

Reproductive and growth performance of Friesian crossbred bulls (Prestasi pembiakan dan pertumbuhan lembu jantan kacukan Friesian)

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Key words: growth, body weight, testosterone, semen, spermatogenesis, puberty

Abstrak

Prestasi pertumbuhan dan pembiakan meliputi umur ketika permulaan spermatogenesis dan baligh pada lembu jantan kacukan Friesian telah dikaji. Keputusan kajian ini boleh digunakan untuk menilai lembu jantan yang akan digunakan untuk pembiakan. Pada umur 36 bulan, berat badan (BW), ketinggian badan (BH), panjang badan (BL) dan lilitan skrotum (SC) lembu jantan masing-masingnya ialah 493.44 kg, 133.68 cm, 160.33 cm dan 35.28 cm. Kajian ini menunjukkan umur lembu berhubung kait secara ketara dengan BW ($r = 92$, $p < 0.01$), BH ($r = 90$, $p < 0.01$), BL ($r = 90$, $p < 0.01$) dan SC ($r = 0.89$, $p < 0.001$). SC juga berhubung kait secara ketara dengan BW ($r = 93$, $p < 0.01$) dan BH ($r = 91$, $p < 0.01$). BW, BH, BL dan SC yang ditunjukkan pada berbagai-bagai peringkat umur dalam kajian ini juga boleh digunakan untuk menilai lembu jantan kacukan Friesian yang akan digunakan untuk pembiakan. Persamaan kuadratik yang dihasilkan daripada ukuran BW, BH dan umur boleh digunakan untuk meramalkan SC jika umur, BW atau BH diketahui. Aras testosteron plasma teraruh-GnRH telah didapati meningkat pada umur 4–24 bulan dan selepas itu mendatar sehingga umur 36 bulan. Pada jenis lembu yang dikaji, walaupun spermatogenesis bermula pada umur 9 bulan, anak lembu jantan mencapai baligh pada umur 11 bulan.

Abstract

This study investigates the growth and reproductive performance which includes the onset of spermatogenesis and puberty of Friesian crossbred bulls, the results of which may become useful in the evaluation of bulls for breeding soundness. At 36 months of age, the bulls attained a mean body weight (BW) of 493.44 kg with mean height at withers (BH), body length (BL) and scrotal circumference (SC) of 133.68 cm, 160.33 cm and 35.28 cm, respectively. The results showed that age was significantly correlated with BW ($r = 92$, $p < 0.01$), BH ($r = 90$, $p < 0.01$), BL ($r = 90$, $p < 0.01$) and SC ($r = 0.89$, $p < 0.01$). The SC was also found to significantly correlate with BW ($r = 93$, $p < 0.01$) and BH ($r = 91$, $p < 0.01$). The BW, BH, BL and SC norms distributed with age in this study would be useful in the evaluation of the Friesian crossbred bulls for breeding soundness. Within the limits of this study for age, BW, BH and breed, from the quadratic equations shown in this paper, it is also possible to predict the SC, which is highly related to fertility, if the age, BW or BH of a bull is known. The

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GnRH-induced plasma testosterone level was found to increase as the bulls grew older from 4–24 months, then it plateaued up to 36 months. Though spermatogenesis began at the age of 9 months in this breed type, the bull calves were found to reach puberty only at the age of 11 months.

Introduction

The demand for beef and milk as a source of protein is expected to continue to rise with the increase in population. In Malaysia, ruminant production has not been able to meet the local demands for beef and milk, and presently could meet only 17.69% of beef and 5.04% of milk demand for Peninsula Malaysia. The cattle population of Malaysia in 1996 was 647 070 of which 57 479 heads were dairy animals (DVS 1996). The self-sufficiency level of beef and milk can be increased mainly by increasing the cattle population. To achieve this objective, Malaysia has been importing Sahiwal-Friesian (50% Friesian) cows to increase dairy cow population and local milk production in the country. In recent years, government agencies such as Department of Veterinary Services (DVS) and Malaysian Agricultural Research and Development Institute (MARDI), and private sectors have embarked their efforts to further upgrade the Sahiwal-Friesian (SF) to 75% Friesian (F) in order to increase the milk yield of the animals. To achieve this, the SF cows have been inseminated with purebred Friesian semen by both farmers and government agencies. It has been reported that milk production of the 75% F (25% Sahiwal and 75% Friesian) cows was higher than the 50% F (Dollah et al. 1995).

Although a lot of research work had been carried out on the 75% F cows to determine and improve their productivity, little work had been done on the males of this breed type. Male animals play a major role in animal production through their effects on pregnancy rates and their transmission of genetic traits. Therefore, the objective of this study was to investigate the growth and reproductive performance which includes the onset of spermatogenesis and

puberty of the Friesian crossbred bulls which consist of 25% Sahiwal and 75% Friesian blood levels.

Materials and methods

Thirty-eight weaned Friesian crossbred male calves (25% Sahiwal and 75% Friesian) between 3 months and 4 months of age were selected based on their growth performance (weaned weight) and their parent's performance. All the calves were housed in a conventional shed and managed under uniform management system. The animals were fed with 35% freshly chopped Guinea grass and 65% concentrate (24% crude protein and 74% total digestible nutrient) on dry matter basis. Water and salt lick were provided *ad libitum*. The animals were weighed and, the body height (BH), body length (BL) and scrotal circumference (SC) were measured once a month.

To determine the GnRH-induced plasma testosterone level of the bulls, the animals were injected intramuscularly with a single dose of a synthetic GnRH (0.01 µg Buserelin, Receptal, Hoechst AG Munchen/kg body weight). Blood samples from the jugular vein were collected into 10 mL heparinised vacuum tubes between 2 hours and 3 hours post-injection. The blood samples were centrifuged immediately and the separated plasma was kept frozen at –20 °C until analysed for testosterone by radioimmunoassay.

Plasma testosterone levels were assayed as described by Ramakrishnan et al. (1993). Plasma testosterone, in unextracted plasma samples, was analysed by a solid-phase ¹²⁵I radioimmunoassay technique using RIA kits purchased from Diagnostic Products Corporation, USA. In this assay, 50 µL of buffer, standard (range from 0.2 ng/mL to 16 ng/mL) or plasma samples

were added in duplicate into 12 mm x 75 mm polypropylene tubes coated with antibody to testosterone. One millilitre of ^{125}I testosterone was then added to each tube and total count tubes, and immediately vortexed for a few seconds. The tubes were later kept in a water bath at 37 °C for 3 h. Following that, all the tubes, except for total count tubes, were decanted and kept inverted until dry. The activity of ^{125}I was then determined for 1 min using gamma counter (ICN, Isoflex gamma spectrometer, USA) and the concentration of testosterone was automatically calculated using an on board software that comes with the counter. The sensitivity of the assay was 0.19 to 16.50 ng/mL and the intra and inter-assay coefficient of variation of the assay were less than 5%.

To determine the onset of spermatogenesis, testicular biopsies were done on two animals randomly selected from the group at monthly intervals beginning from the age of 4 months until 9 months old. Testicular tissues from both the testis of the two bull calves were taken by open surgical technique. Prior to the biopsy, the testicular circumference was measured. Bulls that were biopsied were separated from the group and culled. The testicular specimens were sliced into small pieces and fixed with Bouin's solution. Dehydration was carried out in graded alcohol using the Histokinette 2000. Thin sections were stained with Mayer's Hematoxylin and Eosin Y stain. Using a simple microscope, the slides were observed for spermatozoa in the seminiferous tubules and simultaneously the diameter of about 50 cross sections of the tubules were measured.

At the age of about 8 months, the remaining bull calves were trained for semen collection using an artificial vagina. The interval between the presentation of the bull to the teaser until ejaculation was recorded as ejaculation time. By gross examination, the semen volume was recorded immediately after collection. Microscopic examination of semen for mass

activity, progressive motility, sperm concentration and percent intact acrosome (PIA) was carried out according to Ramakrishnan et al. (1989).

The data collected on semen characteristics, libido and the diameter of the cross sections of seminiferous tubules was analysed statistically using t-test to test the difference between the means.

Results and discussion

The bulls attained a mean body weight (BW) of 493.44 kg with mean height at withers, body length and scrotal circumference of 133.68 cm, 160.33 cm and 35.28 cm, respectively at 36 months of age (*Table 1*). Analysis of the data using regression techniques gives the quadratic equation $Y = A + BX + CX^2$ (where $Y = \text{BW}$, $X = \text{age}$, $A = \text{constant } -34.8712$, $B = 3.8087$ and $C = -0.06134$). This equation may be rewritten as $\text{BW} = -34.8712 + 3.8087X - 0.06134X^2$, for which a BW may be predicted if the age is known. In this study, the correlation coefficient of BW with age was highly significant ($r = 0.92$, $p < 0.01$).

The relationship between the age and BH is described by the quadratic equation $Y = 73.0903 + 3.8087X - 0.0613X^2$, where $Y = \text{BH}$ and $X = \text{age}$. Body height was also significantly correlated with age ($r = 0.90$, $p < 0.01$). The quadratic equation that describes the relationship between BL (Y) and age (X) is $Y = 69.6715 + 5.4242X - 0.0831X^2$. Body length was also significantly correlated with age ($r = 0.90$, $p < 0.01$). A trend for the SC to increase as the age increases was seen in this study. The relationship between the age and SC is described by the quadratic equation $Y = 5.1263 + 1.9481X - 0.03441X^2$, where $Y = \text{SC}$ and $X = \text{age}$. SC was also significantly correlated with age ($r = 0.89$, $p < 0.01$).

The relationship between the BW and SC can be described by the quadratic equation $Y = 4.7662 + 0.1139X - 0.00012X^2$, where $Y = \text{SC}$ and $X = \text{BW}$. Scrotal

Table 1. Mean (\pm SD) body weight, body height, body length, scrotal circumference and GnRH-induced plasma testosterone level in Friesian crossbred bulls

Age (m)	Body weight (kg)	Height at withers (cm)	Body length (cm)	Scrotal - circumference (cm)	Plasma testosterone level (ng/mL)	N
4	90.7 \pm 11.3	86.7 \pm 3.6	92.6 \pm 5.4	14.3 \pm 1.1	0.1 \pm 0.1	12
6	130.9 \pm 36.1	97.3 \pm 5.1	103.8 \pm 8.9	16.9 \pm 2.2	3.5 \pm 2.8	17
12	225.1 \pm 40.2	110.5 \pm 6.6	125.9 \pm 13.4	24.1 \pm 3.6	5.4 \pm 4.9	38
18	330.2 \pm 58.4	122.5 \pm 6.6	139.3 \pm 8.9	29.4 \pm 2.0	7.0 \pm 4.4	13
24	409.8 \pm 51.3	127.3 \pm 3.5	154.7 \pm 8.5	31.4 \pm 3.3	9.9 \pm 2.2	25
30	449.2 \pm 78.9	130.7 \pm 9.9	157.8 \pm 3.5	33.3 \pm 4.7	10.4 \pm 2.9	10
36	493.4 \pm 31.2	133.7 \pm 5.9	160.3 \pm 3.3	35.3 \pm 3.9	9.9 \pm 2.3	15

N = Number of animals

Table 2. Influence of age (months) on libido and semen characteristics of Friesian crossbred bull calves

	11–12(n = 6)	17–18(n = 17)	23–24(n = 53)	29–30(n = 37)	35–36(n = 30)
Ejaculation time (sec)	111.2 \pm 70.4a	40.3 \pm 26.2b	29.5 \pm 14.1c	36.7 \pm 23.9b	43.0 \pm 21.2b
Semen volume (mL)	2.5 \pm 2.2a	3.6 \pm 1.6b	4.2 \pm 1.9c	4.3 \pm 1.9cd	4.9 \pm 2.4d
Mass activity (1–5)	1.0 \pm 0.5a	2.7 \pm 1.3b	2.5 \pm 1.5b	2.9 \pm 1.8b	2.5 \pm 1.4b
Motility (%)	21.7 \pm 19.3a	39.1 \pm 16.7b	43.4 \pm 18.5bc	50.0 \pm 18.1de	47.0 \pm 17.4ce
Concentration (million)	315.2 \pm 210.7a	375.0 \pm 205.2a	718.1 \pm 467.5b	529.4 \pm 306.3c	670.8 \pm 403.4b
PIA	54.8 \pm 37.8a	79.0 \pm 12.9be	81.8 \pm 16.9b	85.8 \pm 8.0c	74.9 \pm 25.5e

Mean (\pm SD) in the same row with different letters are significantly different ($p < 0.05$)

PIA = Percent intact acrosome

circumference was significantly correlated with BW ($r = 93$, $p < 0.001$). The equation that describes the relationship of BH in cm (X) and SC in also cm (Y) was $Y = -43.1287 + 0.7943X - 0.0017X^2$. Scrotal circumference was significantly correlated with BH ($r = 91$, $p < 0.001$).

Within the limits of this study for age, BW, BH, BL and breed, from the quadratic equations it is possible to predict the SC if the BW, BH or age of the bull is known. It has been shown in previous studies that there was a positive correlation between testicular weight, gonadal and extragonadal sperm reserves and sperm production (Amann 1970). Chenoweth and Ball (1980) also reported that scrotal circumference, testicular size and seminal production were highly correlated, especially in bull less than 3 years of age. Studies on rams also showed that scrotal circumference was significantly correlated with semen volume (Abdul Wahid and Yunus 1991) and fertility (Bongso et al.

1982). Since SC was highly related to fertility and sperm production, the results of this study may be used to evaluate potential bulls for breeding soundness when combined with other variables such as sperm motility and morphology. The present study limits itself to Friesian crossbred bulls with the maximum age of 36 months old. Therefore, further studies have to be carried out on older Friesian crossbred bulls to determine whether there is any negative correlation between the SC and other variables mentioned in this paper with the advancement of age.

Although 26 animals were trained for semen collection, semen samples could be successfully collected only from 22 animals. Out of the 22 animals only 13 animals produced semen with more than 50% sperm motility. The young Friesian crossbred bulls showed libido and produced semen at the age of 11 months (Table 2). These findings are comparable to earlier findings of

Murugaiyah and Ramakrishnan (1992) who reported that 50% Friesian crossbred bulls ejaculated semen at the age of 12 months. The age of bull was found to have a significant effect on the mean volume of semen. The analysis indicated increasing trends in semen volume with advancing age of the bulls. Similar findings were also reported by Murugaiyah and Ramakrishnan (1992). A significantly ($p < 0.05$) lower progressive motility and concentration of spermatozoa was noticed in the age groups of 11–12 and 17–18 as compared to the animals in the older age groups. In general, the semen quality of the young bulls was poorer than that of adult bulls. This study indicates that bulls should be continuously evaluated for semen quality and libido up to the age of 24 months before they were culled based on either semen quality or libido.

There was a trend for plasma testosterone level to increase as the bulls grew older from 4–24 months of age and then it plateaued. The present results also showed that the percentage of progressively motile spermatozoa increased with the increase in the plasma testosterone level from 12–36 months of age (*Table 1*). It has been reported that there was a significant correlation between GnRH-induced blood testosterone concentrations and fertility in bulls used for artificial insemination (Andersson 1992). In general, increase in the fertility of bulls is assumed to be related to the higher percentage of progressively motile spermatozoa. Saake and White (1972) also found that the percentage of motility was related to fertility based on 90-day non-return rate. In the light of the above data, the present results indicated that the fertility of the bulls increased with the increase in the GnRH-induced plasma testosterone levels.

Libido is an important characteristic which can affect semen production. Ejaculation time is the most commonly implemented technique to gauge libido in bulls due to its simplicity and quick

assessment. It is generally accepted that testosterone secretion from the testis is the main hormonal influence on male sexual activity (Misra and Sengupta 1965). In the present study, the ejaculation time decreased with the increment of the plasma testosterone level from 12–36 months of age (*Table 2*). This is in agreement with the findings of Mann and Lutwak-Mann (1981) who reported that testosterone level was critical in libido.

Relationship between age, size of semeniferous tubules, testicular size and spermatogenesis in the crossbred bull calves is presented in *Table 3*. There was no significant ($p > 0.05$) difference found in the diameter size of the semeniferous tubules between the right and left testicles. The mean diameter size of the tubules increased from 56.01 microns at 4 months to 169.36 microns at 9 months of age. This was in agreement with a previous study where the size of the tubules of adult cattle was reported to be 0.2 mm in diameter (Peters and Ball 1987). The relationship between the age and size of semeniferous tubules can be described by the quadratic equation $Y = 7.6927 + 7.0715X + 1.1071X^2$, where Y = semeniferous tubules and X = age. The size of semeniferous tubules was significantly correlated with age ($r = 0.90$, $p < 0.01$).

In the present study, spermatozoa were found in the semeniferous tubules at the age of 9 months. However, Arthur (1975) reported that spermatozoa first appeared at $7\frac{1}{2}$ months in temperate cattle. The delay in spermatogenesis in the present study could be due to breed difference. Although spermatogenesis began at the age of 9 months in the bulls in the present work, semen samples with motile spermatozoa could be collected from the bulls only at the age of 11 months using an artificial vagina. From these results it may be concluded that though spermatogenesis commenced at the age of 9 months, the bulls reached puberty only at 11 months of age. This is because according to Wolf et al. (1965) puberty in

Table 3. Influence of age on the size of semeniferous tubules, testicular size (mean \pm SD) and spermatogenesis in Friesian crossbred bull calves

Age (m)	Diameter of semeniferous tubules (mic)			Testicular circumference (cm)			Spermatogenesis	n
	Right testis	Left testis	Mean	Right	Left	Mean		
4	56.0 ± 6.7	58.2 ± 7.8	56.0 ± 6.7	5.9 ± 0.1	6.0 ± 0.1	5.9 ± 0.1	Absent	2
5	62.1 ± 5.2	61.3 ± 5.7	61.7 ± 5.5	6.0 ± 0.2	6.2 ± 0.2	6.1 ± 0.3	Absent	2
6	89.1 ± 17.3	98.2 ± 14.0	93.7 ± 16.4	8.1 ± 0.1	8.1 ± 0.1	8.05 ± 0.1	Absent	2
7	111.4 ± 7.4	112.7 ± 7.9	112.1 ± 7.7	8.8 ± 0.3	9.3 ± 0.3	9.13 ± 0.1	Absent	2
8	128.2 ± 11.2	130.4 ± 12.7	129.3 ± 12.0	9.1 ± 0.1	9.2 ± 0.2	9.28 ± 0.6	Absent	2
9	169.6 ± 19.7	169.2 ± 19.7	169.4 ± 22.1	12.5 ± 0.5	12.8 ± 0.8	12.6 ± 0.7	Present	2

n = Number of animals

the bull occurs only when it first produces an ejaculate containing 50 million sperm of which more than 10% are motile.

Conclusion

Age correlated significantly with body weight, height at withers, body length and scrotal circumference. Similarly scrotal circumference was significantly correlated with body weight and height at withers. Age had significant effect on the semen characteristics of Friesian crossbred bulls. Crossbred bulls should be evaluated for semen quality and libido at least up to the age of 24 months before they were culled based on either semen quality or libido because the semen quality was found to keep on improving till 24 months of age. This study also showed that the GnRH-induced plasma testosterone level increased as the bulls grew older from the age of 4 months to 24 months. Another interesting finding of this study was that although spermatogenesis began at the age of 9 months, the bull calves reached puberty only at the age of 11 months old.

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