Effects of Stocking Density on Some Behavioral and Some Blood Biochemical Parameters in Camel during the Rut Period

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This study investigated the effect of different stocking density on behavior, body condition score and some blood parameters of male camels in the rut period. Thirty-six male dromedary camels in rut period weighing from 350 to 400 kg were randomly divided into three stock densities, 3 [lower stocking density (75%) (LSD)], 4 [normal stocking density (100%) (NSD)], and 5 [higher stocking density (125%) (HSD)] camels on 60 m² of pen, respectively. Behavioral observations were performed for 10 weeks once per week for posture, maintenance, aggressive and social behavior using instantaneous scan sampling. Blood samples were collected at the end of the experiment for some serum blood parameters (Total protein, Albumin, Globulin, and Albumin/Globulin ratio) and blood hormones (Cortisol, Testosterone, T3, T4, and T3/T4 ratio). The results indicated that, in comparison with LSD and NSD, HSD increased the aggressive behavior, standing duration and cortisol level (P < 0.05), while, it decreased the lying, walking, rumination, body condition score, testosterone, T3 and T4 level (P < 0.05). In addition, there were no significant effects on drinking behavior and blood parameters between all the treatments (P > 0.05). Conclusively, HSD in group rearing had a bad effect on aggression, body condition score and sexual performance of male camels in the rut period.

Keywords: Behavior, Blood hormones, Male camel, Rut period, Stocking density.
health, behavior and welfare may be affected by multiple factors including pen size, environmental factors (e.g. temperature, and light), nature of work, type of production and nutrition. In addition, few studies have been conducted to investigate the effects of stocking density of camels in the rut period under group rearing [3]. Therefore, the objective of this study was to investigate the effect of different stocking densities (3 camels on 60 m$^2$, LSD), (4 camels on 60 m$^2$, NSD) and (5 camels on 60 m$^2$, HSD) on behavior and some blood parameters of male camels in rut period. We hypothesized that; the stocking density will affect aggression, body condition score and sexual performance of male camels in the rut period, which has a direct implication on the management of flocks of male camels in the rut period that were kept under intensive conditions.

### Materials and Methods

The procedures were performed under the protocol approved by the Animal Care and Use Committee of Assiut University, Assiut, Egypt.

#### Management

**Animals**

Thirty-six mature clinically healthy male dromedary camels in the rut period. Their ages ranged from 5-7 years old, weighing from 400 to 450 kg. Camels were divided into three groups (Photo 1), 12 camels for each block, each group of camels was housed on 60 m$^2$ pen according to the normal stock density of 15 m$^2$ for each camel; (a) Low stocking density (3 camels on 60 m$^2$ X 3 replicates) (LSD- 75 %), (b) Normal stocking density (4 camels on 60 m$^2$ X 3 replicates) (NSD - 100%) and (c) High stocking density (5 camels on 60 m$^2$ X 3 replicates) (HSD - 125 %).

**Photo 1. Camels divided into three stocking densities, 3 [lower stocking density (75%)(LSD)], 4 [normal stocking density (100%)(NSD)], and 5 [higher stocking density (125%)(HSD)] camels on 60 m$^2$, respectively.**

#### Housing

Camels were assigned and housed in 9-floorpens (3 replicates per group). Each experimental pen had a semi-covered roof and 35 cm sand floor bedding, it was exposed to daylight cycle (12 h) and ventilated with 3 windows of 1m$^2$ through the experimental period.

#### Watering and feeding

Each group of camels had the same size of feed and water troughs. Camels were fed with approximately 3% of its live weight daily [13], the diet consisted of 40% concentrate (11% protein and 60% hay), it was divided into two equal parts, which was offered twice daily in the manger at 8:00-9:00 am and 4:00-5:00 pm, while, barseem as a fed (1.5 kg / day /camel) were offered at 11-12 pm. Feeding quantity and quality remained constant throughout the experiment, water was provided ad libitum.

**Experimental procedure**

**Pre-experimental period**

To get rid of the internal and the external parasites, animals were treated with [(Ivermectin and Clorsulon), Ivomac Super, 1 ml per 50 kg body weight, Merial, France] twice, at 15 days interval, after two weeks from the start of the experiment, animals were observed once (1 h) per week (20 min. morning, 20 min. mid-afternoon and 20 min. afternoon) to adapt the animals for the presence of unfamiliar person and camera. Unhealthy, weak camels and those that showed abnormal behavior such as lameness, biting and kicking were excluded from the study and replaced with healthy ones.

**Experimental period**

Observation of camels’ behavior was done once per week for 10 weeks at the beginning of March (breeding season of camels in Egypt) to
mid-May. Blood samples were collected from venous blood from jugular vein at the end of the experiment.

**Data measurement**

**Behavioral observation**

Ninety hours of video clips by using a digital camera (OPTIKA microscopes SRL, Italy) were recorded for all groups (9 pens x 1 hour x 10 weeks), (20 min x 3 times per day at mornings (5:00-6:00 am), mid afternoon (12:00 am-1:00 pm) and late afternoon (3:00-4:00 pm) per pen).

Camels’ behavior was analyzed by using instantaneous scan sampling technique [14], while, the duration was analyzed by using the focal sampling technique from the recorded video clips, according to predefined ethogram (Table 1).

| TABLE 1. Behavioral ethogram. |
|-------------------------------|
| **Behavior** | **Definition** |
| Posture | |
| Standing | Standing in inactive upright posture on all four feet with no movement. |
| Lying | Body contact with the ground or camel sits in sternal recumbency |
| Walking | Camel does more than 2 complete steps. |
| Maintenance behavior | |
| Drinking | Drink water from water draft. |
| Rumination | A bolus of regurgitating food goes back into his mouth and the camel re-chewed and re-swallowing it again while standing or lying down. |
| Aggressive behavior. | |
| Attack behaviour | Including bite, cross-neck biting, ear-biting or pinching, front-wrestling, knee bite |
| Threatening behaviour | chasing, blowing inflatable from mouth, salivation and making sound by teeth grinding |
| Defensive behaviour | fleeing or running away, neck-away defensive and sideways defensive |
| Social interaction behavior | |
| Displacements number | One camel caused the receiving one to stop eating, remove its head from the manger and back out was summed daily for the entire 20 day observation period to get the average. |
| A displacement index (DI) | Proportion of successful displacements from the feed bunk relative to all displacements in which the animal was involved. (Galindo and Broom, 2000) |

**Blood sampling**

At the end of the experiment, evacuated tubes without anticoagulant were used to collect 10 mL of blood from each animal. Blood samples were centrifuged using 80-2 Electronic Centrifuge (SMIC Inc., China) at 3000 rpm for 15 min. to collect serum. All samples were stored at -20°C until analysis.

Serum triiodothyronine (T3), thyroxine (T4), testosterone and cortisol hormones were estimated by Stat fax-2100 (Awareness technology, INC. USA) and commercial ELISA kits (Assay Max ELISA kits, ASSAYPRO Inc., USA).

Serum total proteins and albumin were assayed by colorimetric method using a commercial kit manufactured by the Egyptian company for biotechnology, Egypt. In addition, serum globulin and Albumin/ Globulin ratio were calculated according to Coles [15].

**Body condition score**

Body condition score was estimated at the end of experiment according to Faye et al. [16].

**Statistical analysis**

Means of the data were analyzed by using SPSS (2001). The normality of the data was analyzed by the Shapiro-Wilk test. The overall effects of the stocking density were analyzed statistically by one-way analysis of variance (ANOVA). Means were compared by Tukey-Kramer test when a significant difference was detected. Statistical significance was declared when the coefficients were at a probability equal to or less than 0.05. Data were presented as Mean ± SE.
Results

Effect of stocking density on some behavioral patterns and the body condition score of male camels in rut period

The effect of stocking density on maintenance, posture, aggression, social interaction behaviors and body condition score were presented in Figures 1, 2, 3 and 4. Compared to LSD and NSD groups, HSD groups had significant increases in aggressive and standing behaviors (P < 0.05), however, it had significant decreases in the daily duration and frequency of lying behavior, locomotion activity (walking), and rumination and the body condition score (P < 0.05). In addition, there were no significant differences in the frequency and the duration of drinking behavior (P > 0.05).

Fig. 1. Shows duration (A) and frequency (B) of maintenance behavior (Rumination and Drinking) of male camels in rut period reared under different stocking densities.

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Fig. 2. Shows frequency (A) and duration (B) of posture (Standing, walking and sitting behaviors) of male camels in rut period reared under different stocking densities.
Fig. 3. Show aggressive behavior (A) and social interaction behavior displacement number (B) and index (C) of male camels in rut period reared under different stocking densities.

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Effect of stocking density on some blood indicators of male camels in rut period

The effect of stocking density on blood biochemicals and hormones levels were presented in Table 2.

Levels of testosterone, T3, T4 and T3 / T4 ratio were decreased in the HSD group in comparison with the LSD and the NSD groups; however, HSD had a significant increase in the cortisol level. On the other hand, there were no significant differences in the measured biochemical blood parameters between all the tested groups.

### TABLE 2. shows different blood parameters of male camels in rut period reared in different stock densities.

| Blood parameters | Stocking density (%)* | Stocking density (%)* | Stocking density (%)* |
|------------------|-----------------------|-----------------------|-----------------------|
|                  | LSD (75%)             | NSD (100%)            | HSD (125%)            |
| Blood Chemicals  |                       |                       |                       |
| Total protein (g/100 ml) | 7.3 ± 0.3         | 7.5 ± 0.4             | 7.2 ± 0.3             |
| Albumin (g/100 ml)      | 2.7 ± 0.2           | 2.8 ± 0.6             | 2.6 ± 0.2             |
| Globulin (g/100 ml)      | 4.6 ± 0.4           | 4.7 ± 0.3             | 4.6 ± 0.4             |
| A/G ratio               | 0.59 ± 0.01         | 0.60 ± 0.01           | 0.57 ± 0.01           |
| Blood hormones         |                       |                       |                       |
| Cortisol (n. mol/l)    | 83.8 ± 2.1b         | 85.9 ± 1.5b           | 92.8 ± 1.9a           |
| Testosterone (n. mol/l)| 1.7 ± 0.3a          | 1.8 ± 0.4b            | 1.2 ± 0.1b            |
| T3 (n. mol/l)          | 1.9 ± 0.3b          | 2.2 ± 0.6b            | 1.6 ± 0.4b            |
| T4 (n. mol/l)          | 3.5 ± 0.8ab         | 3.8 ± 0.7b            | 3.3 ± 0.6b            |
| T4 / T3 ratio          | 1.84 ± 0.2ab        | 1.72 ± 0.3b           | 2.06 ± 0.5a           |

*Mean± SE with different superscripts in the same row differ (P <0.05).

### Discussion

Stocking density has important functions in growth performance, behavior and welfare of camels, which is influenced by various factors such as, number of animals and floor space [17]. Whereas, exposure to various environmental or physiological stressors, such as rut period, disturb the camels’ behavior and temper. Management of the stocking density, as well as isolation, is a beneficial method that helps in relieving the harmful effects of this period, which can be utilized by the camel industry to improve health and increase economic profiles. It should be counted as extra benefit to increase welfare and food safety.

The current experiment was undertaken to investigate the effects of different stocking densities NSD (100 %), HSD (125%) and LSD (75 %)on posture, maintenance, aggressive and social behaviors, body condition score and some blood parameters of male camels in rut period.

High stocking density (125%) showed decreased frequency and duration of ruminating, lying behavior, locomotion activity (except standing duration) and body condition score in comparison with LSD (75%) and NSD (100%) groups. In the same time, many reports revealed that increasing stocking density had a harmful effect on performance and behavior of camels [18,19], however, some researchers reported no effect [20-22].

The obtained result may be linked to the movement activity, which depends mainly on the amount of the space available, reduction of the space availability hinders group individuals from free movement due to the closer presence of other conspecifics [23], they act as a physical barrier, so that, animals are unable to escape [24], leading to reduction in the locomotion and lying. In addition, the decrease in body condition score may be due to reduction of the daily feed intake and gain. Moreover, rumination behavior decreased with HSD group due to high alert behavior, individual vigilance, and low eating time [8,10]. The reduction in the lying time could be due to the relationships between eating and rumination [25].
Standing duration and aggressive behavior significantly increased with HSD, this finding agreed with previously reported findings of [8] and [26], who reported that idly standing time and agonistic behavior were increased linearly with increasing stocking density. The current results may be due to more time spent by the subordinate animal to hide from the dominant one [27]. In addition, HSD increased the aggressive behavior due to less space allowance and increased social interactions [8,9], movement and exercise activity changes [28], violation progress [29], inability to escape [24] and reduction in the daily lying requirements [9,26].

In the present study, the increased serum cortisol level with HSD agreed with [11] and [30], who found that, HSD associated with elevation in the cortisol level. These results can be attributed to adrenal function alterations and elevated cortisol concentrations, which mobilize energy to manage stress, such as the fight/flight response [11].

Testosterone is the most important sex hormone, which regulate most of the reproductive functions such as the activity of sex accessory glands, spermatogenesis and libido of male animals [31], reduction in the testosterone levels in the HSD could be attributed to elevated cortisol level and stress [4].

Thyroid hormones (T3 and T4) play a vital role in controlling thermoregulation and metabolism, they are influenced by stress. In the current study, the reduction in serum T3 and T4 levels with HSD agreed with that reported by [32]. The activity between the thyroid gland and hypothalamus are strongly linked, and the observed decrease in the levels of T3 could be attributed to the influence of HSD related stress on the hypothalamus. When enhanced by stress, the hypothalamus reduced the thyroid stimulating hormone-releasing hormone (TSH-RH), this inhibit the thyrotropes of the anterior pituitary from releasing the TSH. The concentrations of the TSH-RH will be measured in a future study to support this hypothesis.

There was no significant effect of stocking density on serum biochemical parameters. The lack of observable difference between groups may be due to the same amount of feed and the same feeding and watering space provided to all groups.

**Conclusions**

In the current study, the results indicated that, LSD of camels under group rearing in the rut period reduced aggression, social index and cortisol level, with good body condition score and sexual performance. Moreover, LSD could be considered as a protective management practice in male camels to alleviate the negative effects of stress-related rut period. However, further studies should be conducted to examine the mechanisms by which that influences brain function and which brain area is involved.

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**Ethical consideration**

This study was carried out in accordance to the ethical rules for handling the experimental animals, Hygiene Department, Faculty of Veterinary Medicine, Assiut University, Egypt.

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**Conflict of Interest**

Authors have no conflicts of interest to disclose.

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Gauly, M., Erhardt, G., and Dzapo, V., Annual changes in serum levels of thyroid hormones in male llamas (Lama glama) and their correlation with reproduction immunity. J. Camel Pract. Res., 4, 159-1 (1997).

Tأثير كثافة التسکین على بعض العوامل السلوكیة وبعض العوامل الپيوكیمینیة للدم في الإبل خلال فترة التزاوج

بحثت هذه الدراسة في تأثير كثافة التربیة المختلفة على السلوك ودرجة حالة الجسم وبعض المعلّمات الدمویة للجمال في فترة التزاوج. تم تقسيم ستة وثلاثين مجملًا من جمل الجمل العربي في فترة التزاوج يتراوح وزنها من 400 إلى 350 كجم إلى ثلاث مجموعات الأولى تحتوي على ثلاث مجمال تمثل كثافة عادية، والثانية خمس مجمال تمثل كثافة متوسطة، والثالثة اربع مجمال تمثل كثافة عالية. تم توزيع الحصانات في المجموعة الأولى والثانية والثالثة على مساحة 125 و75 و50% من المساحة المخصصة لها. تم إجراء الملاحظات السلوكية لمدة 10 أسابيع واحده في الأسبوع للمساحة والسلوك الغذائي والسلوك العدائي والاجتماعي. تم استخدام أخذ عينات السمن الديفري للسلوك في نهاية التجربة لبعض معلمات الدم في الدم (نسبة البروتين الكلي، الألبومين، الجلوبيولين، الألبومين) من العينات السابقة تصل إلى النتائج ذات الصلة زيادة في مستويات هرمونات الكورتيزون وسلوك الوقوف والسلوك العدائي مع نقص في سلوك السكينة والسلوك العدل. المعدل الإجمالي للعوامل السلوكية لزيادة الكثافة التسکینية من 75% إلى 50% ولكن لوحظ عدم وجود تغيیر معنوي في سلوك الثغب. الخلاصة: زيادة الكثافة في التربیة للجمال في موسم التزاوج عن جمل لكل 125 م يقوم بها 15% من المساواة في السلوک العدائي والدنیا والمسالخ للنمت الجسمی.

الکلمات الدالة: السلوک و هرمونات الدم و ذكر الجمال و موسم التزاوج و الكثافه التسکینیه.