus (Yaniz et al., 2000; Abe and Hoshi, 2008; Ulbrich et al., 2010; Nakahari et al., 2011). Findings of Steinhauer et al. (2004) that the cells in the follicular phase are higher than in the luteal phase were also confirmed in sows (Figure 9). The size of the epithelial cells varies according to Mc Daniel Scalzi and Black (1968) due to the progesterone action. Relative changes in volume of cells alter also the nucleocytoplasmic ratio (NCR) in both cell types in estral cycle. The highest NCR (1 : 1.43) is in the luteal phase of the estral cycle in the *infundibulum* region and the lowest (1 : 1.13) in the *ampulla* of the

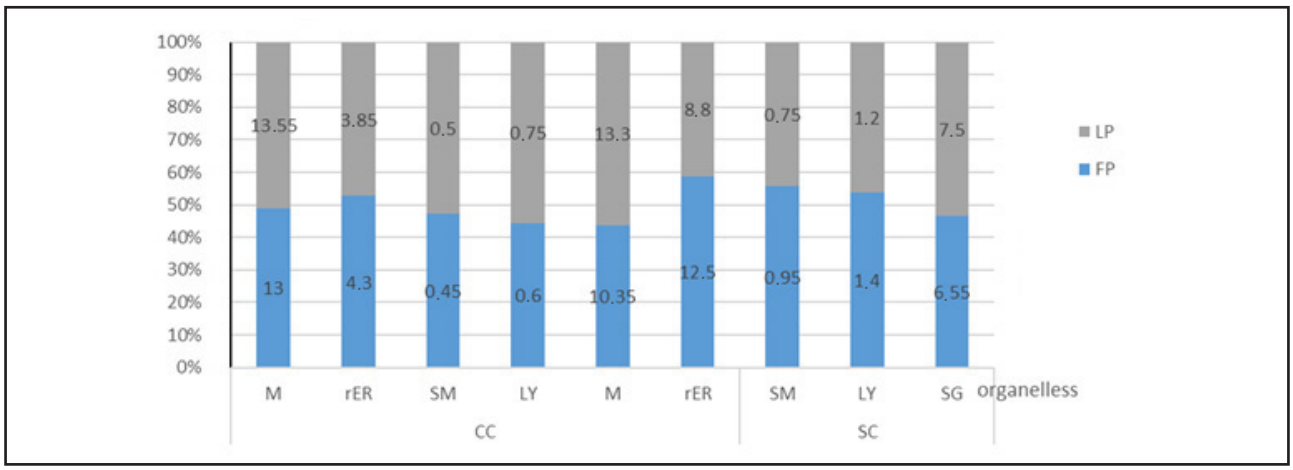

Figure 11 The volume of the organelles in the ciliary and the secretory cells of the *uterine tube* during estral cycle (% of the volume of cytoplasm)
Cc – ciliary cells; Sc – secretory cells; M – mitochondria; rER – rough endoplasmatic reticulum; SM – smooth membranes; LY – lysosomes; SG – secretory granules

oviduct in the luteal phase of the cycle (P <0.01, Figure 10). The increase in secretory activity in the cytoplasm also changes NCR (Uhrin, 1992). In the follicular phase of the estral cycle, the highest ratio was found in the *uterine tube* (1 : 1.35, P <0.05). Šťastná et al. (2013) found the lowest NCR in the proestrus of both cell types in the heifers (1 : 1.16 or 1 : 1.36) since cell nuclei enlarge at this time, but the volume of the cytoplasm does not change over the diestrus. Approximately the same volume of mitochondria was present in ciliary and secretory cells in the luteal phase of the cycle (13.55 vs. 13.3%, Figure 11). Follicular phase was dominated by mitochondria in ciliary cells. In contrast, the rough endoplasmic reticulum and smooth endoplasmic reticulum had a significantly higher volume in secretory cells throughout the cycle (P <0.01). The cell nuclei were localized at different cell levels, depending on the phase of the cycle, with predominantly granulated density nucleoplasm.

4 Conclusions

The thesis describes structural quantitative and qualitative changes in the ovary of sows in the estral cycle. Progressive changes in mucosal structures, particularly the epithelial component of the mucosa have been confirmed. Different qualitative and quantitative changes were observed in ciliary and secretory cells under steroid control. Quantitative and qualitative changes in the estral cycle also indicate that they are under hormonal control and are differentiated at different stages in the cycle. This is related particularly to the secretory cells undergoing more intense changes at both microscopic and submicroscopic level. The numerical differentiation of ciliary and secretory cells in individual parts of the sows' *oviduct* was confirmed as well. Photo documentation supports the description of detected microscopic findings.

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References

ABE, H. (1994) Regional variations in the ultrastructural features of secretory cells in the rat *oviduct*al *epithelium*. *Anatomical Record*, vol. 240, no. 1, pp. 77–85.

ABE, H. and HOSHI, H. (2007) Regional and cyclic variations in the ultrastructural features of secretory cells in the *oviduct*al *epithelium* of the Chinese Meishan pig. *Reproduction in Domestic Animals*, vol. 42, no. 3, pp. 292 – 298.

ABE, H. and HOSHI, H. (2008) Morphometric and ultrastructural changes in ciliated cells of the *oviduct*al *epithelium* in prolific Chinese Meishan and Large White pigs during the oestrous cycle. *Reproduction in Domestic Animals*, vol. 43, no. 1, pp. 66–73.

ABE, H. and OIKAWA, T. (1992) Examination by scanning electron microscopy of *oviduct*al *epithelium* of the prolific Chinese Meishan pig at follicular and luteal phases. *Anatomical Record*, vol. 233, no. 3, pp. 399–408.

ABE, H. et al. (1993) Scanning electron microscopy of goat *oviduct*al epithelial cells at the follicular and luteal phases of the oestrous cycle. *Journal of Anatomy*, vol.183, no. 2, pp. 415–421.

ABE, H. et al. (1999) Ultrastructural features of goat *oviduct*al secretory cells at follicular and luteal phases of the oestrous cycle. *Journal of Anatomy*, vol. 195, no. 4, pp. 515–521.

ABUGHRIEN, B.M. and DORE, M.A. (2000) Ciliogenesis in the *uterine tube* of control and superovulated heifers. *Cells Tissues Organs*, vol. 166, no. 4, pp. 338–48.

BESENFELDER, U. et al. (2012) Role of the Oviduct in Early Embryo Development, *Reproduction in Domestic Animals*, vol. 47, pp. 156–163. doi: 10.1111/j.1439-0531.2012.02070.x. ISSN 0936-6768.

BJORKMAN, N. and FREDRICSSON, B. (1961) The ultrastructure organization and the alkaline phosphatase activity of the epithelial surface of the bovine fallopian tube. *Zeitschrift fur Zellforschung Und Mikroskopiche Anatomie*, vol. 51, no. 5, pp. 589–596. doi:10.1007/bf00335759

BOSCH, P. and WRIGHT, R.W. (2005) The *oviduct*al sperm reservoir in domestic mammals. *Archivos de Medicina Veterinaria*, vol. 37, no. 2, pp. 95–104. doi:10.4067/s0301-732x2005000200002

BRENNER, R.M. (1969) Renewal of *oviduct* cilia during the menstrual cycle of rhesus monkey. *Fertility and Sterility*, vol. 20, no. 4, pp. 599–611. doi:10.1016/s0015-0282(16)37086-8

BULLÓN, F. et al. (1980) Ultrastructure of the *oviduct*al mucosa of the rat. III. Basal and Peg cells. *International journal of fertility*, vol. 25, no. 4, pp. 293–297.

ELLINGTON, J.E. (1991) The bovine *oviduct* and its role in reproduction – a review of the literature. *The Cornell veterinarian*, vol. 81, no. 3, pp. 313–328.

HACKETT, A.J. and HAFS, H.D.(1969: Biochemical and Morphological Changes in Bovine Tubular Genitalia during the Estrous Cycle. *Journal of Animal Science*, vol. 29, no. 1, pp. 35–38. doi:10.2527/jas1969.29135x

HADEK, R. (1955) The secondary process in the sheep's *oviduct*. *Anatomical Record*, vol. 12, pp. 187 – 192.

HAFEZ, E.S.E. (1987) Reproduction in farm animals. 5th ed., Philadelphia, PA: Lea and Febiger.

KENNGOTT, R..A. and SINOWATZ F. (2007) Prenatal development of the bovine *oviduct*. *Anatomia, Histologia, Embryologia: Journal of Veterinary Medicine Series C*, vol. 36, no. 4, pp. 272–283. doi:10.1111/j.1439-0264.2006.00762.x

KOELLE, S. et al. (2010) Newaspects of gamete transport, fertilization,and mbryonic development in the *oviduct* gained by means of live cell imaging. *Theriogenology*, vol. 73, no. 6, pp. 786–795. doi:10.1016/j.theriogenology.2009.11.002

LAUSCHOVA, I. (2003) Secretory cells and morphological manifestation of secretion in the mouse *oviduct*. *Scripta Medica*, vol. 76, no. 4, pp. 203 – 224.

LEWIS, A.W. and BERARDINELLI, J.G. (2001) Gross anatomical and histomorphometric characteristics of the *oviduct* and

uterus during the pubertal transition in sheep. *Journal of Animal Science*, vol. 79, no. 1, pp. 167–175. doi:10.2527/2001.791167x

McDANIEL, J. W. et al. (1968) Influence of ovarian hormones on histology and histochemistry of the bovine *oviduct*. *Journal of Dairy Science*, vol. 51, no. 5, pp. 754–761. doi:10.3168/jds.s0022-0302(68)87067-5

MENEZO, Y. and GUERIN, P. (1997) The mammalian *oviduct*: biochemistry and physiology. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, vol. 73, no. 1, pp. 99–104. doi:10.1016/s0301-2115(97)02729-2

MILLONING, G. (1962) Further observation on a phosphate buffer for osmium solution in fixation. *Electron Microscopy*, vol. 2, pp. 1–8. doi:10.1016/b978-1-4832-2795-5.50066-6

MRÁZ, P. and POLÓNYI, J. (1988) Metódy v elektrónovej mikroskopii živočíšnych tkanív. Bratislava: VEDA, vyd. SAV,1988.

NAKAHARI, T. et al. (2011) The regulation of ciliary beat frequency by ovarian steroids in the guinea pig Fallopian tube: interactions between oestradiol and progesterone. *Biomedical Research*, vol. 32, no. 5, pp. 321–328. doi:10.2220/biomedres.32.321

NATARAJAN, T. et al. (2003) Ultrastructural studies of *oviduct* in buffalo (*Bubalus bubalis*). *Indian Journal of Animal Sciences*, vol. 73, pp. 522 – 523.

PRICHARD, J.F. et al. (1992) *In vitro* co-culture of early stage caprine embryos with *oviduct* and uterine epithelial cells. *Human Reproduction*, vol. 7, no. 4, pp. 553–557. doi:10.1093/oxfordjournals.humrep.a137689

SANGHA, G.K. et al. (2003) Changes in proteins, total lipids, cholesterol, phospholipids and phosphatase enzymes in *oviduct*al flushings of goat. *Indian Journal of Animal Sciences*, vol. 73, pp. 848 – 850.

SENGER, P.L. (2003) Pathways to pregnancy and parturition. 2nd ed., New York: Current Conceptions Inc. 373 p.

SHARMA, R. K. et al. (2015) Scanning and transmission electron microscopic analysis of *ampulla*ry segment of *oviduct* during estrous cycle in caprines. *Scanning*, vol. 37, no. 1, pp. 36–41. doi:10.1002/sca.21176

SHARMA, R.K. et al. (2013) Topographic and ultrastructural variations in Steinhauer NO, Boos A, Gunzel-Apel AR. 2004. Morphological changes and proliferative activity in the *oviduct*al *epithelium* during hormonally defined stages of oestrous cycle in the bitch. *Reproduction in Domestic Animals*, vol. 39, no. 2, pp. 110–119. doi:10.1111/j.1439-0531.2004.00490.x

SHIRISH, D. and CHAKRAVARTI, S. (2011) Manual of Obstetrics. 3rd ed., Elsevier. pp. 1–16. ISBN 9788131225561.

SHIRLEY, B. and REEDER, R.L. (1996) Cyclic changes in the *ampulla* of the rat *oviduct*. *Journal of Experimental Zoology*, vol. 276, no.2, pp. 164–173. doi.org/10.1002/(SICI)1097-010X(19961001)276:2<164::AID-JEZ10>3.0.CO;2-K

STEINHAUER, N.O. et al. (2004) Morphological changes and proliferative activity in the *oviduct*al *epithelium* during hormonally defined stages of oestrous cycle in the bitch. *Reproduction in Domestic Animals*, vol. 39, no. 2, pp. 110–119. doi:10.1111/j.1439-0531.2004.00490.x

ŠŤASTNÁ, D et al. (2013) Morphological changes of *oviduct* in postnatal development and in oestrous cycle of heifers. *Acta Fytotechnica et Zootechnica*, vol. 16, no. 4, pp. 90–98.

ŠŤASTNÝ, P. and LACKOVÁ, D. (1987) Morphological analysis of the changes in *oviduct* during the sexual cycle of gilts (Morfologická analýza zmien vajcovodu v pohlavnom cykle prasničiek). *Živočíšna výroba*, vol. 32, pp. 797–809.

TIENTHAI, P. et al. (2009) Light and scanning electron microscopic studies of *oviduct*al *epithelium* in Thai swamp buffalo (*Bubalus bubalis*) at the follicular and luteal phases. *Reproduction in Domestic Animals*, vol. 44, no. 3, pp. 450–455. doi:10.1111/j.1439-0531.2008.01111.x

UHRÍN, V. (1992) *Funkčná morfológia epitelov vajcovodu a maternice kravy*. Bratislava: Slovak Academic Press, spol. s.r.o., ISBN 80-85665-05-0.

ULBRICH, S.E. et al. (2010) *In vitro* systems for intercepting early embryo-maternal cross-talk in the bovine *oviduct*. *Theriogenology*, vol. 73, no. 6, pp. 802–816. doi:10.1016/j.theriogenology.2009.09.036

VACEK, Z. (1974) *Histológia a histologická technica*. Martin: OSVETA.

VERHAGE, H.G. et al. (1979) Cyclic changes in ciliation, secretion and cell height of the *oviduct*al *epithelium* in women. *American Journal of Anatomy*, vol. 156, no. 4, pp. 505–521. doi:10.1002/aja.1001560405

WEIBEL, E.R. et al. (1966) Practical stereological methods for morphometric citology. *The Journal of Cell Biology*, vol. 30, no. 1, pp. 23–38. doi:10.1083/jcb.30.1.23

YÁNIZ, J.L. et al. (2000) Study of the functional anatomy of bovine *oviduct*al mucosa. *The Anatomical Record: An Official Publication of the American Association of Anatomists*, vol. 260, no. 3, pp. 268–278.

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Original Paper

Nutritional and phytogenic properties of pawpaw (*Carica papaya*) leaf meal on blood characteristics of growing rabbits

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The study aimed to examine the effect of pawpaw (*Carica papaya*) leaf meal diets on blood characteristics of rabbits. A total of 48, male rabbits were randomly divided into four experimental groups of twelve animals each, with four rabbits constituting a replicate. Each group was assigned to one of the experimental diets containing pawpaw leaf meal (PLM) at 0% (control), 15%, 30% and 45% for 56 days in a completely randomized design (CRD). Blood samples for analysis were obtained from each replicate and data obtained were analyzed statistically. Results on chemical composition of the PLM revealed 87.67% DM, 17.30% CP, 12.86% CF, 8.88% ash, 0.81% EE 47.82% NFE and 2348.05 Kcal/kg ME. PLM at 15% inclusion increased (P <0.05) the packed cell volume (PCV) and haemoglobin (Hb) when compared with the control. PLM at 30 and 45% resulted to improved mean cell volume (MCV) concentration. The concentrations of white blood cells were increased (P <0.05) at the treatment (15%, 30% and 45%) groups. Red blood cell, mean cell volume, mean cell haemoglobin concentration, creatinine, AST, ALP, sodium, potassium and chloride remained similar (P >0.05) across the treatments. Total protein was however best (P <0.05) at 45% supplementation. The results indicated that PLM enhanced haemopoiesis and health status of the experimental rabbits and therefore should be incorporated into rabbit feeding to enhance blood formation and health status of the animals.

Keywords: rabbits, pawpaw leaf, phytogenic compound, haematology and serum biochemistry

1 Introduction

Phytogenic compounds have recently received great attention in animal nutrition due to their growth-promoting and medicinal properties. Their uses in animal production have been on increase mainly by the belief that they are free from chemical additives and absence of toxic influence on the animals. However, incidences of toxicity have been reported by Edeh (2013) and Ogbuewu et al. (2014) among animals fed diets containing phytogenic compounds. Hence, the need to ascertain the nutritional and phytogenic properties of this plant materials before recommending their use in animal production. The clinical examination of blood becomes necessary since it provides reliable information about feed toxicity on animals fed such diets.

Pawpaw is known with diverse names, in Nigeria, it is known as "Okwuru bekee" by Igbos, "Ibepe" in Yoruba and "Gwada" by Hausas. Pawpaw is a herbaceous plant, with a single stem growing from 5 to 10 m tall, with spirally arranged leaves confined to the top of the trunk. The leaves are spirally arranged, clustered near the trunk apex, hollow, greenish or purplish-green, 50–75 cm in diameter, palmate, deeply 7-lobed, prominently veined and broadly toothed. The flowers appear on the axils of the leaves, maturing into large fruit. The fruit is ripe when it feels soft and its skin has turned yellowish-brown to orange shade. Pawpaw though a native of America is easily grown in Africa. Pawpaw Aravind et al. (2013) was reported to be a powerhouse of nutrients. The leaves contain carbohydrates, minerals and vitamins, lipids and proteins (Patil et al., 2014). The leaves are high in papain, volatile oil, terpenoids, folic acid, vitamins B_1, B_2, B_{12}, A, C and E, alkaloids (carpaine and pseudocarpaine), saponins, anthraquinones, cardiac glycosides, glucosinolate

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(benzylglucosinolate), choline, flavonoids, calcium, magnesium, sodium, potassium, manganese and iron. Pawpaw aids in digestion of protein at acid, alkaline or neutral medium due to the presence of papain, a natural enzyme, which also helps in the cleansing of gastro intestinal tract (Jiwuba, 2018). Earlier reports by Otsuki et al. (2010) and Nguyen et al. (2013) indicated that the leaves can prevent and kill cancer cells. It was also reported (Chávez-Quinta et al., 2011; Aravind et al., 2013; Nguyen et al., 2014; Patil et al., 2014) to have antimalarial, antiplasmodial, anthelmintic, antiparasitic, antibacterial, antiviral, antifungal, anti-inflammatory, digestive stimulant and antihypertensive activities. These thus indicated the dual properties of pawpaw leaves as a nutritional agent and medicinal agent; hence highlighting its nutritional and ethno-veterinary properties. Despite, these properties, pawpaw leaf meal has not been extensively considered as a reliable feedstuff for rabbits. The study was therefore, designed to evaluate the blood characteristics of growing rabbits fed varying levels of pawpaw leaf meal containing diets with a view of ascertaining nutritional and ethno-veterinary properties for improved rabbit performance.

2 Material and methods

2.1 Location of experiment

The experiment was carried out at the Rabbit Unit, Federal College of Agriculture, Ishiagu, Ebonyi state, Nigeria. The College is located at about three kilometers (3 km) away from Ishiagu main town. The College is situated at latitude 5.56 °N and longitude 7.31 °E, with an average rainfall of 1653 mm and a prevailing temperature condition of 28.5 °C and relative humidity of about 80% (Jiwuba et al., 2016a).

2.2 Sources and processing of experimental material

Fresh leaves of pawpaw were harvested within the College environment and air dried for some days to a moisture content of about 10%. The air dried leaves were processed and milled using 5 mm hammer mill. Other feed ingredients were procured from Farm associate, Enugu, Enugu State, Nigeria.

2.3 Experimental animals and management

Forty eight growing rabbits weighing averagely 477.01 g were randomly divided into four experimental groups of twelve animals each, with four rabbits constituting a replicate. Each rabbit was housed in a standard hutch measuring 120 by 150 cm and raised 120 cm above the ground level. The four treatment groups were assigned the four diets in a Completely Randomized Design (CRD). Each rabbit received an assigned diet for 56 days. Each animal was vaccinated against prevalent disease. They were also dewormed using kepromec (Ivermectin) at the rate of 0.1 ml per rabbit subcutaneously and given accaricide bath using Roys' Amitraz 20 at the rate of 1ml in 2 litre water prior to the experiment.

Experimental diet.Four (4) experimental diets were formulated and designated as T_1, T_2, T_3 and T_4 to contain pawpaw leaf meal (PLM) at 0%, 15%, 30% and 45%, respectively (Table 1). Treatment T_1 did not contain PLM thereby serving as the control diet.

2.4 Data collection

Blood samples (5 ml) were drawn from each animal on the last day of the study. The rabbits were bled through the ear marginal vein. The samples were separated into two lots and used for biochemical and haematological

Table 1 Percentage Composition of the experimental diets

Ingredient	T_1 (0%)	T_2 (15%)	T_3 (30%)	T_4 (45%)
Maize	43.00	42.00	40.00	38.00
Wheat offal	13.00	9.00	5.00	00.00
PKC	21.00	13.00	6.00	00.00
Fish meal	1.00	1.00	1.00	1.00
Soya bean meal	18.00	16.00	14.00	12.00
Pawpaw leaf meal	0.00	15.00	30.00	45.00
Bone meal	2.00	2.00	2.00	2.00
Lime stone	1.00	1.00	1.00	1.00
Premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25

studies. An initial 2.5 ml was collected from each sample in labelled sterile universal bottle containing 1.0 mg/ml ethyldiamine tetracetic acid and used for haematological analysis. Another 2.5 ml was collected over anti-coagulant free bottle. The blood was allowed to clot at room temperature and serum separated by centrifuging within three hours of collection. Serum biochemistry and haematological parameters were measured using Beckman Coulter Ac-T10 Laboratory Haematology Blood Analyzer and Bayer DCA 2000+ HbA1c analyzer, respectively. Mean cells haemoglobin (MCH), MCV and mean cell haemoglobin concentrations (MCHC) were calculated.

2.5 Chemical analysis

All feeds and experimental material (PLM) were analyzed for proximate compositions using the method of AOAC (2000). The nitrogen free extract was derived by subtracting the sum of other proximate components, crude protein, crude fat, ash, crude fibre on dry weight basis from 100. Metabolizable energy calculated using the formula; ME = (3.5 × crude protein) + (8.5 × crude fat) + (3.5 × Nitrogen Free Extract) × 10.

2.6 Data analysis

The results were analyzed using the Statistical Package for Social Science Window 17.0. One-way analysis of variance (ANOVA) was employed to determine the means and standard error. Significant differences between the treatment means were separated using the Duncan Multiple New Range Test.

3 Results and disscussion

3.1 Proximate composition

The results of the proximate analysis of experimental diets and PLM are shown in Table 2. The dry matter (DM) range of 90.46–92.91% in this study compared well with the range of 90.95–93.29% reported by Jiwuba et al. (2016a) for weaner rabbits. The crude protein (CP) values in this study ranged between 16.70 and 17.66% which falls within the requirement for growing rabbits (16% CP) as recommended by NRC (1977) and Lebas (2013) and 16–18% as recommended by Fielding (1991). The crude fibre (CF) range of 15.40–16.45% reported in this study failed to follow a particular trend but however compared with the recommended values of 14–18% and 14–16% reported by Gidenne and Lebas (2002) and Mayer (1955) for growing rabbits. Adequate supply of dietary fibre reduces digestive problems, promotes intestinal motility and enhances growth in weaned rabbits. Hence, diets low in fibre promotes an increased incidence of intestinal problems, like enterotoxaemia and lower growth rates (Mayer, 1955; Gidenne and Jehl, 1999). The energy values reported in this study also failed to follow a particular trend. The reported range of 2,550.05–2,604.10 kcal/kg in this study is in agreement with the recommended values of 2,500.00kcal/kg, 2,400–2,800 kcal/kg and 2,500–2,800 Kcal/kg as recommended by NRC (1977), Pond et al. (1995) and Aduku and Olukosi (1990) respectively for growing rabbits. Rabbits however adjust their feed intake as a function of their dietary energy concentration. The ash values of 3.29–8.09% reported in this study followed a particular pattern increasing with increasing levels of PLM; an indication that the mineral requirements of the rabbits were met, since ash is a reflection of the mineral content of a diet. The highest value of ash observed in T_4 may be attributed to high levels of minerals which abound in pawpaw leaves. It is worthy to note that the values for the control diet compared favourably well with the treatment groups. The results of the proximate analysis of the PLM revealed 87.67% DM, 17.30% CP, 12.86% CF, 8.8 8% ash, 0.81% ether extract (EE), 47.82% NFE and 2,348.05 kcal/kg metabolisable energy. The DM content of 87.67% reported in this study is lower than 93.02% reported by Ganzon-Naret (2015), but however compared with 89.60% reported by Nath and Dutta (2016) for the same leaf meal. The crude protein value of 17.30% reported in this study is in agreement with 25.30, 32.60, 21.36 and 13.10% reported by Unigwe

Table 2 Proximate composition of the experimental diets and pawpaw leaf meal

Parameters (%)	Dietary levels (%)				
	T_1	T_2	T_3	T_4	PLM
Dry matter	92.12	90.82	90.46	92.91	87.67
Crude protein	17.44	16.70	16.84	16.70	17.30
Crude fibre	16.20	16.45	15.75	15.40	12.86
Ash	3.29	4.41	5.78	8.09	8.88
Ether extracts	2.30	3.11	2.75	2.65	0.81
Nitrogen free extract	52.89	50.15	49.34	50.07	47.82
Metabolizable energy (kcal/kg)	2,587.05	2,604.10	2,550.05	2,562.20	2,348.05

et al. (2014), Ogbuokiri et al. (2014), Ganzon-Naret (2015) and Nath and Dutta (2016) for the same leaf meal respectively. The differences in the proximate composition of the PLM maybe attributed to season, age, level of development of the leaves, processing methods, soil fertility and location of the study.

The haematological characteristics of growing rabbits fed diets containing pawpaw leaf meal is presented in Table 3. Packed cell volume (PCV), haemoglobin (Hb), mean cell volume (MCV), and white blood cell (WBC) differed significantly (p <0.05) across the treatment groups. Red blood cell (RBC), Mean cell haemoglobin concentration (MCHC) and mean cell haemoglobin (MCH) were statistically (P >0.05) similar across the treatments. PCV was significantly higher among the treatment groups (T_2, T_3 and T_4) in comparison with the control (T_1). T_2 also differed (p <0.05) from other treatments while T_3 and T_4 showed no significant (p >0.05) difference. The PCV range of 38.61–44.83% obtained in this study is in agreement with the normal range of 33.00–50.00% for growing rabbits as reported by Burke (1994). T_1 recorded the lowest value of 38.61% while T_2 recorded the highest value of 44.83%. The improved PCV recorded for the treatment groups may be attributed to the biologically active compounds which may have enhanced nutrient utilization or oxygen carrying capacity of the blood. However, the normal range of PCV reported in this study indicated absence or tolerable level of anti-nutrients. This further indicated that the diets were nourishing, non-toxic and influenced adequate blood supply. The haemoglobin (Hb) however, followed a similar pattern with the PCV, with T_2 having the highest and best value of 14.20 g/dl while T_1 had the lowest value. However, the improved haemoglobin concentration of rabbits on the treatment groups may imply that the dietary proteins were of high quality. Jiwuba (2018) indicated that Pawpaw leaves serve as a major source of papain, a protease used effectively as a natural digestive aid which breaks down protein. This may have enhanced the protein digestibility, availability and utilization by the experimental animals. PCV and Hb are generally used as an indicator of feed toxicity and nutritional status of animals. The values for MCV were higher (p <0.05) in T_3 and T_4 (74.60 and 75.59 fl) respectively than T_1 and T_2. The range of MCV (66.77–75.59 fl) reported in this study fell within the normal range (50 to 75 fl) for apparently healthy rabbit as reported by Burke (1994). The range is however lower than 78.97–88.97 fl reported by Jiwuba et al. (2016a) for weaner rabbits fed Gmelina arborea leaf meal, but highly comparable with range of 68.02 to 74.91

Table 3 Haematological characteristics of growing rabbits fed diets containing pawpaw leaf meal

Parameters (%)	Dietary levels (%)				
	T_1	T_2	T_3	T_4	SEM
Packed cell volume (%)	38.61^c	44.83^a	40.48^b	41.50^b	0.85
Haemoglobin (g/dl)	10.60^c	14.20^a	11.60^b	11.50^b	0.51
Red blood cell (X10$^{12/L}$)	5.34	6.71	5.42	5.49	0.21
Mean cell haemoglobin (pg)	19.80	21.16	21.40	20.95	0.23
Mean cell volume (fl)	72.30^b	66.77^c	74.60^a	75.59^a	3.65
Mean cell haemoglobin conc. (%)	27.14	31.01	28.61	27.16	1.18
White blood cell (X10$^{12/L}$)	4.50^b	8.30^a	9.50^a	8.70^a	0.73

a–c means within a row with different superscripts are significantly (P <0.05) different

Table 4 Serum biochemistry of growing rabbits fed diets containing pawpaw leaf meal

Parameters (%)	Dietary levels (%)				
	T_1	T_2	T_3	T_4	SEM
Total Protein (g/dl)	6.13^b	6.91^{ab}	6.93^{ab}	7.32^a	0.14
Creatinine (mg/dl)	1.72	1.86	1.75	1.71	0.11
AST (iu/l)	33.50	35.50	32.50	30.50	1.77
ALP (iu/l)	115.29	118.02	111.21	113.25	3.14
Sodium (mmol/L)	131.66	133.12	133.66	141.05	3.13
Potassium (mmol/L)	3.47	3.81	3.85	4.46	0.31
Chloride (mmol/l)	96.87	96.96	105.47	107.05	3.38

fl reported by Jiwuba et al. (2016b) for growing rabbits fed *Moringa oleifera* leaf meal. The differences among the treatments maybe attributed to differences in the activity of bone marrow and some haemopoietic factors influencing the capacity of bone marrow to produce red blood cells. The medicinal properties of the pawpaw leaf meal (PLM) maybe due to phytogenic compounds like chamopapain, chitinase, papain, lycopene, pectin, alkaloids (carpaine and pseudocarpaine), saponins, tannins, anthraquinones, cardiac glycosides, glucosinolate (benzylglucosinolate), choline, flavonoids (Ayoola and Adeyeye, 2010; Boshra and Tajul, 2013). These compounds have been reported (Aravind et al., 2013; Boshra and Tajul, 2013) to have antibacterial, anticoagulant, anti- rheumatoid arthritis, antiparasitic, antiviral, antifungal, anti-inflammatory, antihypertensive anti-sickling activities and promote digestive and lung health. These together with the antioxidant properties which remove free radicals from the body may have resulted to the significant improvement of the white blood cells (WBC) among the T_2, T_3 and T_4 groups in comparison with the T_1. Furthermore, the white blood cell count (WBC) ranged from 4.50 to 9.50 × $X10^{12/L}$, the values were within the range of 4.5–11 × 10 g/l reported by RAR (2009) for apparently healthy growing rabbits. These results indicated that the animals were healthy; hence decreased WBC count below the normal range (leukocytopenia) is an indication of allergic conditions and certain parasitism, while elevated values (leukocytosis) indicate the existence of a recent infection (Jiwuba et al., 2016b).

The serum biochemistry of growing rabbits fed diets containing pawpaw leaf meal (Table 4) showed no significant (P >0.05) difference across the groups, except protein. The significantly (P <0.05) higher total protein observed in T_4 in comparison with T_1 maybe attributed to presence papain which may have enhanced protein digestion and utilization among the rabbits. The non-significant (P >0.05) difference observed for creatinine, AST, ALP, sodium, potassium and chloride is an indication that the physiological status of the rabbits were not influenced by the experimental diets. Furthermore, all the parameter measured fell within the normal physiological range for apparently healthy rabbit as reported by Benson and Paul-Murphy (1999), Bradley (2001) and Putwain (2008). This further indicated that the PLM may have supported the proper functioning of the liver, maintained optimal osmolality and enhanced digestion and absorption of the minerals.

4 Conclusions

This study showed a good performance and normal physiological functioning of growing rabbits fed pawpaw leaf meal containing diets. It can be therefore, concluded that dietary supplementation of PLM in male rabbits showed no adverse effect on blood characteristics of the growing rabbits and therefore recommended for the production of healthy rabbits.

References

A.O.A.C. (2000) *Official Methods of Analysis of AOAC INTERNATIONAL (OMA)*. 6th ed., Washington: AOAC.

ADUKU, A.O. and OLUKOSI, J.O. (1990) *Rabbit management in the tropics: production, processing. utilization, marketing economics, practice, research and future prospects.* Abuja FCT: GU Publication..

ARAVIND. G. et al. (2013) Traditional and Medicinal Uses of *Carica papaya*. *Journal of Medicinal Plants Studies*, vol. 1, no. 1, pp. 7–15.

AYOOLA, P.B. and ADEYEYE, A. (2010) Phytochemical and Nutrient Evaluation of *Carica papaya* (Pawpaw) Leaves. *IJRRAS,* vol. 5, no. 3, pp. 325–328.

BENSON, K.G. and PAUL-MURPHY, J. (1999) Clinical pathology of the domestic rabbit. Acquisition and interpretation of samples. *Vet Clin North Am Exot Anim Pract.*, vol. 2, no. 3, pp. 539–551.

BOSHRA, V. and TAJUL, A.Y. (2013) Papaya – An Innovative Raw Material for Food and Pharmaceutical Processing Industry. *Health and the Environment Journal*, vol. 4, no. 1, pp. 68–75.

BRADLEY, T.A. (2001) *What every veterinarian needs to know about rabbits. Zoological Education Network, Lake Worth,* pp. 42–45.

BURKE, J. (1994) Clinical care and medicine of pet rabbit. In: Proceedings of the Michigan Veterinary Conference, pp. 49–77.

CHÁVEZ-QUINTAL, P. et al. (2011) Antifungal Activity in Ethanolic Extracts of *Carica papaya* L. cv. Maradol Leaves and Seeds. *Indian J Microbiol.*, vol. 51, no. 1, pp. 54–60.

EDEH, H.O. (2013) *Physiological response of broiler birds to oral supplementation with aloe vera and neem leave extracts:* MSc. Thesis. Nsukka: University of Nigeria.

GANZON-NARET, E. S. (2015) Effects of incorporated swamp cabbage (*Ipomea aquatica*) and papaya (*Carica papaya*) leaf meals at different dietary levels in order to replace fish meal protein in practical diets for sea bass (*Lates calcarifer*). *ABAH Bioflux*, vol. 7, no. 1, pp. 93–102.

GIDENNE, T. and JEHL, N. (1999) Zootechnical response of the growing rabbit face to a decrease in fiber supply, for diets rich in digestible fibre. In: J.M. Perez (Ed), *8ème J. Rech. Cunicoles Fr., ITAVI éditions, 9–10 Juin, Paris,* pp. 109–113.

GIDENNE, T. and LEBAS, F. (2002) Role of dietary fibre in rabbit nutrition and in digestive troubles prevention. In: *2nd Rabbit Congress of the Americas, Habana City, Cuba, June 19–22, 2002.*

JIWUBA, P.C. et al. (2016a) Haematological and serum biochemical indices of weaner rabbits fed varying levels of dried Gmelina arborea leaf meal. *International Blood Research & Reviews,* no. 6, pp. 1–8.

JIWUBA, P.C. et al. (2016b) Haematological and Serum Biochemical Indices of Growing Rabbits Fed Diets Containing Varying Levels of *Moringa oleifera* Leaf Meal. *British Biotechnology Journal*, vol. 15, no. 2, pp. 1–7.

JIWUBA, P.C. (2018) Effect of pawpaw (*Carica papaya*) leaf meal on productive parameters of growing rabbits. *Agricultural science and technology*, vol. 10, no. 2, pp.102–106. doi: https://doi.org/10.15547/ast.2018.02.022

LEBAS, F. (2013) Feeding strategy for small and medium scale rabbit units. In: *3rd Conference of Asian Rabbit Production Association – Bali Indonesi, 27–29 August 2013,* pp. 1–15.

MAYER, J. (1955) Nutrition of rabbits. In: Tropical Agricultural Series C.T.A. London: Macmillan Education Ltd., pp. 39–50.

NATH, R. and DUTTA, M. (2016) Phytochemical and Proximate Analysis of Papaya (*Carica papaya*) Leaves. *Sch J Agric Vet Sci*, vol. 3, no. 2, pp. 85–87.

NGUYEN, T.T. et al. (2013) Anticancer activity of *Carica papaya*: a review. *Mol Nutr Food Res.*, vol. 57, no. 1, pp. 153–164. doi: https://10.1002/mnfr.201200388

NRC. (1977) *Nutrient requirements of rabbits. National Research council.* Washington: National Academy of Science.

OGBUEWU, I.P. et al. (2014). Responses of pubertal rabbits to dietary supplementation of ginger rhizome powder. *Nig J Anim Prod*, vol. 41, pp. 53– 60.

OGBUOKIRI, U.D.E. et al. (2014) Effect of pawpaw leaf (*Carica papaya*, Linn.) meal on some performance attributes of starter broiler chicks. *Journal of Animal and Veterinary Advances*, no. 4, pp, 826–832.

OTSUKI, N. et al. (2010) Aqueous extract of *Carica papaya* leaves exhibits anti-tumor activity and immunomodulatory effects. *Ethnopharmacol*, no. 127, pp. 760–767.

PATIL, T. et al. (2014) *Carica papaya* Leaf Extracts – An Ethnomedicinal Boon. *International Journal of Pharmacognosy and Phytochemical Research*, vol. 6, no. 2, pp. 260–265.

POND, W.G., CHURCH, D.C. and POND, K.R. (1995) *Basic animal nutrition and feeding.* 4th ed., New York: John Wiley and Sons, pp. 495–504.

PUTWAIN, S. (2008) Clinical pathology updates: haematology and biochemistry of the rabbit. *UK Vet Publications*, vol. 13, no. 6, pp. 75–77.

RESEARCH Animal Resource (RAR). (2009). *Reference values for laboratory animals: Normal Haemotological values.* [Online]. Minneapolis: RAR, University of Minnesota. Retrieved 2019-02-28 from http://www.ahe.umn.edu.rar.refvalues.html

UNIGWE, C.R. et al. (2014) The Nutritive Profile of Sun-Dried Paw-Paw (*Carica papaya*) Leaf Meal and its Effect on the Growth Performance of Broiler Chickens. *Int. J. Pure Appl. Sci. Technol*., vol. 20, no. 2, pp.72–78.

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Short communication

Impact of various moisture regime on selected growth-production characteristics of *Medicago sativa* L. and *Trifolium pratense* L.

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The aim of the experiment was to find out the impact of different moisture regime on selected indicators of the growth and production process of *Medicago sativa* L. cv. Zuzana and *Trifolium pratense* L. cv. Poľana. The pot experiment was carried out at the Department of Grassland Ecosystems and Forage Crops, FAFR SUA in Nitra in 2015. There were evaluated two treatments of irrigation: 1st – irrigation once a week and 2nd – irrigation twice a week with a single dose of 300 ml of water per pot. The results of the experiment showed a positive effect on the height of *Medicago sativa* L. and *Trifolium pratense* L. plants ($p = 0.006$ and $p = 0.316$), the number of stems ($p = 0.001$ and $p = 0.002$), dry phytomass production ($p = 0.016$ and $p = 0.154$) and the quantity of harvest residues of evaluated legume forages ($p = 0.100$ and $p = 0.146$) with a general more visible effect under irrigation twice a week. By comparison of both species, irrigation twice a week was more effective for *Medicago sativa* L. on plant height, number of stems and weight of above-ground phytomass, whereas for *Trifolium pratense* L. only on the weight of harvest residues compared to irrigation once a week.

Keywords: legume forages, growth, production, water deficit

1 Introduction

The amount of available water and the temperature are determined not only for the maximum length of the growing season, but also for the growing spectrum of crops and their final harvest. In growing areas with permanent or periodically occurring drought has water stress becomes an important external factor limiting the effective implementation of plant production. The water deficit itself greatly limits the physiological activity of the plants and with it the associated phytomass formation (Krivosudská and Filová, 2016). Although it is predominantly dependent on meteorological conditions, it is also related to the tolerance and resistance properties of plants to drought (Brestič and Olšovská, 2001). According to several authors (Lichner et al., 1983; Gejguš et al., 1998; Skládanka et al., 2014) are diverse the requirements of legumes for habitat conditions and specified on either with the strong emphasis on the soil or moisture conditions.

Medicago sativa L., the most important leguminous with a beneficial protein composition (Bíro et al. 2006), feels a lack of moisture especially in the early stages of development (Porvaz, 2001). *Medicago sativa* L. drought resistance is quite strong, though not typical signs of dry-loving plants and consumes twice as much water than cereals and about a one third more than *Trifolium pratense* L. (Procházka, 2003). Its transpiration coefficient is in the range 500–900, in dry and warm areas up to 1,600–1,900 (Holúbek et al., 2007). As a result, the yield of *Medicago sativa* L. is very variable and, in general, hay production varies from 3–4 to 10–15 t/ha (Říha, 2009). *Trifolium pratense* L., which approximates by a nutrient content to the nutritional value of a *Medicago sativa* L. (Gálik et al., 2011), requires more humid conditions and needs approximately 500–700 litres of water to produce 1 kg of dry matter. The increased need for water mainly occurs in the period after 1st cut (Abberton and Marshall, 2005).

The aim of the experiment was to find out the impact of the various moisture regimes on the selected growth

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and production characteristics of *Medicago sativa* L. and *Trifolium pratense* L.

2 Material and methods

Pot experiment, realized at the Department of Grassland Ecosystems and Forage Crops of FAFR SUA in Nitra, was established on 21 April 2015 by sowing seeds of *Medicago sativa* L. cv. Zuzana and *Trifolium pratense* L. cv. Poľana into rooting pot. After 5 weeks from sowing (26 May 2015), the emerged plants were torn by to the final number of 5 plants per container. Planting of plants from rooting pots into containers with volume 5 dm^3 (in 3 replicates) took place on 3 June 2015. The chemical composition of the used soil (universal gardening substrate made from a mixture of quality peat, mineral combined fertilizer with trace elements and finely ground dolomitic limestone, the content of combustible substances min. 35%, electrical conductivity max. 1.0 mS/cm, and particles above 10 mm max. 5%) is documented in Table 1. Additional fertilization was not realised. After planting, the plants were irrigated with 300 ml of water per container once a week (on Monday – "Irrigation 1-time") and twice a week (on Monday and Thursday – "Irrigation 2-times") without the possibility of water getting from atmospheric precipitation (rain). Average daily temperatures (°C) during growing season in year 2015 are presented in Table 2.

The measurements and samplings were determined by the "beginning of plant flowering" phenophase. Then, the first measurement and sampling of the phytomass were carried out on 29 July 2015, the second measurement and sampling were approximately 2 months later (on 1 October 2015). In the above-mentioned terms, the height of the plants was measured at each replication, and then the crop was sheared by a scissors at a height of 50 mm.

The harvested phytomass was used to determine the yield in g/m^2. After shearing of above ground phytomass, the number of stems was determined in each container, with subsequent conversion to pieces (pcs) per/m^2.

At the end of the experiment (after the 2nd measurement and shearing), the containers were placed in a drier to kill the plants. Subsequently, the stubble was separated and the roots cleaned from the soil. After drying, their weight – expressed as post-harvest residues in g/m^2 – was determined.

Data were statistically evaluated in STATISTICA 7.1 Complete CZ (StatSoft Inc. 2005) using one-way analysis of variance (ANOVA). To assess differences between variants was used the Fischer´s LSD test at $P \leq 0.05$.

3 Results and discussion

The average height of *Medicago sativa* L. and *Trifolium pratense* L. plants in the individual harvests at the determined level of irrigation is presented in Figure 1. We can state the significant influence of irrigation ($p = 0.006$) on the plants height of *Medicago sativa* L. and insignificant influence of irrigation ($p = 0.316$) on *Trifolium pratense* L. height on the basis of the found values. At irrigation once a week, the plants were on average about 141 mm (*Medicago sativa* L.) and 46 mm (*Trifolium pratense* L.) lower compared with irrigation twice a week. Similarly, there were also differences between cuts, whereas under the conditions of 1 irrigation dose per week (irrigation 1-time) were minimal the differences in the height of the *Medicago sativa* L. plants (16 mm) in favour of the 1st cut. The higher plants were characteristic in the 2nd cut

Table 1 Agrochemical composition of soil used in experiment

N_t	P*	K*	Mg*	Ca*	Na*	C_{ox}	pH
mg/kg						g/kg	
4,067.07	71.53	538.78	716.29	6,720.00	556.52	44.10	6.70

* Content of pure nutrients; used analytical methods: N_t – Kjeldahl method, P, Mg – spectrophotometry, K, Ca, Na – atomic emission spectrometry, C_{ox} – determined by the wet combustion Tyurin method in modification by Nikitin, pH – exchangeable in 1 M KCl

Table 2 Average daily temperatures (°C) during growing season in year 2015

Day	Month							
	III	IV	V	VI	VII	VIII	IX	X
1–10	4.0	6.1	15.8	21.7	24.1	25.3	17.8	13.3
11–20	5.9	10.6	14.4	17.9	22.2	21.4	18.6	8.5
21–31	8.3	11.8	13.9	16.2	22.6	21.3	13.1	8.9
1–31	6.3	10.4	15.1	19.9	23.6	23.5	17.5	10.5

Source: Bulletin Meteorology and Climatology (2015); modified

for the *Trifolium pratense* L. (by 113 mm). Irrigation twice a week was likely to created more suitable conditions, especially for the growth of *Medicago sativa* L. stems. It was particularly visible in the second cut, when *Medicago sativa* L. was significantly higher (by 102 mm) compared to the 1st cut in this irrigation variant. Although *Trifolium pratense* L. is more demanding to moisture than *Medicago sativa* L. (Holúbek et al., 2007; Skládanka et al., 2014), to 2 irrigations per week reacted by increasing of height only in the 1st cut (by 92 mm compared to irrigation once a week). Possible reasons for this condition could be a more favourable temperature conditions for the *Trifolium pratense* L. in the period to the 1st cut. An optimum day temperature for *Trifolium pratense* L. is of

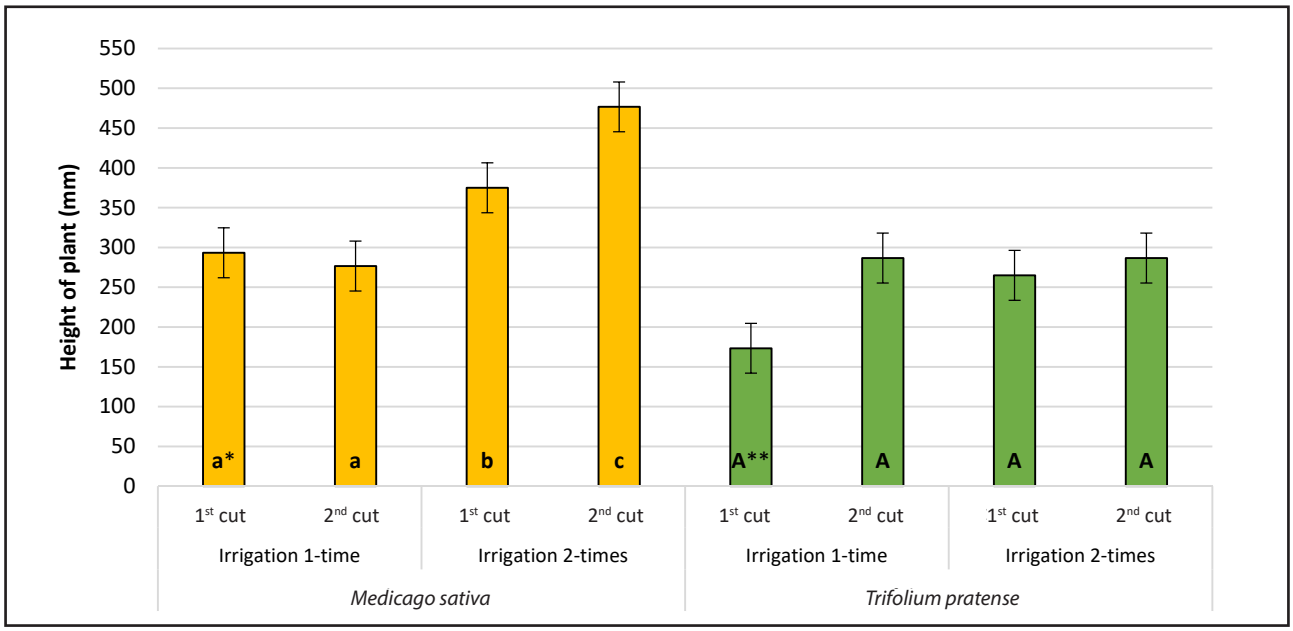

Figure 1 Average plant height (mm) of *Medicago sativa* L. and *Trifolium pratense* L. in cut at individual levels of irrigation
The different letters at an average values mean statistically significant difference (Fischer´s LSD test, $P \leq 0.05$) for *Medicago sativa* L. (*) and *Trifolium pratense* L. (**).The error bars in upper part of columns indicate standard deviation

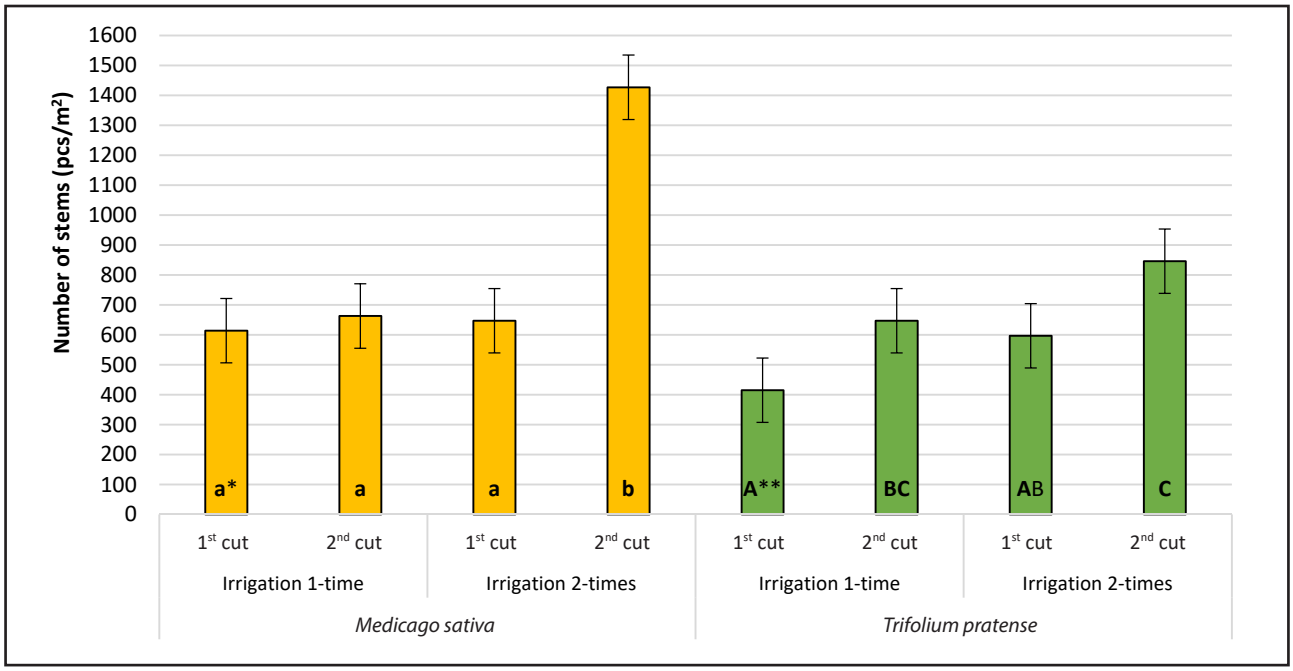

Figure 2 Average number of stems of *Medicago sativa* L. and *Trifolium pratense* L. (pcs/m²) in cut at individual levels of irrigation
The different letters at an average values mean statistically significant difference (Fischer´s LSD test, $P \leq 0.05$) for *Medicago sativa* L. (*) and *Trifolium pratense* L. (**).The error bars in upper part of columns indicate standard deviation

about 18 °C (20–30 °C for *Medicago sativa* L.) according to several authors (Holúbek et al., 2007; Radovic et al., 2009).

One of the criteria for evaluate the condition of perennial forage crops is the coppice density, which depends on the number of plants, respectively stems per unit area (m^2). The values of this indicator are shown in Figure 2.

Similarly to the previous case, there was demonstrated a significant effect of the irrigation ($p = 0.001$ – *Medicago sativa* L. and $p = 0{,}002$ – *Trifolium pratense* L.). *Medicago sativa* L. and *Trifolium pratense* L. had in average 639 stems/m^2 and 531 stems/m^2 under irrigation once a week, respectively. While irrigation twice a week encouraged plant growth and we found out in average 1,037 stems/m^2 (*Medicago sativa* L.) and 722 stems/m^2 (*Trifolium pratense* L.). According to Lichner et al. (1990) *Medicago sativa* L. created a "sparse" coppice and *Trifolium pratense* L. "very sparse" (irrigation 1-time). In the case of irrigation twice a week was found out, on average, the "dense" coppice of *Medicago sativa* L. and the "sparse" coppice of *Trifolium pratense* L. The lack of water was also negatively reflected among the cuts within the different irrigation treatments of the evaluated species of legume forages. In both treatments we found denser crops in the term of the 2nd cut. The number of stems of *Medicago sativa* L. was insignificantly increased by an average of 50 pcs/m^2 (from 614 pcs/m^2 to 664 pcs/m^2) under irrigation once a week, whereas in the case of more frequent irrigation (twice a week), the coppice density has increased on average by 780 pcs/m^2, i.e. approximately 2.2-times (from 647 pcs/m^2 to 1,427 pcs/m^2). Likewise, *Trifolium pratense* L. was denser in the 2nd cut with the minimum difference between the irrigation treatments, but in favour of irrigation twice a week (by 232 pcs/m^2 – Irrigation 1 and 249 pcs/m^2 – Irrigation 2-times).

The part of biological control of perennial forage crops is also finding out the production of above-ground phytomass. According to several authors (Brestič and Olšovská, 2001; Safarnejad, 2008; Farissi et al., 2014) phytomass formation is associated with plant physiological activity, which can be significantly limited by the water deficit itself. From the values presented in Figure 3 can see differences in the amount of above-ground phytomass not only between individual cuts, but also between irrigation treatments ($p = 0.016$ – *Medicago sativa* L.; $p = 0.154$ – *Trifolium pratense* L.). Total production of dry above-ground phytomass of *Medicago sativa* L. was 131.39 g/m^2 under irrigation once a week, while for irrigation twice a week the total yield of phytomass was 233.96 g/m^2. Comparison of the total dry above-ground phytomass weight of evaluated legume forages showed that *Trifolium pratense* L. was more productive (about 99.92 g/m^2 – Irrigation 1-time and 74.40 g/m^2 – Irrigation 2-times) than *Medicago sativa* L. Similarly to the height of plants (Figure 1), in this case, the water deficit was more pronounced in the 2nd cut by reducing the production of *Medicago sativa* L. by 18.55 g/m^2 in comparison with the 1st cut under irrigation once a week (for *Trifolium pratense* L. only about 0.50 g/m^2). This is consistent with the claim of Míka et al. (1997), who state that, despite the deep root system of *Medicago sativa* L. is characterized by

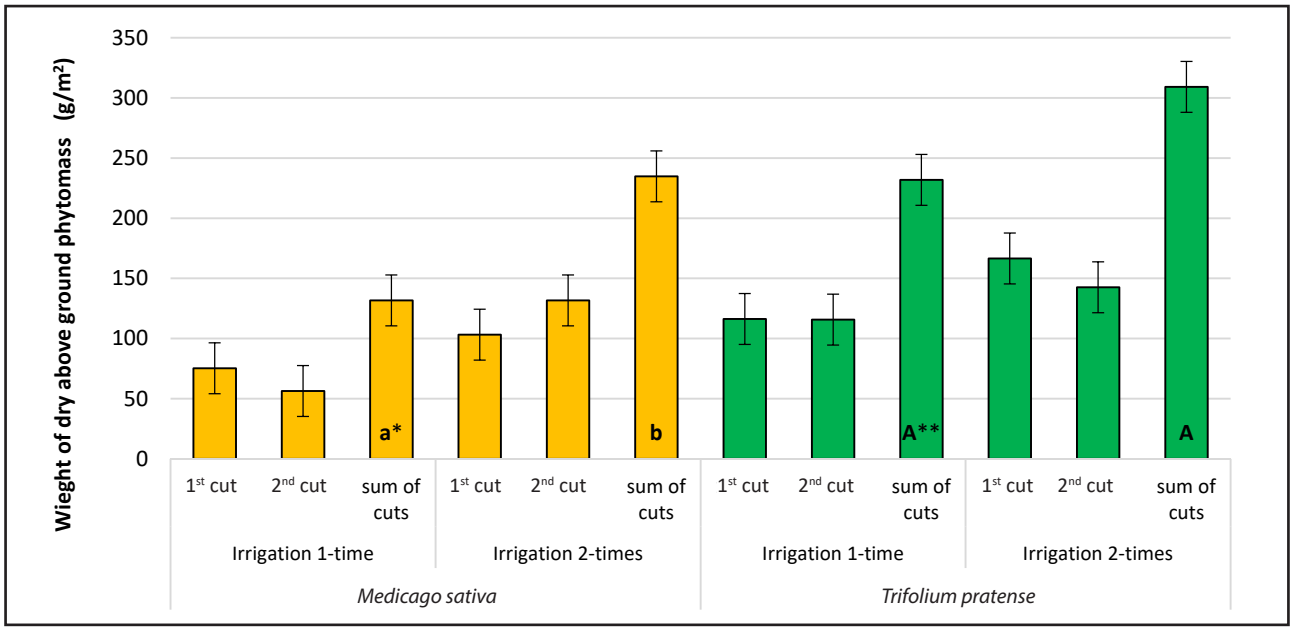

Figure 3 Average weight of dry above ground phytomass of *Medicago sativa* L. and *Trifolium pratense* L. (g/m^2) in cut at individual levels of irrigation

The different letters at an average values mean statistically significant difference (Fischer´s LSD test, $P \leq 0.05$) for *Medicago sativa* L. (*) and *Trifolium pratense* L. (**).The error bars in upper part of columns indicate standard deviation

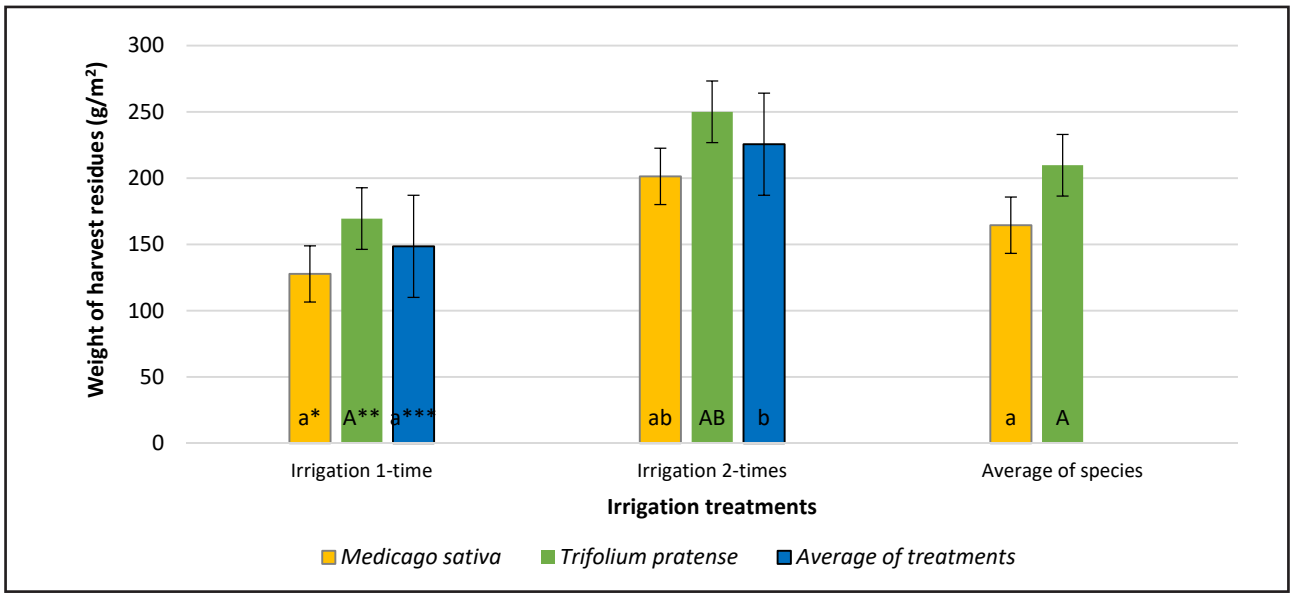

Figure 4 Average weight of harvest residues of *Medicago sativa* L. and *Trifolium pratense* L. (g/m²) at individual levels of irrigation
The different letters at an average values mean statistically significant difference (Fischer´s LSD test, $P \leq 0.05$) for *Medicago sativa* L. (*), *Trifolium pratense* L. (**) and average of treatments (***).The error bars in upper part of columns indicate standard deviation

a decrease in phytomass production in the case of water deficit.

The water stress affects the physiological processes of plants and it is visible not only on the above-ground part of plants but also significantly affects the root system. According to Bláha et al. (2003) and McKenna et al. (2018), in the case of a long-term water deficiency, is inhibited the formation of a root system with initial extension to depth but with limited formation of lateral roots. The root system is greatly reduced, root hairs and growth stop with continued water stress. We can conclude a positive and in average insignificant effect of irrigation on the amount of post-harvest residues (roots + stubble) of *Medicago sativa* L. ($p = 0.100$) and *Trifolium pratense* L. ($p = 0.146$) based on the values presented in Figure 4. There were created 127.75 g/m² and 169.51 g/m² of post-harvest residues for *Medicago sativa* L. and *Trifolium pratense* L. under irrigation once a week, respectively. An increase in the frequency of irrigation (irrigation 2-times) resulted to an increase in post-harvest residues by 73.57 g/m² (*Medicago sativa* L.) and by 80.52 g/m² (*Trifolium pratense* L.). However, this increase for individual species was statistically insignificant ($p = 0.148$). From a general point of view it can be stated that on average a larger amount of post-harvest residues produced a *Trifolium pratense* L. (209.77 g/m²) compared to a *Medicago sativa* L. (164.54 g/m²).

4 Conclusions

Based on the results of the pot experiment, it can be stated that when irrigated twice per week, *Medicago sativa* L. plants were significantly higher, produced a larger number of stems (denser stand) and also was found greater weight of the above-ground phytomass and post-harvest residues in comparison with irrigation once a week. For *Trifolium pratense* L. were these growth-production parameters equally positively influenced by more frequent irrigation (twice per week). From a practical point of view it can be stated that by eliminating water stress, the growth-production process is promoted not only for the water of more demanding species (*Trifolium pratense* L.), but also for relatively more resistant species of clovers (*Medicago sativa* L.). However, these results also need to be verified in natural (field) conditions, where they interact, respectively may interact several factors.

References

ABBERTON, M. T. and MARSHALL, A. H. (2005) Progress in breeding perennial clovers for temperate agriculture: centenary review. In *Journal of Agricultural Science*. vol. 143, no. 2–3, pp. 117–135. https://doi.org/10.1017/S0021859605005101

BÍRO, D., MICHÁLKOVÁ, J. and JURÁČEK, M. (2006) Changes in amino acid composition of *Medicago sativa* during the preservation process. *Animal nutrition 2006: Proteins.* Brno: MZLU, pp. 22–26 (in Slovak).

BLÁHA, L. et al. (2003) *Plant and stress*. Praha: VÚRV, p. 156 (in Czech).

BRESTIČ, M. and OLŠOVSKÁ, K. (2001) *Water stress of plants: causes, consequences, perspectives.* Nitra: SPU (in Slovak).

FARISSI, M. et al. (2014) Water deficit effect on yield and forage quality of *Medicago sativa* populations under field conditions in Marrakesh area. In *Annals of West University of Timisoara, ser. Biology,* vol. 17, no. 1, pp. 1–8.

GÁLIK, B. et al. (2011) *Nutritional characteristics of feeds.* Nitra: SPU (in Slovak).

GEJGUŠ, J. et al. (1998) Impact of climatic factors on phenophases in *Medicago sativa* and *Trifolium pratense* in the East Slovak lowlands. In: *Proceedings of scientific works 14.* Michalovce: OVÚA, pp. 179–185 (in Slovak).

HOLÚBEK, R. et al. (2007) *Fodder crops production – management of the cultivation and use of forage.* Nitra: SUA (in Slovak).

KRIVOSUDSKÁ, E. and FILOVÁ, A. (2016) Physiological responses of genotypes soybean to simulated drought stress. In *Acta fytotechn zootechn*, vol. 19, no. 4, pp. 157–162. https://doi.org/10.15414/afz.2016.19.04.157-162

LICHNER S. et al. (1990) *Instructions for exercises from forage crops production.* Bratislava: Príroda (in Slovak).

LICHNER, S. et al. (1983) *Fodder crops production*. Nitra: VŠP (in Slovak).

McKENNA, P. et al. (2018) The use of red clover (*Trifolium pratense*) in soil fertility-building: A Review. *Field Crops Research.* vol. 221, pp. 38–49. https://doi.org/10.1016/j.fcr.2018.02.006

MÍKA, V. et al. (1997) *Fodders quality.* Praha: Ústav zemědělských a potravinářských informací (in Czech).

PORVAZ, P. (2001) *The production potential of the Medicago sativa L. in the different systems of founding.* Doctoral thesis. Michalovce: OVÚA (in Slovak).

PROCHÁZKA, S. (2003) *Botany. Morphology a physiology of plants.* Brno: Mendel University (in Czech).

RADOVIC, J. et al. (2009) Alfalfa-most important perennial forage legume in animal husbandry. *Biotechnology in Animal Husbandry,* vol. 25, no. 5–6, pp. 465–475. Retrieved 2019-01-25 from http://www.doiserbia.nb.rs/img/doi/1450-9156/2009/1450-91560906465R.pdf

ŘÍHA, P. (2009) Recommended varieties of alfalfa, white clover and perennial ryegrass. *Pícninářské listy*, vol. 16, pp. 5–8 (in Czech).

SAFARNEJAD, A. (2008) Morphological and biochemical response to osmotic stress in alfalfa (*Medicago sativa* L.). *Pak. J. Bot.*, vol. 40, no. 2, pp. 735–746. Retrieved 2019-02-12 from http://www.pakbs.org/pjbot/PDFs/40(2)/PJB40(2)735.pdf

SKLÁDANKA, J. et al. (2014) *Fodder crops production.* Brno: Mendel University (in Czech).

SLOVAK HYDROMETEOROLOGICAL INSTITUTE (2015) *Bulletin Meteorology and Climatology.* Retrieved 2019-01-10 from http://www.shmu.sk/sk/?page=1613 (in Slovak).

STATSOFT, Inc. (2005). *STATISTICA Cz* [Software system for data analysis], version 7.1. Www.StatSoft.Cz

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Original Paper

Growth response, cost benefit, carcass characteristics and organoleptic properties of pigs fed biscuit dough as a replacement for maize

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The growth performance, economic indices, carcass cuts and organoleptic properties of pigs (large white × landrace, $n = 30$) fed biscuit dough as a replacement for maize was examined in a 49-day feeding trial. Weaned pigs were randomly allotted to five dietary groups of six pigs each. A maize-soybean meal based diet served as the control (D1) while diets D2, D3, D4, and D5 had 12.5%, 25.0%, 37.5% and 50.0% biscuit dough respectively as a replacement for maize. The feed conversion ratio (FCR), feed cost per kg, feed cost per kg weight gain, profit per kg weight and economic efficiency of gain (EEG) were significantly influenced (P <0.05) by the dietary treatments. The feed cost per kg and feed cost per kg weight gain reduced linearly from D1-D5, unlike the profit and EEG which increased linearly. The carcass primal cuts including jowl, boston butt, loin, spare rib, belly, ham, trotters, head and picnic shoulder were significantly different (P <0.05). The organoleptic properties (meat colour, texture and overall acceptability) were significantly influenced (P <0.05) by the dietary treatments. In conclusion, feeding up to 37.5% biscuit dough as a replacement for maize improved the feed conversion ratio. Nevertheless, the use of biscuit dough up to 50% would result in reduced cost of production, higher profit margin, economic efficiency of gain, greater loin, belly, ham and overall consumer acceptability. Therefore, the use of biscuit dough by pig farmers would improve pig performance and enhance profitability up to 50.0% replacement for maize.

Keywords: biscuit dough, carcass characteristics, cost, growth performance, sensory properties

1 Introduction

Pig production holds a prominent place in the economy of many developing countries (Steinfeld, 2003), especially where there is no religious taboo or sentiments as it represents one of the means of correcting animal protein shortage because of their fast rate of production and quick return on investment. Pig production, unfortunately, is adversely affected by fluctuations in the supply of good quality feed due to the inadequate local production of feedstuffs, cost of conventional feedstuffs and unavailability of some ingredients year round.

In view of this, research efforts have been geared towards the search for available alternatives (Arowora and Tewe, 2003) including discarded cashew nut meal (Akande et al., 2015), *Jatropha curcas* seed and kernel meal (Oladunjoye et al., 2014; Ojediran et al., 2014), cassava peels, brewer's dried grain, rice husk, pineapple waste, palm kernel meal, sorghum spent grains, among others (Shittu et al., 2016). However, the attendant anti-nutritional factors, cost of processing and seasonal availability (Ojediran et al., 2017) of some of these alternatives have prompted the possibilities of using industrial wastes devoid of anti-nutrients such as biscuit dough.

Biscuit dough is an agro-industrial waste product found in substantial quantities in biscuit producing industries. According to Shittu et al. (2016), biscuit dough, a palatable, high energy feedstuff is made up of biscuit components such as wheat flour, skimmed milk powder, vegetable fat, sugar, salt and flavour material but failed to raise the first time and are yet to undergone baking. Biscuit dough could be an economical feed source for monogastrics, because it is not been used in the bakery for production of cookies. Bakery by-products including biscuit dough has been identified to be one of the non-conventional feed resources, however, biscuit waste has been given attention by various researchers (Eniolorunda

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et al., 2011 and Adeyemo et al., 2013) because it has higher metabolizable energy (ME) than corn grain (NRC, 1998).

Biscuit dough is relatively cheap compared with maize because it is considered a waste product from the bakery. The use of biscuit dough as a replacement for maize has potential to reduce the competition between man and animal for conventional feed sources and also to reduce agro-industrial waste. Meanwhile, information on the potential of biscuit waste as a good ingredient for monogastric animals is available, but, there is dearth of information on the potential of biscuit dough (Shittu et al., 2016).

This study examined the growth response, economic indices, carcass characteristics and organoleptic properties of weaned pigs fed graded levels of biscuit dough as a replacement for maize.

2 Material and methods

2.1 Experimental Site, Birds and Management

The experiment was carried out at the Piggery Unit of the Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso. Ogbomoso lies on longitude 4° 15[1] East of the Greenwich Meridian and Latitude 8° 07[1] North of the equator. The latitude is between 300 and 600 meters above sea level. The mean annual temperature is about 27 °C while that of average rainfall is 1,247mm. The vegetation of the study area is in the derived savannah zone (Ojedapo et al., 2009).

2.2 Experiment pigs and management

Thirty (30) male weaned pigs (Large white × Landrace) weighing 4.09 ±0.21kg were acclimatized and fed with weaner ration of 22% CP for a week prior to the commencement of the experiment. The weaned pigs were randomly allotted to five dietary groups of six pigs each. Each weaned pig was housed individually in a concrete pen. The animals had access to feed and water *ad libitum*. The experiment was conducted for a period of 49 days.

2.3 Collection of test ingredients and the experimental diets

The test ingredient was purchased from a reputable feed mill. It was sun-dried and milled before been mixed with other feed ingredients. Five experimental diets were

Table 1 Gross composition of the experimental diets

Ingredients (%)	D1	D2	D3	D4	D5
Maize	56.00	49.00	42.00	35.00	28.00
Biscuit dough	0.00	7.00	14.00	21.00	28.00
Soybean meal	25.50	24.50	23.50	21.70	19.50
Fish Meal	5.50	4.00	2.80	2.00	1.00
Cassava peel meal	9.00	9.00	9.00	9.00	9.00
Palm kernel cake	2.00	4.50	6.70	9.30	12.50
Limestone	0.60	0.60	0.60	0.60	0.60
DiCalcium phosphate	0.60	0.60	0.60	0.60	0.60
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.05	0.05	0.05	0.05	0.05
Premix	0.20	0.20	0.20	0.20	0.20
Salt	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00
Calculated nutrients					
Metabolizable energy (Kcal/kg)	3,014.04	2,966.49	2,919.54	2,873.39	2,826.83
Crude Protein	21.03	20.70	20.54	20.38	20.02
Ether Extract	3.78	3.72	3.68	3.68	3.68
Crude Fiber	3.77	4.16	4.51	4.87	5.29
Calcium	0.59	0.56	0.54	0.53	0.50
Lysine	1.42	1.30	1.19	1.08	0.96
Methionine	0.45	0.42	0.38	0.35	0.32

formulated with a crude protein content of between 20–21% and metabolizable energy ranging from 2,800–3,000 ME/Kcal/kg in a Maize-soybean meal based diet as shown in Table 1. Biscuit dough was used to replace maize in the control (D1) diet at 12.5%, 25%, 37.5% and 50% in treatments D2, D3, D4 and D5 respectively.

2.4 Data collection

Data were collected on growth parameters including feed intake and weight gain while the feed conversion ratio was calculated. Feed intake was measured individually on a daily basis as the differential between feed offered and feed left while the weight gain was taken weekly using a sensitive electronic scale. The feed to gain ratio was calculated as average feed intake divided by average weight gain. Economic indices were calculated thus (as described by Ojediran et al., 2017):

- Feed cost/kg = sum (quantity of each ingredient × unit cost of each ingredient) % / 100.
- Feed cost per kg weight gain = feed cost/kg × total feed intake (kg)/total weight gain.
- Income per kg weight gain = Selling price/kg × final weight per pig/total weight gain (kg).
- Profit per kg weight gain = Income per kg weight – feed cost/kg weight gain.
- Economic efficiency of growth (EEG) = (profit per kg weight gain/feed cost per kg weight gain) × 100.

At the end of the experiment, 3 pigs were randomly selected from each treatment for carcass evaluation and were starved overnight for 12 hours but allowed access to water *ad libitum*. The pigs were slaughtered by severing the jugular veins. The carcasses were later cut into primal cuts and weighed using Kerro electronic compact scale, model BL30001E. The weights of cut parts were expressed as the percentages of live weight of each pig.

Organoleptic evaluation was carried out on colour, flavour, tenderness, juiciness and overall acceptability of meat samples from each slaughtered pig per replicate. It involved 10 untrained panelists but usual meat consumers. Meat samples were taken from the ham of the carcasses. They were served to the panelist as coded samples. The descriptor was quantified on a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely).

2.5 Statistical analysis

Data were subjected to analysis of variance (ANOVA) in a completely randomized design using SAS (2000) software package and means were separated using Duncan multiple range test of the same package.

3 Results and discussion

The proximate composition and metabolizable energy of biscuit dough were shown in Table 2. The biscuit dough had a dry matter content of 91.02%, 19.40% crude protein, 4.18% crude fibre, 3.87% ether extract, 7.00% ash, 65.55% nitrogen-free extract and 2808.61 Kcal/kg metabolizable energy. The proximate composition of the biscuit dough meal obtained was similar to that reported by Shittu et al. (2016). This may be because of the similar source of the biscuit dough. The biscuit dough had not been baked into biscuit and this may be responsible for the difference in proximate values when compared to the report of Adeyemo et al. (2013) for biscuit waste. Although, Eugene et al. (2008) had earlier reported that heat processing could decrease dry matter, crude protein, ash, ether extract, carbohydrate and energy values.

Table 2 Proximate composition and metabolizable energy of biscuit dough

Parameters (%)	Biscuit dough
Dry matter	91.02
Crude protein	19.40
Crude fibre	4.18
Ether extract	3.87
Ash	7.00
Nitrogen free extract	65.55
Metabolizable energy (Kcal/kg)	2,808.61

The growth parameters of weaned pigs fed graded levels of biscuit dough as a replacement for maize is shown in Table 3. There were no significant differences (P >0.05) in the growth parameters except the feed conversion ratio (FCR). The FCR of pigs fed D3 and D5 were significantly different (P <0.05) while those fed other diets were comparable. Pigs fed D3 had the least FCR while those on D5 had the highest. The non-significant final weight, average daily gain and feed intake suggests that the test ingredients were well tolerated. This corroborates the report of Adeyemo et al. (2013) that biscuit waste has no anti-nutritional factor and could make a good replacement for maize and other cereal grains in livestock feed. This is similar to the observation of Ajasin et al. (2010). Contrary, to the report of Shittu et al. (2016), the pigs had better feed conversion ratio at diet 3 (25% inclusion of biscuit dough), although, comparable to those fed up to diet 4 (37.5% BD).

The economic indices (Table 4) showed that feed cost per kg, feed cost per kg weight gain, profit per kg weight and economic efficiency of gain (EEG) was significantly

influenced (P <0.05) by the dietary treatments. The feed cost per kg and feed cost per kg weight gain reduced linearly from D1–D5, unlike the profit and EEG which increased linearly. The cost of producing a kilogramme of the control diet is highest (₦179.03) while that of D5 is the lowest (₦116.04). The feed cost per kg weight gain is least for pigs on D5 (₦214.25), while those fed D1 (₦353.83) had the highest. Although, values obtained for pigs on D3–D5 were not significantly different (P >0.05). On the contrary, the profit per weight gain ranges from ₦251.23 (D1) to ₦368.80 (D5). Similarly, the economic efficiency of gain was least (72.89) for pigs fed D1 while those fed D5 (152.07) had the highest, although, a significant difference was not observed for pigs fed D3–D5.The result on economic indices is similar to the report of Ojediran et al. (2016) who fortified low crude protein (LCP) diets with lysine. As also observed by Shittu et al. (2016), it costs more to produce the control diet than other diets. Although, a linear decrease across the dietary treatment showed that feed cost per kg reduced as the biscuit dough increases. Hence, reduced cost of feed ingredients will improve production by resource-poor farmers and impact significantly on the will to purchase livestock products by consumers (Nkukwana, 2014). Similarly, feed cost per kg weight gain also had a similar trend as feed cost per kg. Moreover, the profit per kg weight gain and economic efficiency of gain increased with increased biscuit dough. This showed that the use of biscuit dough bears an important and applied implication for a commercial investment in pig production.

Primal cuts of grower pigs fed graded levels of biscuit dough are shown in Table 5. The jowl, boston butt, loin, spare rib, belly, ham, trotters, head and picnic shoulder were significantly different (P <0.05). Pigs fed the control diet (D1) had the highest value (2.88) for jowl while those fed D5 (1.72) had the least similar (P >0.05) to those fed D3. The boston butt of pigs fed D3 (12.75) was highest and comparable to those fed D1 (11.94) and D4 (11.34). The loin and spare rib of pigs fed D4 was highest while those fed D1 had the least. The belly of pigs fed D2–D5 was significantly different from those. The values obtained for Ham ranges from 12.95 (D1) to 14.11 (D2). Pigs fed T1 had the highest value for Trotters (2.33) and head (10.44). The earlier report by Manu et al. (2015) indicated that primal cuts of grower-finisher pigs were not influenced by feeding diets containing biscuit dough. This is also similar to the observation of Adeyemo et al. (2013) when broilers were fed biscuit waste. Conversely, both reports were contrary to the observations in this study.

Table 6 shows the organoleptic properties of grower pigs fed biscuit dough. The analysis of the panellist response showed that the colour, texture and overall acceptability were significantly influenced (P <0.05) but

Table 3 Growth Performance of weaned pigs fed graded levels of biscuit dough

	Dietary treatments					
Parameters	D1	D2	D3	D4	D5	SEM
Initial weight (kg)	4.09	4.11	4.08	4.11	4.06	0.21
Final weight (kg)	23.25	22.92	24.33	23.17	23.00	0.62
Total weight gain (kg)	19.16	18.81	20.26	19.05	18.94	0.46
Average daily gain (kg)	0.39	0.38	0.41	0.39	0.39	0.01
Average daily feed intake (kg)	0.76	0.75	0.77	0.75	0.80	0.01
Feed conversion ratio	1.97^{ab}	1.96^{ab}	1.86^{b}	1.92^{ab}	2.08^{a}	0.03

a–b mean within the row lacking common superscript differ (p <0.05); SEM – standard error of means; kg – kilogram

Table 4 Economics indices of weaned pigs fed graded levels of biscuit dough

	Dietary treatments					
Parameters	D1	D2	D3	D4	D5	SEM
Feed cost/kg (₦)	179.03^{a}	160.17^{b}	144.16^{c}	131.23d	116.04e	5.96
Feed cost/kg wt gain (₦)	353.83^{a}	313.54^{b}	268.37^{c}	252.01^{c}	241.25^{c}	12.00
Income/kg wt gain (₦)	605.17	609.08	600.79	607.56	607.05	4.65
Profit/kg wt gain (₦)	251.34^{c}	295.54^{bc}	332.42^{ab}	355.56^{ab}	365.80^{a}	13.61
EEG	72.89^{b}	94.19^{b}	124.12^{a}	141.30^{a}	152.07^{a}	8.57

a–b mean within the row lacking common superscript differ (p <0.05); SEM – standard error of means; kg – kilogram; wt – weight; ₦ – Naira (₦360 – 1USD$); EEG – economic efficiency of gain

Table 5 Primal cuts of growing pigs fed graded levels of biscuit dough (% live weight)

	Dietary treatments					
Primal cuts	D1	D2	D3	D4	D5	SEM
Jowl	2.88^{a}	2.28^{b}	1.77^{c}	2.38^{b}	1.72^{c}	0.12
Buston butt	11.94^{ab}	10.56^{b}	12.75^{a}	11.34^{ab}	10.57^{b}	0.32
Loin	11.30^{b}	13.04^{ab}	13.21^{ab}	13.60^{a}	13.15^{ab}	0.31
Spare rib	2.39^{c}	3.62^{ab}	2.73^{bc}	3.92^{a}	2.89^{bc}	0.19
Belly	3.57^{b}	4.43^{a}	4.81^{a}	4.65^{a}	4.62^{a}	0.13
Ham	12.95^{b}	14.11^{a}	13.88^{ab}	13.12^{b}	13.36^{ab}	0.16
Trotters	2.33^{a}	1.80^{c}	1.94^{bc}	2.12^{ab}	1.81^{c}	0.06
Head	10.44^{a}	9.32^{b}	9.69^{b}	9.09^{b}	9.47^{b}	0.15
Picnic shoulder	11.01	11.04	9.89	10.48	11.17	0.21

a-b-c Mean within the row lacking common superscript differ (p <0.05); SEM – standard error of means

Table 6 Organoleptic properties of weaned pigs fed graded levels of biscuit dough

	Dietary treatments					
Parameters	D1	D2	D3	D4	D5	SEM
Colour	6.80^{ab}	7.00^{a}	6.00^{ab}	6.40^{ab}	5.60^{b}	0.19
Flavour	5.40	4.40	5.80	4.80	4.20	0.24
Tenderness	5.00	4.40	6.20	6.20	6.40	0.33
Juiciness	5.60	5.00	6.80	5.20	5.00	0.31
Texture	4.60^{b}	5.40^{ab}	7.20^{a}	5.00^{b}	6.0^{ab}	0.32
Overall acceptability	5.80^{b}	6.50^{ab}	7.80^{a}	7.20^{a}	8.00^{a}	0.27

a-b Mean within the row lacking common superscript differ (p <0.05); SEM – standard error of means

they were indifferent (p >0.05) to the flavour, tenderness and juiciness of samples provided. The sensory panel rating for colour (P <0.05) ranged from 5.60–7.00 where D5 (5.60) had the lowest grade and D2 (7.00) had the highest grade for colour. In this study, the colour of the meat had significantly been affected by the different level of biscuit dough in the diets of weaned pigs. There was also a significant difference in the texture and overall acceptability between the various treatment although high score was given to treatment 3 (25% biscuit dough) for texture and overall acceptability. This study shows that meat texture and overall acceptance steadily improved from D1–D3, after which they slightly deteriorated at D4 with a subsequent increase at D5. The organoleptic properties of the weaned pigs fed biscuit dough showed that the meat colour, texture and overall acceptance were influenced. Meat colour is an important property that influences consumer preference. Karthika et al. (2016) reported that colour could be influenced by myoglobin content, composition, muscle physical state and meat structure. Meat overall acceptability reflects the consumers preference. The palatable nature and composition (Shittu et al., 2016) of the biscuit dough may have influenced the overall rating by the panellist because increased acceptability correlates with the linear increase of biscuit dough.

4 Conclusions

Conclusively, the best feed conversion ratio was observed at 25.00% biscuit dough replacement for maize though comparable to those fed 37.50%. Nevertheless, the use of biscuit dough up to 50% would result in reduced cost of production, higher profit margin, economic efficiency of gain, greater loin, belly, ham and overall acceptance.

References

ADEYEMO, G. O. et al. (2013) Effect of dietary biscuit waste on performance and carcass characteristics of broilers. *Food Science and Quality Management*, vol.12, pp. 1–10.

AJASIN, F. O. et al. (2010) The feeding value of biscuit waste as a replacement for Maize in the diet of growing-finishing snails (*Archachatina marginata*). *Journal of America Science*, vol. 6, no. 2, pp. 1–5.

AKANDE, T. O. et al. (2015) Cashew reject meal in diets of laying chickens: nutritional and economic suitability. *Journal of Animal Science and Technology*, vol. 57, 17 p. doi: https://doi.org/10.1186/s40781-015-0051-7

AROWORA K. A.and TEWE, O. O. (2003) Serum biochemical parameters, apparent nutrient utilization and economy of production of growing pigs fed cassava based fibrous diets. Trop. *Journal of Animal Science*, vol. 6, pp. 35–45.

ENIOLORUNDA, O. O. et al. (2011) Performance and carcass characteristics of Yankasa ram fed with variable levels of biscuit waste and *Leucaena leucocephala* based diets. *African Journal of Biotechnology*, vol. 10, no. 22, pp. 4619–4623. doi: https://doi.org/10.5897/AJB10.1539

EUGENE, N. O. et al. (2008) Effect of heat processing on the proximate compositions and energy value of selected Nigerian staple foods from oil producing areas of Niger Delta. *Biokemistri*, vol. 20, no. 1, pp. 1–9. doi: http://dx.doi.org/10.4314/biokem.v20i1.56431

KARTHIKA, S., et al. (2016) Sensory attributes of Namakkal Quail-1 meat. In *International Journal of Advanced Veterinary Science and Technology*, vol. 5, pp. 266–269. doi: https://doi.org/10.23953/cloud.ijavst.174

MANU, F. et al. (2015) Nutrient composition, pest and microbial status and effects of discarded biscuits on the growth performance, carcass characteristics and economic profiles of growing-finishing pigs. *African Journal of Food, Agriculture, Nutrition and Development*, vol. 15, no. 4, pp. 10241–10254.

NATIONAL RESEARCH COUNCIL (NRC, 1998) Nutrient Requirements of Swine. *Nutrient Requirements of Domestic Animals 10th Revised Edition*. Washinton: National Academy of Science, U.S.A. pp. 8–118. doi: https://doi.org/10.17226/6016

NKUKWANA, T. (2014) Poultry production for food security: The South African perspective. *Farmlink Africa*, vol. 4, no. 3, pp. 15–17.

OJEDAPO, L. O. et al. (2009) The influence of strain and sex on carcass on three commercial strains reared in cage system. *Tropical Journal of Animal Science*, vol. 11, pp. 1–7.

OJEDIRAN, T. K. et al. (2014) Nutritional evaluation of processed *Jatropha curcas* kernel meals: Effect on Growth Performance of Broiler Chicks. *Journal of Animal Science* Advances, vol. 4, no. 11, pp. 1110–1121. doi: https://doi.org/10.5455/jasa.20141115115449

OJEDIRAN, T. K. et al. (2016) Response and economic indices of broilers on low crude protein diets fortified with lysine. *American Journal of Experimental Agriculture*, vol. 10, pp. 1–7. DOI: https://doi.org/10.9734/AJEA/2016/22243

OJEDIRAN, T. K. et al. (2017) Growth performance, flock uniformity and economic indices of broiler chickens fed low crude protein diets supplemented with lysine. *Arch. Zootec.,* vol. 66, no. 256, pp. 543–550. DOI: https://doi.org/10.21071/az.v66i256.2770

OLADUNJOYE, I. O. et al. (2014) Effects of inclusion level and length of fermentation on the utilization of Jatropha (*Jatropha curcas*) seed cake by broiler chickens. *International Journal of Current Microbiology and Applied Sciences*, vol. 3, no. 7, pp. 44–54.

SAS (2000). SAS Institute SAS/STAT Guide for personal computers version and Edition Cary North Carolina.

SHITTU, M. D. et al. (2016). Replacement value of biscuit dough for maize on performance and nutrient utilization of broiler chickens. *International Journal of Science, Environment and Technology*, vol. 5, no. 3, pp. 1057–1065.

STEINFELD, H. (2003). Economic constraints on production of animal source foods for nutrition in developing countries. *The Journal of Nutrition*, vol. 133, no. 11, pp. 4054–4061. doi: https://doi.org/10.1093/jn/133.11.4054S

