# TESTOSTERONE AND DIHYDROTESTOSTERONE PRODUCTION IN GENETICALLY FURLESS AND FURRED MALE RABBITS AND EFFECTS ON GROWTH

**Jackson R.H., Lukefahr S.D.*, Stanko R.L., Flores D.O.**

Department of Animal and Wildlife Sciences, MSC 228, Texas A&M University, Kingsville, TX 78363, USA
*Corresponding author: s-lukefahr@tamuk.edu

## ABSTRACT

Rabbits are highly prone to heat stress in arid and tropical environments where a tremendous potential exists in lesser developed countries for small-scale meat rabbit projects to alleviate hunger and poverty. One solution may be the breeding of rare, genetically furless rabbits, which is inherited as a simple autosomal recessive gene. To elucidate the physiological mechanism of furless gene expression, our hypothesis reflected the model of androgenic alopecia in humans (i.e., male patterned baldness) involving elevated levels of testosterone (T) and dihydrotestosterone (DHT), which could possibly account for the growth advantage in furless male rabbits as reported ifrom previous trials. The primary aim of this investigation was to determine if a difference exists between furred and furless male rabbits for mean serum T and DHT concentration, and secondly if a relationship exists between T and DHT with growth performance. Data were collected in summers over a 3-year period (2005 to 2007). Matings were made between a homozygous furless parent and a heterozygous furred parent, which produced 35 litters. Ninety-six weaned female and male fryers (range in initial age of 42 to 56 d with mean body weight of 809 g) were randomly assigned to growing cages containing two or three either furless or furred fryers from different litters. Following a 42-d growing period, blood was drawn from males (n=40) to evaluate serum T and DHT levels. Models consisted of the fixed effect of treatment (furred vs. furless) and random effects of batch (i.e., a contemporary group effect of litters born within 1 wk), litter nested within batch, and residual error. Data showed a large range of T values from 23 to 7,534 pg/ml (SD of 1,106 and CV of 102%), as well as for DHT with values ranging from 23 to 1,308 pg/ml (SD of 217 and CV of 94.9%). Results from ANOVA revealed that furred compared to furless male rabbits had similar least-squares means for T of 1,154±364 and 1,020±364 pg/ml (P=0.7560) and likewise for DHT of 235±57 and 221±57 pg/ml (P=0.8563), respectively. Furless male rabbits tended (P=0.1022) to have more rapid ADG than furred male rabbits (23.9 vs. 21.3 g/d) and have numerically (P=0.1409) heavier final body weights (1,913 vs. 1,782 g). The residual correlation between T and DHT was small and negative (r=-0.30; P>0.05). The residual correlation between T and ADG was -0.19 (P>0.05) and between DHT and ADG was -0.45 (P<0.05). Our experimental results do not support the androgenic alopecia model to account for major growth differences between furred and furless male rabbits in our population.

**Key words**: Rabbits, Furless, Growth, Dihydrotestosterone, Testosterone.

## INTRODUCTION

The majority of lesser-developed countries are located in arid and tropical regions. It is generally known that heat-humidity stress is detrimental to rabbit production (Finzi *et al.*, 2000), resulting in reduced feed appetite, decreased libido and fertility, slow growth and development, *etc*. Heat and humidity related problems could potentially be partially alleviated by using genetically furless rabbits. At the last World Rabbit Congress, Rogers *et al.* (2004) presented a preliminary report on performance of furless and furred rabbits. The furless condition is due to an autosomal recessive gene. In a 3-yr experiment, Jackson *et al*. (2006) reported that furless rabbits generally had better heat tolerance than their furred cohorts, and, in terms of growth, naked male rabbits had significantly heavier body

weights by 243 g than furred male rabbits (at approx. 80 d of age); however, a significant difference was not detected between females groups. This difference could be due to increased levels of anabolic steroids in furless male rabbits. In humans, androgenic alopecia (male pattern baldness) is caused by increased levels of anabolic steroids, such as dihydrotestosterone and testosterone (Garrett and Grisham, 1997; Choi *et al.*, 2001). Dihydrotestosterone is an androgen derived from testosterone. Gerrard and Grant (2003) reported that androgens increase muscle protein synthesis.

Our objective was to elucidate the physiological mechanism involving steroid gonadal hormones that may account for previously reported superior growth performance in furless compared to furred male rabbits.

## MATERIALS AND METHODS

### Study site, animals, housing, and diets

The experiment was conducted in summers (June-September) of 2005 to 2007 under subtropical and semiarid conditions at Texas A&M University–Kingsville (2736N, 9757W). Rabbits were housed in commercial pens (76.2 x 76.2 x 45.0 cm) fitted with automatic water valves, and fed *ad libitum* a commercial pelleted diet (Nutrena Rabbit Pellets, Cargill-Nutrena Feeds Division, Minneapolis, MN).

Matings involved one furred (naked), heterozygous parent (Nn) and one furless, homozygous parent (nn), designed to produce an expected 1:1 ratio of furred to furless offspring of equal viability (Jackson *et al.*, 2006). Fryers (n=96) from 35 litters were weaned when the youngest litter in a mating batch reached minimum age of 42 d (range from 42 to 56 d with mean BW of 809 g). On a within-litter basis, furless and furred weanlings were randomly assigned to separate growing pens consisting of only two or three rabbits (Table 1). In 2006, data on male rabbits were lost. Body weights were recorded bi-weekly over a 6-wk period from which ADG was calculated. Ear length and width, core body temperature, and respiration counts were also measured, although not reported in this paper due to limited space.

**Table 1:** Distribution of furless and furred weanling rabbits produced in the experiment

| Year | Batch | No. of litters | Furless | | Furred | | Total |
|---|---|---|---|---|---|---|---|
| | | | Females | Males | Females | Males | |
| 2005 | 1 | 5 | 2 | 5 | 4 | 6 | 17 |
| 2006 | 2 | 5 | 4 | 0 | 6 | 0 | 10 |
| | 3 | 6 | 4 | 5 | 3 | 8 | 20 |
| 2007 | 4 | 5 | 3 | 5 | 4 | 4 | 16 |
| | 5 | 7 | 0 | 3 | 0 | 4 | 7 |
| | 6 | 7 | 5 | 6 | 7 | 8 | 26 |
| | Total | 35 | 8 | 24 | 24 | 30 | 96 |

### Testosterone and dihydrotestosterone analyses

For each age-batch group, following 6-wk trials, jugular vein blood samples were collected from only male rabbits (total of 40 rabbits, 20 furless and 20 furred) by trained personnel via an insertion of a 20-gauge needle mounted to a 12 cc syringe. Blood was placed in 7cc vacutainers and kept on ice until centrifugation (2,000 x g; 20 min). Blood serum was separated from cells and extracted using disposable pipettes placed in 1.7 cc micro-centrifuge tubes, and frozen at -20°C until radio immunoassay (RIA) and ELISA were performed. Serum concentrations of testosterone were quantified by RIA (DSL-4000, Diagnostics Systems Laboratories, Inc., Webster, TX) following double-extraction procedures. Serum samples were thawed and 500 µL were pipetted and placed into glass test tubes with screw caps. Then 4 ml of methyl tertiary-butyl ether (MTBE) was added to each tube and vortexed for 20 min. Tubes were then placed in a dry ice and methanol bath so that the upper organic layer could be separated from frozen serum and placed in test tubes (12 x 75 mm) and NTBE

was evaporated under air. This extraction procedure was repeated and extracted sample pairs were combined. Extracted samples were re-hydrated with 90 µl of PBS-1% BSA (phosphate buffered saline and bovine serum albumin) and 50 µl was then placed in antibody coated test tubes provided by the radioimmunoassay kit. To determine dihydrotestosterone concentration, serum samples were analyzed using ELISA (11-DHH-280, Alpco Diagnostics®, Salem, NH). Following serum extractions, the ELISA was performed according to manufacturer's instructions, where 50 µl of each calibrator, control, and sample were placed into corresponding wells of the well plate. Then 100 µl of enzyme conjugate was placed into each sample and calibrator well. The well plate was then incubated on a plate shaker for 1 h, and subsequently washed using a plate washer (using the washing solution that was enclosed with ELISA). After the wash cycle had been completed, 150 µl of TMB (tetramethylbenzidine) substrate was added to each well and incubated on the plate shaker for an additional 15 min. Then 50 µl of stopping solution was added to each well and the plate was read using a colormetric procedure at 450 nm to determine serum dihydrotestosterone concentration.

**Statistical analysis**

Data were subjected to analysis of variance using General Linear Model procedures (SAS, 2003). Our primary experimental hypothesis states that mean testosterone and dihydrotestosterone levels are significantly different between furless and furred male rabbits, and secondly that a relationship exists between growth rate and testosterone and dihydrotestosterone levels. Employing ANOVA procedures, an F test was used to detect significance at the $P<0.05$ level. The model used for analyses of data was as follows:

$$Y_{ijkl} = \mu + a_i + l_{ij} + T_k + \varepsilon_{ijkl}$$

where $Y_{ijkl}$ is the observed trait value; $\mu$ is the overall mean; $a_i$ is the random effect of the $i^{th}$ age-batch group; $l_{ij}$ is the random effect of the $j^{th}$ litter nested within the $i^{th}$ batch group; $T_k$ is the fixed effect of the $k^{th}$ treatment class (furless vs. furred); and $\varepsilon_{ijkl}$ is the random error (assumed to be normally and independently distributed; 0, $\sigma^2_\epsilon$).

In preliminary data analyses, a random pen nested within treatment source was included in the above model. However, spurious results were obtained due to statistical confounding among random and fixed effects; hence, the pen source which had variances closest to zero was eliminated from the model.

## RESULTS AND DISCUSSION

For testosterone, the data showed a large range of values from 23 to 7,534 pg/ml (SD of 1,106 and CV of 102%), as well as for dihydrotestosterone with values ranging from 23 to 1,308 pg/ml (SD of 217 and CV of 94.9%). Histograms for both hormones are presented in Figures 1 and 2 that clearly illustrate the large spread of values. In addition, data are highly skewed to the right with skewness coefficients of 1.26 and 1.30 for testosterone and dihydrotestosterone, respectively. Two records with values exceeding three standard deviations from the means were eliminated from the data set, and the data were then reanalyzed. In addition, transformation of data to natural logs was performed to compare ANOVA results with those obtained from original, non-transformed data. Despite the variability in the data, results from ANOVA revealed that furred compared to furless male rabbits had similar ($P=0.7560$) least-squares means for testosterone of 1,154±364 and 1,020±364 pg/ml, and likewise ($P=0.8563$) for dihydrotestosterone with means of 235±57 and 221±57 pg/ml, respectively. Upon transformation of data to natural logs and subsequent reanalysis of data, the results were statistically similar. For testosterone and dihydrotestosterone, least-squares geometric means (converted from logs back to original units of measurement) were 696 and 530 pg/ml and for dihydrotestosterone were 147 and 135 pg/ml for furred and furless male rabbits, respectively. Although these means are lower than those from non-transformed values, mean differences were not important between furred and furless male rabbits ($P=0.5616$ and $P=0.8240$ for testosterone and dihydrotestosterone).

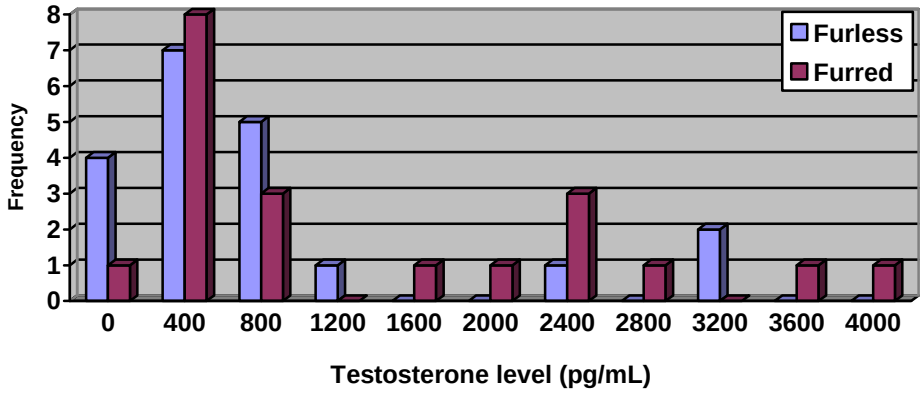


**Figure 1**: Testosterone levels in furless and furred rabbits at approximately 100 days of age

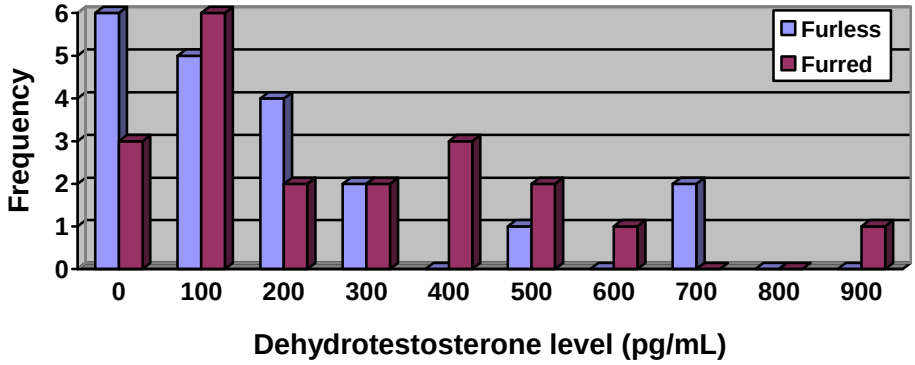


**Figure 2**: Dihydotestosterone levels in furless and furred rabbits at approximately 100 days of age

Lau and Saksena (1979) reported testosterone and dihydrotestosterone means of 1.0 and 0.5 ng/ml in mature male rabbits. Ben Saad and Maurel (2004) reported a testosterone mean of 920 pg/ml in wild male rabbits. These results are consistent with our testosterone mean; however, our mean for dihydro-testosterone is lower, perhaps because we sampled young males. Of relevance, Berger *et al.* (1979) reported that testosterone and dihydrotestosterone levels reach a maxima between 60 and 90 d of age. For growth traits involving the same sample of 40 rabbits used for testosterone and dihydrotestosterone analyses, furless male rabbits tended (P=0.1022) to have more rapid ADG than furred male rabbits (23.9 vs. 21.3 g/d) and numerically (P=0.1409) heavier final body weights (1,913 vs. 1,782). With a larger sample size (n=143 fryers), Jackson *et al.* (2006) previously reported highly significant differences for these growth traits, as well as for higher feed intake and lower core body temperature and respiration rate.

In addition, the residual correlation between serum concentrations of testosterone and dihydrotestosterone was small and negative (r = -0.30; P>0.05). The residual correlation between testosterone and ADG was -0.19 (P>0.05) and between dihydrotestosterone and ADG was -0.45 (P<0.05). Examination of scatter plots between ADG and these androgen hormones neither revealed strong linear nor curvilinear patterns.

According to Ellis *et al.* (2001), androgenic alopecia in human males may be due to a mutation of the androgen receptor gene. In agreement, Zittmann and Nieschlag (2003) reported that both dihydrotestosterone and testosterone impart their effects on gene expression at the level of androgen receptors. As well, other hormones could account for the major growth advantage in our furless compared to furred male rabbits. In rats, Colón *et al.* (2005) demonstrated that growth hormone levels elicited a 4.2-fold increase in dihydrotestosterone concentration, and that insulin-like growth factors-I and II can stimulate increases in dihydrotestosterone concentration.

## CONCLUSIONS

Our experimental results do not support the androgenic alopecia model. Presumably, some other physiological mechanism exists that can explain why furless male rabbits have superior growth performance compared to furred male rabbits in our population, but that does not affect females. Further research is warranted to shed light on this elusive mechanism.

## REFERENCES

Ben Saad M.M., Maurel D.I. 2004. Reciprocal interaction between seasonal testis and thyroid activity in Zembra Island wild rabbits (*Oryctolagus cuniculus*): Effects of castration, thyroidectomy, temperature, and photoperiod. *Biol. Reprod., 70, 1001-009*.

Berger M., Corre M., Jean-Faucher C., De Turckheim M., Veyssiere G., Jean C. 1979. Changes in the testosterone to dihydrotestosterone ratio in plasma and testes of maturing rabbits. *Endocrinology, 104, 1450-1454*.

Choi M.H., Yoo Y.S., Chung B.C. 2001. Biochemical roles of testosterone and epitestosterone to 5α-reductase as indicators of male-pattern baldness. *J. Invest. Dermatol., 116, 57-61*.

Colón E., Svechnikov K.V., Carlsson-Skwirut C., Bang P., Soder O. 2005. Stimulation of steroidogenesis in immature rat leydig cells evoked by interleukin-1α is potentiated by growth hormone and insulin-like growth factors. *Endocrinology, 146, 221-230*.

Ellis J.A., Stebbing M., Harrap S.B. 2001. Polymorphism of the androgen receptor gene is associated with male pattern baldness. *J. Invest. Dermatol., 116, 452-455*.

Finzi A., Ciorba P., Margarit R. 2000. A cooling system able to reduce the relative humidity. *In: Proc.7th World Rabbit Congress, 2000 July, Valencia, Spain.*

Garrett R.H., Grisham C.M. 1997. Principles of Biochemistry with a Human Focus. (1st ed.). *Brooks/Cole Thompson Learning, USA.*

Gerrard D.E., Grant A.L. 2003. Principles of Animal Growth and Development. (1st ed.). *Kendall/Hunt Publishing Company, Dubuque, IA.*

Jackson R., Rogers A.D., Lukefahr S.D. 2006. Inheritance of the naked gene and associations with postweaning performance and thermotolerance characters in fryer rabbits from an $F_2$ generation. *World Rabbit Sci., 14, 147-155*.

Lau I.F., Saksena S.K. 1979. Steroids in the rete testis fluid of fertile male rabbits. *Arch. Androl., 2, 49-52*.

Rogers A.D., Lukefahr S.D., Jackson R. 2004. Effects of the naked gene on postweaning performance and thermotolerance characters in fryer rabbits. *In: Proc. 8th World Rabbit Congress, 2004 September, Puebla, Mexico, http://www.dcam.upv.es/8wrc*.

SAS 2003. SAS/STAT User's Guide (Release 9.1). *SAS Inst. Inc*., Cary, NC, USA.

Zittmann M., Nieschlag E. 2003. The CAG repeat polymorphism with the androgen receptor gene and maleness. *J. Androl., 26, 76-83*.