

## Age at puberty in rabbit – bucks (New Zealand and Chinchilla) fed mistletoe leaf meal by the penile smear techniques in a humid tropical environment

Iso Ekong Iso <sup>1,\*</sup> and Ukam Victor Efut <sup>2</sup>

<sup>1</sup> Department of Animal Science, University of Cross River State, Nigeria.

<sup>2</sup> Department of Agricultural Education, Cross River State College of Education, Akamkpa-Nigeria.

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### Abstract

The study evaluated the effects of mistletoe (*Viscum album* L.) leaf meal inclusions on growth rate, age and body weight at puberty in rabbit-bucks in a humid tropical environment using penile smear technique. Thirty-five rabbit bucks with mean age and body weight of  $72 \pm 5.14$  days and  $680.2g \pm 6.08$  were used for the six months study. Animals were randomized in a  $3 \times 2$  factorial design with three (3) diets levels of mistletoe leaf meal inclusion and two (2) levels of mistletoe on host tree ("A" pear and "B" citrus – (sweet orange). The diets had highly significant ( $P < 0.05$ ) effect on growth and body weight at puberty of the rabbit bucks. The study revealed the overall mean age and live weights at puberty were  $118 \pm 4.48$  days and  $1689 \pm 70.68g$  respectively. Results suggest that puberty was affected more by chronological age than physiological development of the rabbits. However, it also shows that, nutrition can be related to the age at puberty in animal since age of puberty is directly related to body weight when fed balanced diet. The study showed that mistletoe leaf meal is a good feed source for rabbit production. It is therefore recommended that for improved growth, up to 15% mistletoe leaf meal should be included in rabbit diets, especially during dry season of forage scarcity.

**Keywords:** Puberty; Bucks; Penile Smear; Humid tropics.

### 1. Introduction

Puberty at an early age and its detection are important landmarks in the reproductive life of animals (Agiang, 1990). Productivity and growth are one of the major goals in livestock production in achieving the objectives and meeting protein needs of the growing population and industrial sectors that requires livestock products (Fielding, 1991), (Gillespie, 1992). Puberty has been seen as the period of post-natal development, when a male or a female animal is first able to release gametes. In males it is marked by the onset of spermatogenesis and androgen production, while in females, it is the occurrence of the first estrus or ovulation or estrus (Osinowo, 2006), (Egbunike, 1995) viewed puberty in farm animals as the phase of bodily development when the gonads secrete hormones in quantities sufficient to provoke and accelerate growth of the genital organs and the manifestation of secondary sexual characteristics. (Bearden, 1992) reported that reproductive morphology and puberty in bucks is based on a number of behavioural and physiological changes such as body conformation, increased in aggressiveness, sexual desire, rapid growth of the penis, testes and separation of the glans penis from the prepuce for the extension of the penis. Studies showed that many factors affect puberty such as environmental stress, poor health and under nutrition delays onset of puberty with impairment of testicular development (Lebas *et al.*, 1997), (Allen & Lamming, 1961) reported that nutrition is the variable factor as age at puberty is inversely related to plane of nutrition (Iso, 2002), (Iso, 2010). Age at puberty is a major determinant of lifetime reproductive efficiency in farm animals, however, the cost of achieving this varies among animals. Early attainment of puberty in farm animals is one of the important characteristics in achieving optimum reproductive performance in farm animals, (Abe & Abiola, 2008).

\* Corresponding author: Iso Ekong Iso

Mistletoe (*Viscum album* L.) plant is an ever-green broad leaves that grows as an epiphyte on various tree crops in the tropical and temperate regions. The plant has been identified with different names by tribes in Nigeria thus: Yoruba's (Afomo), Efiks in Cross River (Ndot Eyong) and the Ibo's (Awuruse). The plant possessed a well-developed root system called haustaria (sinkers, stems, fruits and leaves) at maturity. The haustaria serves as partial –root for attachment to the host plant where it derived nutrients and photosynthesized (Edmonds *et al.*, 2000). In tropical and temperate regions, the plant has been identified and used in human herbal medicines base on the leaves and the young twigs for tumors, diabetes, hypertension and epilepsy (Duke, 1985). However, animals in the forest feed on the foliage and fruits (Edmonds *et al.*, 2000).

This study was carried out to investigate the effects of mistletoe (*Viscum album* L.) leaf meal inclusions on growth, and weight age at puberty of Rabbit bucks in humid environment. base on its availability all year round.

## 2. Material and Methods

Thirty-five New Zealand white and Chinchilla bucks were used for the study. The mean ( $\pm$ SEM) age and weight of the rabbits at the beginning of the experiment were  $72 \pm 5.14$  days and  $680.29g \pm 6.08$  respectively. There were only one group namely males in a randomized 3 x 2 factorial design. The animals in each group were fed with 18% crude protein formulated diets with mistletoe (*Viscum album* L.) leaf meal inclusion (Table 1). They were fed in the morning between 8.00 and 9.00hrs and in the evening 4.00 and 5.00h. Water was supplied *ad libitum*. Body weight were taken every 7 days from the start of the experiment throughout the 140 days of the study. Weight gain during the experiment was obtained to deduce Average Daily Gain (ADG).

Investigations of puberty was based on the age, body weight when at least single sperm cell was present in preputial swabs. Smears were taken from the prepuce from the 30<sup>th</sup> days of age as literature has it that spermatogenesis begins between 40-50 days in temperate regions, (Bearden and Fuquary 1992).

The smears were taken at an interval of three days by gently and carefully rotating a moistened cotton wool (Johnson and Johnson Limited, slough) in the preputial pouch, between the orifice and the glans penis and smearing the adherent material unto a glass slide. The slide was examined with a microscope with magnification of x 40 without staining to check for the presence of sperm cells (Vandenbergh, 1971). Three consecutive examinations were carried out from each buck and mean age and live weight were regarded as the age and liveweight at puberty (Egbinike, 1979). Bucks with smears containing sperm cells were excluded from subsequent examination. Percentage positive smears sperm cell was calculated for ages and their corresponding live weights at puberty and similarly compared and means separated using Duncan's Multiple Range Test (DMRT) according to (SAS, 1995).

**Table 1** Composition of experimental diet using two host mistletoe (*Viscum album* L.) inclusions

Ingredients	Host (pear) MLM "A"				Host (citrus) MLM "B"		
	1	2	3	4	5	6	7
	(0%)	(5%)	(10%)	(15%)	(5%)	(10%)	(15%)
Maize	49.99	49.99	44.99	39.99	49.99	44.99	39.99
Soya bean meal	31.01	31.01	31.01	31.01	31.01	31.01	31.01
Rice bran	5.00	-	-	-	-	-	-
MLM "A"	-	5.00	10.00	15.00	-	-	-
MLM "B"	-	-	-	-	5.00	10.00	15.00
Wheat offal	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin/ Mineral	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total	100	100	100	100	100	100	100

Vitamin mineral premix per kg contained; vit. A 4,000,000 IU, vit D3, 1,200,000 i.u. 3,200 i.u, k, 800mg, vit B<sub>1</sub>, 800mg, D<sub>2</sub>2,200mg; vit. B<sub>6</sub>, 480mg; vit. B<sub>12</sub>, 4.8mg; Biotin, 12mg, pantothenic acid, 2,800mg; folic acid, 240mg, zn, 20,000mg; co 180mg; iodine, 800mg; selenium, 40mg, Anti-oxidant, 2.400mg; coco<sub>3</sub> 1000mg. MLM "A" mistletoe leaf meal on pear MLM "B" mistletoe leaf meal on citrus (sweet orange).

### 3. Results

**Table 2** Development of puberty in Male rabbits (New Zealand x Chinchilla) as indicated by the age when penile smears first contained sperm

Age at penile smear (Days)	n	Sperm tve(n)	Sperm -ve(n)	Sperm tve (%)
61-70	35	0	35	0
71-80	35	0	35	0
81-90	35	0	35	0
91-100	35	0	35	0
101-110	35	8	27	22 <sup>a</sup>
111-120	27	16	11	59 <sup>b</sup>
121-130	11	11	0	100 <sup>c</sup>

n = 35, a, b, c values along the same vertical column bearing different superscripts differ significantly ( $p < 0.05$ ).

**Table 3** Development of puberty in the 35 males' rabbit (New Zealand x Chinchilla) as indicated by live weight when penile smears first contained sperm

Weight at penile smear (g)	n	Sperm +ve	Sperm -ve(n)	Sperm tve (%)
1001-1200	35	0	35	0
1021-1400	35	0	35	0
1401-1600	35	12	23	34.29 <sup>a</sup>
1601-1800	23	13	10	56.52 <sup>b</sup>
1801-2000	10	10	0	100 <sup>c</sup>

a, b, c, values along the same vertical column bearing different superscripts differ significantly ( $p < 0.05$ )

**Table 4** The distribution of age at puberty (sperm positive penile smear) of the male rabbits (New Zealand x Chinchilla)

n	Age at puberty (Days)	n	Mean sperm $\pm$ SEM	Sperm tve %
35	101-110	8	108 $\pm$ 0.45	23.00 <sup>a</sup>
35	111-120	16	118 $\pm$ 0.60	46.00 <sup>b</sup>
35	121-130	11	127 $\pm$ 0.67	31.00 <sup>c</sup>
23	Mean $\pm$ SEM	35	118 $\pm$ 0.58	

a, b, c, values along the same vertical column bearing different superscripts differ significantly ( $p > 0.05$ )

The ages and live weights at puberty of the rabbit bucks as judged from the time when penile smears first contained sperm cells ranged from 101-130 days (Table 2), and 1401-2000g (Table 3) respectively. A summary of the ages and live weight at puberty in this study are as shown in (Tables, 4,5 and 6). In the study results as shown in table 4, the first group of eight bucks attained puberty earlier than the others at an average age of 108  $\pm$  0.45 days (range 101 – 110 days,  $p < 0.05$ ).

However, the highest number of rabbits sixteen (16) attained puberty. Puberty representing 46% with an average age 118  $\pm$  0.60 days (range 111-120 days) when penile smears first contained sperm cell, while eleven (11) bucks attained puberty at the age of 127  $\pm$  0.57 days.

**Table 5** The distribution of live weight at puberty (sperm positive penile smear) of the male rabbits (New Zealand x Chinchilla)

Weight at puberty(g)	n	mean $\pm$ SEM	Sperm +ve (%)
1401-1600	12	1550 $\pm$ 14.70	34.30a
1601-1800	13	1670 $\pm$ 21.20	37.10b
1801-2000	10	1848 $\pm$ 8.90	28.60c
Mean $\pm$ SEM	35	1689 $\pm$ 19.07(g)	

a,b,c, values along the same vertical column bearing different superscripts differ significantly ( $p < 0.05$ ).

**Table 6** Means of weights of the Rabbit Bucks' Parameter MLM "A" Treatment MLM "B"

	1	2	3	4	5	6	7	SEM
	%	5%	10%	15%	5%	10%	15%	
Initial weight (g/rabbit)	655	695	693	689	654	673	703	-
Final live weight (g/rabbit)	1629 <sup>d</sup>	1997 <sup>b</sup>	1788 <sup>c</sup>	2231 <sup>a</sup>	1673 <sup>cd</sup>	1917 <sup>bc</sup>	1719 <sup>c</sup>	74.56
Weekly weight gain (g /rabbit)	5430 <sup>c</sup>	66.57 <sup>b</sup>	59.60 <sup>b</sup>	74.37 <sup>a</sup>	55.77 <sup>c</sup>	63.90 <sup>b</sup>	57.30 <sup>c</sup>	2.48
Daily weight gain (g/rabbit)	7.76 <sup>c</sup>	9.51 <sup>b</sup>	8.51 <sup>c</sup>	10.62 <sup>c</sup>	7.96 <sup>c</sup>	9.13 <sup>bc</sup>	8.19 <sup>c</sup>	0.35

a, b, c, means with different superscript indicate significant ( $p < 0.05$ ) differences.

The life weight at puberty showed that three major and significant groups were observed, at 1550 $\pm$  14.70, 1670 $\pm$  21.20 and 1848  $\pm$  8.70g (Table 5). Pooled data for the thirty-five (35) bucks rabbit showed an overall mean ( $\pm$  SEM) age and live weight at puberty when penile smears first contained sperm 118  $\pm$  4.48 days and 1689g  $\pm$  70.68 respectively in a humid tropical environment.

#### 4. Discussion

The results in this study showed that the nutritional inclusions of mistletoe leaf meal inclusions have influence on the growth of the bucks compared to the control. The Average Daily Weight Gain (ADWTG) and Final Live Weight (FLWT) of the bucks fed mistletoe leaf meal inclusions were higher than the control diet. Hence, the potential for growth rate of the rabbits can be fully exploited through adequate feeding of mistletoe leaf meal and concentrate feed stuff or mixed feed regime. It has been observed that the diets showed significant effects on body weight at puberty. This agrees with reports by (Lofstedt, 2005) that animals with rapid growth rate before puberty usually attained puberty at a younger age but at a higher body weight than the slower growing animals. The early attainment of puberty of some bucks observed in this study supports the general view that animals reared in the same environment in mixed sexes of males and females attain puberty at an early age (Nelson *et al.*, 1982). In the same vein, this could be as a result of constant stimulus due to the presence of females in other cells of the hutch.

Also, it could be attributed to the genetical superiority of individual buck compared to the rest of the bucks with respect to pubertal development.

In this study, the overall mean of puberty was attained at 118  $\pm$  4.48 days and 1689g  $\pm$  70.68 of mature body weight. The observation in this study suggest that with good nutrition and constant stimulus of both sexes of animal in same environment, puberty could be attained at a reasonable body weight and age.

#### 5. Conclusion

The results of this study suggest that mistletoe leaf meal inclusions up to 15% in rabbit bucks' diet enhanced growth rate and showed a significant ( $p > 0.05$ ) effect physiologically on body weight and age at puberty when sperm first appear using penile smear method. Hence the study has help in defining the age (118 $\pm$  4.48 days) and body weight (1689g  $\pm$  70.68) at puberty in rabbit bucks fed mistletoe leaf inclusions. However, the significant ( $p > 0.05$ ) pubertal

attainment and physiological development in terms of morphology of the testes sizes and weight as observed during the studies may suggest fertility, good quality and quantity sperm production from the bucks

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## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

### *Statement of ethical approval*

The research was conducted in accordance with ethical standards for animal research. All procedures involving the rabbit bucks adhered to the principles of humane treatment and welfare ensuring minimal distress and discomfort. The rabbits were housed in appropriate environmental conditions and received adequate care throughout the study. Informed consent was also obtained for any necessary interventions, and all measures were taken to ensure the validity and reliability of the research findings while prioritizing animal welfare. This research contributes valuable insights into the reproductive physiology of rabbit bucks and supports sustainable practices in animal husbandry.

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