Variation in Fecal Testosterone Hormone Concentration with Season and Harem Size in Misaki Feral Horses

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ABSTRACT. On Misaki peninsula, Japan, fecal samples were collected from 14 Misaki stallions at monthly intervals for 12 consecutive months. The fecal testosterone concentration was measured by radioimmunoassay. We examined monthly fecal testosterone hormone patterns and the relationship between fecal testosterone concentration and breeding season and later harem size. Marked monthly variations in fecal testosterone concentration were observed. The fecal testosterone concentration began rising in March; the highest mean monthly concentration, 2.87 ± 0.18 ng/g, was found in April, and the level remained elevated until the end of August and thereafter decreased. A significant correlation was found between the fecal testosterone concentrations and harem size in both the breeding and non-breeding season among the 14 stallions. It is therefore possible that the testosterone levels in feces, instead of blood, correlate very well with harem size in Misaki stallions. Our findings emphasized that the fecal testosterone concentration can be a powerful indicator for monitoring of endocrine status in wild stallions.

KEY WORDS: fecal testosterone level, feral horse, harem size, radioimmunoassay.

An understanding of endocrinology is basic to reproductive behavior, and testosterone is the hormone principally responsible for male behavior. Annual variations in testosterone concentration in the peripheral plasma of wild and domesticated stallions have been noted [2, 3, 21]. Moreover, the testosterone concentrations of mature dominant stallions are higher during the breeding season than in the non-breeding season [19]. The highest concentration during the breeding season leads to seasonal changes in libido [2]; the libido peak occurs at the start of the breeding season [5] and is followed by a marked increase in mating rates [8]. Approximately 100 free roaming feral horses have occupied parts of southern Japan for up to 300 years. The basic social unit in feral horse groups consists of one stallion, a few mares and their foals [4, 10, 15, 17]. In Misaki area, most stallions formed harem groups with smaller numbers of mares, with an average of 2 [12]. The harem group is therefore considered to be the basic reproductive unit in the feral horse population [14], and the reproductive success of a stallion is positively correlated with the number of adult mares in his harem group [15, 18]. Young stallions become sexually mature by 2 years of age [13], but are not treated as rivals by adult stallions until they are three years old [20]. In Misaki area, most stallions form their harem groups at an age ranging between 4 and 6 years [9], and most new harem groups are formed at the beginning of the breeding season [11]. Changes in a stallion’s harem size or individual differences in harem size among stallions may be influenced by factors that affect the reproductive success of the stallion, such as body weight and fighting ability [1], age [9] or dominance and aggressive behavior [16]. Our previous study emphasized the relationship of hormones to these changes in harem size through measurements of the plasma testosterone concentrations by radioimmunoassay in Misaki horses [12]. However, feral horses can be extremely dangerous and difficult to capture, making collection of blood samples difficult. Hence, fecal hormone examination is an attractive option because of the relative ease in obtaining samples. We therefore aimed to 1) measure changes in the fecal concentration of testosterone throughout the year and determine the time of peak concentration and to 2) determine the relation between fecal testosterone concentration and breeding season and subsequently harem size.

During the study period, the number of adult males (≥ 3 years) was 14; these stallions inhabit ranges over an area of about 5 km² on Cape Toi, the southeast end of Kyushu Island. Individual horses are readily identifiable by their coat color, sex, body size, marking, age and a number branded on the hip.

Fecal samples were collected every month for 1 year from the 14 stallions, which were 3 years of age or older. Fecal samples were collected at irregular intervals, but an attempt was made to collect at least 4 samples from each stallion every month during the breeding season (April to August) and non-breeding season (September to March). When more than one sample was collected from a single individual during the same sampling interval, the values were averaged for a mean value per individual per interval. Hence, the data for each stallion was transformed into one sample per month for each data point to evaluate the variation in fecal testosterone concentration in association with season and harem size in horses as shown in Table 1. Each sample, consisting of about 50 g of fecal material,
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was collected in a plastic bag and frozen at –20°C within 6 hr. The sample remained frozen until the assay was performed. The position of each stallion was checked at least twice a month and marked on field maps to observe each group composition and determine its stability throughout the year. The fecal samples were thawed and dried in an oven at 100°C for 1.5 hr. Distilled water (1.5 mL) was added to 0.25 g of dried, crushed feces in an extraction tube with a Teflon-sealed cap, and the sample was mixed well before addition of 5 mL of diethyl ether. After a 10 min extraction with a vortex mixer, the ether layer was collected by snap-freezing and decantation in a tube and then dried at 42°C. The wall of the test tube was rinsed with 0.5 mL ether, which was then evaporated under a nitrogen gas stream. The residue was redissolved in 2 mL of ethanol and vortex-mixed for 10 min, transferred to an assay vial and then dried under nitrogen at < 50°C. This method was similar to that described by Hirata and Mori [6].

Testosterone concentrations were measured by radioimmunoassay with a specific antibody for testosterone, which was extracted from fecal samples. We used the method of Hotchkiss et al. [7]. Statistical analysis was performed on an Apple Macintosh S.E. running the StatView software. The results are expressed as means of all values ± the standard error of the mean. The Spearman’s rank correlation coefficient test was used for the correlation between the concentration of fecal testosterone and harem size in both the breeding and non-breeding season. P values of less than 0.05 were considered to denote statistical significance.

We calculated the monthly mean fecal testosterone concentrations of all 14 stallions for 12 consecutive months (Fig. 1). The highest concentration (mean ± S.E.) of fecal testosterone, 2.87 ± 0.18 ng/g, was found in April, while the lowest concentration (mean ± S.E.) of fecal testosterone, 1.69 ± 0.07 ng/g, was found in December.

Table 1. Relationship between fecal testosterone concentrations and harem size (number of mares/harem)

<table>
<thead>
<tr>
<th>Stallion name</th>
<th>Stallion age</th>
<th>Harem size</th>
<th>Testosterone concentration (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(year)</td>
<td>Breeding season</td>
<td>Non-breeding season</td>
</tr>
<tr>
<td>M7</td>
<td>4</td>
<td>0</td>
<td>1.48 ± 0.23</td>
</tr>
<tr>
<td>M11</td>
<td>4</td>
<td>0</td>
<td>1.67 ± 0.17</td>
</tr>
<tr>
<td>M12</td>
<td>6</td>
<td>0</td>
<td>1.81 ± 0.13</td>
</tr>
<tr>
<td>M20</td>
<td>7</td>
<td>1</td>
<td>2.29 ± 0.15</td>
</tr>
<tr>
<td>M24</td>
<td>8</td>
<td>1</td>
<td>2.44 ± 0.11</td>
</tr>
<tr>
<td>M56</td>
<td>14</td>
<td>1</td>
<td>2.44 ± 0.18</td>
</tr>
<tr>
<td>M57</td>
<td>4</td>
<td>1</td>
<td>2.51 ± 0.25</td>
</tr>
<tr>
<td>M2</td>
<td>10</td>
<td>2</td>
<td>2.67 ± 0.24</td>
</tr>
<tr>
<td>M8</td>
<td>5</td>
<td>2</td>
<td>2.59 ± 0.17</td>
</tr>
<tr>
<td>M36</td>
<td>7</td>
<td>2</td>
<td>2.60 ± 0.30</td>
</tr>
<tr>
<td>M6</td>
<td>10</td>
<td>3</td>
<td>3.30 ± 0.35</td>
</tr>
<tr>
<td>M23</td>
<td>5</td>
<td>3</td>
<td>2.69 ± 0.35</td>
</tr>
<tr>
<td>M16</td>
<td>8</td>
<td>4</td>
<td>3.44 ± 0.15</td>
</tr>
<tr>
<td>M42</td>
<td>7</td>
<td>4</td>
<td>3.10 ± 0.25</td>
</tr>
</tbody>
</table>

A significant correlation was found between the fecal testosterone concentrations and harem size in the breeding (represented as the averaged value from April to August) and non-breeding season (represented as the averaged value from September to March—Spearman r_s=0.970 and 0.976, n=14 and 14, P<0.0005 and P<0.0004, respectively).

Fig. 1. The seasonal changes in fecal testosterone concentration in Misaki stallions. The highest concentration (mean ± S.E.) of fecal testosterone, 2.87 ± 0.18 ng/g, was found in April, while the lowest concentration (mean ± S.E.) of fecal testosterone, 1.69 ± 0.07 ng/g, was found in December.
Fecal testosterone and harem size in the feral horse

1.69 ± 0.07 ng/g, was found in December.

We examined the relationship between fecal testosterone concentration and harem size in the breeding and non-breeding season (Table 1). A significant correlation was found between the fecal testosterone concentration and harem size in both the breeding and non-breeding season in all the stallions analyzed in this study (Spearman r=0.970 and 0.976, n=14 and 14, P<0.0005 and P<0.0004, respectively).

In Misaki area during the breeding season (from the beginning of April until the end of August), stallions developed stable groups consisting of one stallion, one or more adult mares, with an average of 2, and their foals. These smaller harems would tend to be more stable than a larger harem, and so the mares tended to be associated with the same stallions every year [8]. Furthermore, the grasslands usually increased in grazing capacity during the period from April to August and developed a comfortable microclimate for the life activities and breeding of Misaki horses. On the other hand, after September (the beginning of non-breeding season), when the grass productivity of the grasslands tends to decrease, most of horses generally separated into numerous small groups [9]. Most of the mares at this time are in seasonal anoestrus; the stallions are therefore not sexually active and have lower testosterone levels. This suggests that seasonal changes in fecal testosterone are correlated with the seasonal frequencies of mating and seasonal variation in libido. In the present study, we found that the highest mean concentration of fecal testosterone was 2.87 ± 0.18 ng/g (in April). These results are consistent with those of Floris et al. [5], who found the same results by measuring seasonal plasma testosterone concentrations and reported that the greatest libido occurs at the start of the breeding season. Hence, we concluded that the fecal testosterone concentration reflects the endocrine status very well in Misaki stallions.

Furthermore, we found that fecal testosterone concentration was closely associated with harem size not only in the breeding season but also in the non-breeding season. During the breeding and non-breeding season, the differences in the fecal testosterone concentration increased and decreased with the increase and decrease in the number of mares. Teasing mares in estrus results in the increase in testosterone due to the increase in LH that occurs during teasing; therefore, stallions around mares in estrus have increased concentrations of fecal testosterone during the breeding season. On the other hand, the presence of stable mares with the same stallions throughout the year may be a social stimulus influencing the fecal testosterone concentration during the non-breeding season. Hence, the noninvasive method of fecal testosterone analysis can be used successfully to examine reproduction in feral horses.

In conclusion, our results suggest that the fecal, instead of blood, testosterone concentrations correlate very well with the season and harem size. Furthermore, it can be emphasized that the fecal testosterone concentration can be a powerful indicator for monitoring of the endocrine status in wild stallions. This is surprising as fecal testosterone analysis would undoubtedly be a valuable research tool in feral horses, especially in species in which low-stress, noninvasive sampling methods are desirable.

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REFERENCES


17. Miller, R. 1981. Male aggression, dominance and breeding


