UNIVERSITI PUTRA MALAYSIA

COMPARISON OF GROWTH AND REPRODUCTIVE RESPONSES OF CALVES TO AN INTRATESTICULAR INJECTION OF ZINC TANNATE AND CASTRATION

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By

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LIST OF ABBREVIATIONS

BC	Burdizzo-castrated
Bo	Zero Count
CMO-BSA	Carboxymethyloxime-Bovine Serum Albumin
cpm	count per minute
CT	Control
h	hour
HS	Haemorrhagic Septicaemia
kg	kilogram
KK	Kedah-Kelantan
LSC	Liquid Scintillation Counter
ml	mililitre
MW	Molecular Weight
ng	nanogram
NSB	Non Specific Binding
PGB	Phosphate Gelatin Buffer
PKC	Palm Kernel Cake
PPO	Diphenyloxazole
RIA	Radioimmunoassay
SC	Scrotal Circumference
ST	Seminiferous Tubules
TC	Total Count
UPM	Universiti Pertanian Malaysia
ZT	Zinc Tannate Injected

μCi	microcurie
μl	microlitre

Abstract of thesis submitted to the Senate of Universiti Pertanian Malaysia in fulfilment of the requirements for the degree of Master of Science.

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APRIL 1992

Chairman : Professor M.R. Jainudeen

Faculty : Veterinary Medicine and Animal Science

A study was carried out to demonstrate that intratesticular injection of 6% zinc tannate to indigenous Kedah-Kelantan (KK) calves will result in better growth rates and superior carcass characteristics than calves castrated with burdizzo and that it would selectively destroy the spermatogenic cells and not the interstitial cells (Leydig cells). Thirty KK male calves aged 9-10 months old were selected and assigned to three treatment groups with 10 animals in each group. Animals in group 1 were castrated with a burdizzo (BC), group 2 animals were injected intratesticularly with 6% zinc tannate (ZT) and intact animals in group 3 as a control group (CT). The dose of 6% zinc tannate injected was based on scrotal circumference. All the animals were slaughtered after 368 days post-treatment.

There was no significant difference ($P>0.05$) in average daily weight gain of animals injected with zinc tannate (0.38 $\pm$ 0.007 kg), castrated (0.34 $\pm$ 0.07 kg) and intact (0.43 $\pm$ 0.007 kg) animals; but intact animals showed higher ($P<0.05$) gain than the castrated animals. Zinc tannate animals had lower daily feed intake and better feed conversion ratio than castrated and intact animals. No significant differences were observed in chilled dressing percent, muscle and bone percent and muscle:bone ratio of the three treatment groups but a higher percentage of fat was found in castrated animals.

This study revealed that zinc tannate is an irreversible chemical sterilant. Histological section of testes indicated complete destruction of spermatogenic cells with intact Leydig cells in the interstitium. No spermatozoa were observed in the ejaculates and the animals produced high levels of testosterone hormone.

It is concluded that zinc tannate could be used as a substitute for burdizzo castration and as an alternate procedure for the preparation of teaser bulls.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia bagi memenuhi syarat-syarat untuk Ijazah Master Sains.

PERBANDINGAN TINDAKBALAS TUMBESARAN DAN PEMBIAKAN ANAK LEMBU TERHADAP SUNTIKAN ZINK TANNAT INTRATESTIKULAR DAN PENGEMILIAN

Oleh

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Satu kajian telah dijalankan untuk menunjukkan bahawa suntikan 6% zink tannat secara intratestis terhadap anak-anak lembu tempatan Kedah-Kelantan (KK) akan menjadikan kadar pertumbuhan dan ciri karkas lebih baik daripada anak-anak lembu yang dikembiri menggunakan burdizzo. Bahan kimia ini juga akan hanya memusnahkan sel-sel spermatogenik dan tidak sel-sel interstis (sel Leydig). Tiga puluh anak lembu jantan KK berumur di antara 9-10 bulan dipilih dan diagih-agihkan kepada 3 kumpulan rawatan yang mempunyai 10 ekor lembu bagi tiap-tiap satu kumpulan. Lembu-lembu dalam kumpulan 1 dikembiri dengan burdizzo (BC), kumpulan 2 disuntik secara intratestis dengan 6% zink tannat (ZT) dan lembu dalam kumpulan 3 tidak diberikan apa-apa rawatan (CT). Dos 6% zink tannat yang disuntik berdasarkan kepada

lilitan skrotum. Kesemua lembu-lembu ini disembelih selepas 368 hari rawatan.

Tidak terdapat perbezaan yang ketara ($P>0.05$) dalam purata pertambahan berat badan lembu-lembu ZT (0.38 $\pm$ 0.007 kg) dengan lembu-lembu BC (0.34 $\pm$ 0.07 kg) dan CT (0.43 $\pm$ 0.007 kg). Lembu-lembu CT menunjukkan pertumbuhan yang tinggi ($P<0.05$) daripada lembu-lembu BC. Lembu-lembu ZT menunjukkan pengambilan makanan harian yang rendah dan nisbah pertukaran makanan yang lebih baik daripada lembu-lembu BC dan CT. Tidak ada perbezaan yang ketara pada peratus lapah, peratus daging dan tulang dan nisbah daging:tulang pada ketiga-tiga kumpulan tetapi peratus lemak lebih tinggi pada lembu BC.

Kajian ini menunjukkan bahawa zink tannat ialah bahan kimia sterilan yang tidak berbalik. Hirisan histologi testis menunjukkan pemusnahan sel-sel spermatogenik tanpa membabitkan sel-sel Leydig dalam interstitis. Pancutan semen tidak mengandungi spermatozoa dan lembu-lembu menghasilkan aras hormon testosteron yang tinggi.

Kesimpulan dari kajian ini ialah zink tannat boleh digunakan sebagai ganti kepada kembirian secara burdizzo dan sebagai satu cara bagi penyediaan lembu penggiat.

CHAPTER I

INTRODUCTION

There is a need to upgrade the productivity of indigenous cattle in Malaysia to meet the national objective of attaining self-sufficiency in beef besides importation of exotic breeds.

The Kedah-Kelantan (KK) cattle, indigenous to Malaysia and comprising about 80% of the total cattle population, represent an important and major economic resource for beef cattle production in Malaysia. This breed is relatively small in size with a lower average daily gain compared with Brahman and Brahman cross cattle.

Palm kernel cake, a by-product of the palm oil industry is used extensively in the feedlotting of beef. Palm kernel cake supplies both protein and energy and with its high fibre content is a suitable feed for ruminants. The trend towards leaner beef carcasses and more efficient feedlot performance has focussed attention on the effect of sex condition on feedlot efficiency, age at castration and carcass quality and meatiness. Present day consumer demand the production of edible lean meat with a minimum of fat.

The effect of castration is to modify the secondary sex characteristics of an animal, resulting in a differing carcass characteristics where the amount and distribution of fat in the carcass is altered. Animals can be castrated either physically or chemically. An animal castrated by a physical method will lose its spermatogenic and androgenic functions as compared with animal castrated chemically to destroy the spermatogenic function only.

Therefore, the study was conducted by injecting zinc tannate 6% solution intratesticularly to Kedah-Kelantan cattle to test the following hypotheses :

1. A 6% zinc tannate solution injected intratesticularly affects the spermatogenic function and not the endocrine function of the bull testis.

2. The growth performance and carcass characteristics of zinc tannate treated animals and entire bulls are similar.

CHAPTER II

LITERATURE REVIEW

Growth

Growth is often measured as liveweight gain over time. Liveweight is a commercially useful measure of growth only if it is highly predictive of the amount of desirable edible product (Berg and Butterfield, 1976).

There are differences in relative rate of growth and feed utilisation among breeds of different mature size. Animals of large mature size (Holstein) gained more rapidly on less feed than animals of smaller mature size (Hereford) (Kidwell and McCormick, 1959). Such differences may be due to length of feeding period, weight, manner of computing feed efficiency, age, state of maturity and/or degree of finish at slaughter. The indigeneous Kedah-Kelantan cattle of Malaysia, are relatively small in size with a lower average daily gain compared with Brahman and Brahman cross cattle (Clayton, 1983).

Sex and age of the animals have an influence on growth rate through differences in testosterone levels of the animal (Gortsema *et al.*, 1974). Growth rate of bulls and steers were similar at preweaning but there were significant differences in growth rate at postweaning. This difference was due to the change of testosterone level before and after puberty (Bailey *et al.*, 1966; Gortsema *et al.*, 1974). Before puberty, the level of testosterone in bulls and steers was not significantly different but were significantly different at puberty.

Puberty

Puberty may be defined as the time when a bull is capable of participating in reproduction (Amann, 1983) or when a male produces sufficient spermatozoa to impregnate a female (Amann and Schanbacher, 1983). Puberty may also be defined as the time when testes start to produce sperm (Oxender, 1974). As the onset of spermatogenesis cannot be easily measured, puberty in bulls has been defined as the age at which the first ejaculate contained a minimum of 50×10^6 spermatozoa with more than 10% showing progressive motility (Barber and Almquist, 1975; Oyedipe *et al.*, 1981).

Puberty in bulls is characterised by an episodic LH secretion, a gradual increase in blood testosterone, rapid testicular growth and the initiation of spermatogenesis (Lacroix and Pelletier, 1979; Schanbacher, 1981; Amann and Schanbacher, 1983). Puberty is not synonymous with sexual maturity or adult status which occur months or years later (Amann, 1981).

Hormonal Control of Secondary Sexual Characters and Puberty

Normally growth and development of the reproductive tract and gonads are gradual processes and is comparatively slow before puberty. These organs do not exhibit any functional activity until puberty is reached. The progressive development of the reproductive organs is dependant on age and body weight and to a certain extent on heredity (Sane and Despande, 1982).

Profound changes occur in hypothalamic, pituitary and gonadal functions at puberty (Amann, 1983). The hypothalamus is believed to play a key role in initiating puberty because the pituitary gland, gonads and steroid-dependent target tissues each are competent and ready to respond to their respective tropic hormones

prior to puberty (Davidson, 1974). Puberty occurs when an animal becomes desensitised to the feedback inhibition imposed on the hypothalamic-pituitary complex by gonadal steroids (Ramirez and McCaan, 1963). Basically, puberty is the result of a gradual adjustment between increasing gonadotrophic activity and the ability of the gonads to simultaneously assume steroidogenesis and gametogenesis (Hafez, 1987).

Age at Puberty and Sexual Maturity

Numerous environmental factors, both internal and external influence the central nervous system to modulate the endocrine system and, thereby, alter the chronological age at which a given animal reaches puberty (Amann and Schanbacher, 1983). Several factors were found to influence the onset of puberty and sexual maturity, such as genetics, breed and season of birth, nutritional, bodyweight, climate, hormone and social factors (Amann and Schanbacher, 1983). Attainment of puberty does not qualify the male for service since sexual maturity is reached sometime later (Amann, 1981; Sane and Deshpande, 1982). Bull calves of exotic breeds usually reach puberty by 12 months of age (Hafez, 1987). Tomar, as cited by Sane and Despande (1982) stated that zebu calves usually attain puberty at 16 - 18 months of age. It was concluded that the indigenous breeds of bulls (Bos indicus) attained puberty later than exotic breeds (Bos taurus) (Oyedipe *et al.*, 1981).

Prepubertal Period

The prepubertal period culminates with release of the first spermatozoa from the seminiferous tubule (Amann, 1983). It starts at 10 to 12 weeks for a well-fed Holstein bull which was characterised by profound changes of hypothalamic, pituitary and gonadal function that culminate in puberty (Amann, 1983). Castration of prepubertal males results in suppression of development.

Testes

The reproductive system of the male can be divided into three parts: (1) The testes or male gonads (primary sex organ), which lie outside the abdomen within the scrotum; (2) accessory sex organs; and (3) penis (external genitalia or organs of copulation).

Histology of Testes

The testes can be considered to have three functional compartments. The interstitial tissue compartments which include the Leydig cells, surrounds the seminiferous tubules and bathes the seminiferous tubules with a fluid rich in testosterone. Leydig cells are large, polyhedral cells occurring in clusters and are found in association with the lymphatics and blood capillaries in the interstitial compartments of the testis (Setchell, 1978). Leydig cells are active in the early embryo, regress during later development, and reactivate during the onset of puberty (Hooker, 1970).

The other two compartments are within the seminiferous tubules : A basal compartment that contains spermatogonia which

divide by mitosis and an adluminal compartment that contains a special isolated environment in which spermatocytes undergo meiosis and spermatids differentiate into spermatozoa. The two compartments are separated by junctional complexes that function as the major component of the blood-testis barrier (Fawcett *et al.*, 1970). Sertoli cells, which rest upon the lamina propria of seminiferous tubules provide the only communication link across the blood-testis barrier (Amann and Schanbacher, 1983). Sertoli cells have a pivotal role in the hormonal control of spermatogenesis. Because of the blood-testis barrier, the action of follicle stimulating hormone (FSH) on spermatogenesis is indirect, via the Sertoli cells, rather than directly on the germ cells (Schanbacher, 1979).

Functions of Testes

The two basic function of the testes are : (a) secretion of testosterone and other hormones through the process of steroidogenesis; and (b) production of spermatozoa through the process of spermatogenesis. These two functions occur in the Leydig cells and seminiferous tubules respectively. Steroid secretion and spermatozoa production are dependent on the separate actions of the two gonadotropins, luteinizing hormone (LH) and FSH but both processes are closely related in that adequate levels of testosterone are essential for the normal production and maturation of spermatozoa (Amann and Schanbacher, 1983).

Testicular Hormone

Hormones produced by the testes are called androgens. Androgens have a specific function in the maintenance of the testes, reproductive tract and secondary sexual characteristics of the male. Testosterone and androstenedione are the primary androgens produced by the testes. Testosterone is necessary for spermatogenesis, continued function of the reproductive tract glands, growth of the testes and scrotum, growth and distribution of body hair, body configuration and male aggresive behaviour. Numerous investigators have reported that bovine spermatogenesis is associated with increased serum concentration of testosterone (Rawlings *et al.*, 1972; Secchiari *et al.*, 1976; Schanbacher, 1979).

Metabolically, androgens stimulate protein metabolism and induce a positive nitrogen balance which can increase the rate of growth. Castration of prepuberal animals results in the failure of the reproductive tract to mature and failure of secondary sexual characteristics to appear. Castration after puberty causes regression and atrophy of the structures of the reproductive tract and secondary characteristics (Oxender, 1974).

Leydig cells in bulls increase rapidly in size from 3.5 to 6.5 months of age (Hooker, 1970). At 6.5 months, many of the Leydig cells are secretory and at 28 months nearly all Leydig cells are secretory which indicates that the androgen secreting cells in the bull testes become progressively more active during the time sperm production is also increasing.

Plasma concentration of testosterone gradually increased during the first month of life and showed a sharp increase between 4 to 6 months of age (Lindner, 1969). This increment probably represents the rapid endocrine maturity of calves. There is a large variation in

androgen secretion among bulls within ages with testosterone concentration in peripheral plasma increasing erratically from birth to 11 months of age, dropping abruptly at 12 months (Rawling *et al.*, 1972). But in a zebu (Kedah-Kelantan) bulls, after 12 months of age, plasma testosterone increased rapidly (Ismaya, 1987). In contrast, serum concentration of testosterone was low in bull calves at birth, remained low (< 0.6 ng/ml) until 5 months of age, and increased to peak concentration of 10.14 ng/ml at 11 months of age (Schanbacher, 1979). At 6.5 months, *Bos taurus* bulls showed a substantial rise in the plasma concentration of testosterone reaching 1.48 ng/ml (Secchiari *et al.*, 1976). Testosterone concentration increased linearly and significantly from 2 to 6 months of age but remained at a steady level around 3 ng/ml until 12 months of age (Bedair and Thibier, 1979). They also found that the level of testosterone in the castrated bull was less than 0.03 ng/ml.

There is an episodic pattern of testosterone secretion during a 24 h period in bull (Gombe *et al.*, 1973; Smith *et al.*, 1973), ram (Katongole *et al.*, 1974) and man (Rowe *et al.*, 1974). Peripheral testosterone concentration have also been described as showing an oscillatory pattern between basal and maximal values throughout the 24 h period after 6.5 months of age (Secchiari *et al.*, 1976). Other studies reported that testosterone concentration increased linearly between 8 to 20 months of age in Brahman bulls (Field *et al.*, 1982), between 1 to 2 years of age in Brahman and Sahiwal cross bulls (Wildeus *et al.*, 1984) and in young bull calves (Thun *et al.*, 1980; Amann and Walker, 1983). In sexually mature 15 months old beef bulls, basal serum testosterone concentration was 0.8 $\pm$ 0.1 ng/ml interspersed with pulses of 11.3 $\pm$ 0.7 ng/ml occured once every 7.1 $\pm$ 1.3 h (Melson *et al.*, 1986). This discrepancy might be due to species

and breed differences, seasonal variation, nutritional factors and differences in frequency of sampling and number of animals (Lunstra *et al.*, 1978; Lacroix and Pelletier, 1979). Also, season of birth was found to influence testosterone profiles (Amann and Walker, 1983). However, there is no evidence of seasonal effect, as bulls born at different time showed a similar testosterone pattern during development (Secchiari *et al.*, 1979). Restraint of untrained bulls caused a marked decrease in the blood levels of LH as well as of testosterone (Nancy *et al.*, 1977). Restraint also significantly ($P<0.01$) depressed mean 24 h levels of testosterone from 3.6 $\pm$ 0.5 to 1.6 $\pm$ 0.20 ng/ml, the number of episodic peaks from 3.4 $\pm$ 0.3 to 1.4 $\pm$ 0.2 (Post, 1978).

The testosterone level of bull calves that had been injected intratesticularly with 3 ml of 6% zinc tannate solution increased from 1.41 ng/ml prior to treatment to 5.68 ng/ml by 24 weeks post-treatment (Anon, 1983). The levels in the intact bull calves and castrated bull calves were 6.36 and 0.20 ng/ml at that time. When bull calves were injected with 5 ml of a 6% solution of zinc tannate, the plasma testosterone before treatment fell considerably during the 8 weeks following treatment (3.2 ng/ml) but recovered to a mean value of 2.05 ng/ml at 18 weeks post-treatment . In order to optimise testosterone production the authors conclude that there is an advantage in using a dose of 3 ml 6% zinc tannate in animals whose circumference lies between 16.0 cm and 17.0 cm.

Spermatogenesis

Spermatogenesis is the process by which a bull produces ~ 140 spermatozoa per g of testicular parenchyma for many years following puberty (Amann, 1983). It is a process that involves both production