RESEARCH ARTICLE

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Seasonal Changes in Plasma Testosterone and Biochemical Parameters of Male Donkey (*Equus asinus*) in Northern Algeria

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Abstract

This present study was aimed to determine the changes of plasma testosterone in North Algerian donkeys, the correlation coefficients between T concentrations and biochemical parameters were estimated. Blood samples were withdrawn in the morning from the jugular vein into a tube containing EDTA. A total of 24 mature healthy donkeys were selected randomly under condition of free stabling period. T concentrations in male donkeys were measured by enzyme-linked immunosorbent assay kit. AST, ALT and ALP concentration were measured according to the manufacturer recommendation. As regards the concentration of Ch and Tg, the assay was carried out using the automatic biochemical analyzer. The results indicated that T concentration values were significantly ($P<0.05$) higher in winter and autumn (2.468±0.66 ng/ml and 2.785±0.49 ng/ml, respectively) seasons than in spring and summer (0.95±0.343 ng/ml and 0.745±0.236 ng/ml, respectively) seasons. Likewise, Ch and Tg values were significantly ($P<0.05$) higher in winter and autumn seasons than in spring and summer seasons. Also, it is also noteworthy that ALT values were significantly ($P<0.05$) higher in winter and autumn seasons than in spring and summer seasons. Whereas, AST values were significantly ($P<0.05$) lower in summer than in winter, autumn and spring seasons. As regards ALP, values mean were practically similar in all seasons. Our finding suggests that the sexual activity probably occurs during winter and autumn in local donkeys of Northern Algeria. In addition, our results of Ch and Tg levels are correlated with season, which may be a good indication of the reproductive performance in donkeys.

Keywords: Testosterone, biochemical parameters, season, donkeys, Algeria.

INTRODUCTION

The domestic donkey (*Equus asinus*) represents a unique equine species that descended from wild donkeys (*Equus africanus*), and that evolved in inhospitable, mountainous and arid desert environments (Epstein, 1984). The common donkey is mainly kept as a domestic companion animal in Europe and North America, but is commonly used as a working animal in developing countries. The number of donkeys worldwide considerably decreased with the advent of motorization in transport and work, which constitutes a risk of extinction of the species in the worldwide (Vlaeva et al., 2017; Kugler et al., 2008). It appears a good knowledge of the reproductive function seems to be a very essential tool to preserve and reintroduce the donkey populations.

The mechanisms regulating reproductive function are based on the permanent relationship between the central nervous system and the gonads, which is provided by the steroidal and gonadotrophic hormones. However, this hormonal regulation is influenced by different environmental factors such as age; season (Vasantha et al., 2016). Seasonal variations of equides are due to the change of day duration throughout the year. The seasonally reproductive animal species, notably donkeys, express seasonal variations in their sexual activity. It is known in the literature that the testosterone is a steroid hormone that is the main key to the spermatogenesis and control males' sexual behavior; and its dosage is a best tool to determination of the reproductive season. Despite the scientific controversy on seasonality in donkeys, it likely could be influenced by photoperiod and other factors such as nutrition and temperature.

In other hand, biochemical parameters are commonly used for assessing the physiological or pathological condition of animals, however, the values of these parameters vary between populations, sex, age, nutrition and season (Longodor et al., 2020; Tesfaye et al., 2014; Girardi et al., 2014). It has been reported that transaminase enzyme activity (AST and ALT) is a good indicator of semen quality (Corteel et al., 1980). Moreover, a relationship was found between ALT and ALP enzymatic activity and reproductive function (Longodor et al., 2020; Hussein et al., 2017). Numerous studies that have shown that testosterone has a strong influence on hepatocytes and induces more variation in enzyme functions (Azani et al., 2018; Charni-Natan et al., 2019). In addition, it was reported that male androgens increase blood cholesterol (Guyton, 1981).

To our knowledge, no study has been done to describe the seasonal testosterone (T) concentrations of the domestic donkey *(Equus asinus*) in north Algeria. We hypothesized that total cholesterol (Ch), triglyceride (Tg), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP)

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could influence T concentrations in domestic donkeys. Therefore, this present study was aimed to determine the changes of plasma testosterone in North Algerian donkeys, the correlation coefficients between T concentrations and biochemical parameters were estimated.

MATERIALS AND METHODS

This research was approved by the Scientific Council of the Faculty of Nature and Life Sciences (Report of Faculty Scientific Council #05 dated October 30, 2018), University of Bejaia, Algeria). Concerning the ethical aspects, the experimental procedure was performed according to good veterinary practice under farm conditions.

Study area and animals

The study was carried out from January 2020 to February 2021 in Northern Algeria, Bejaia (36° 43' N, 5° 04' E) and Jijel (36° 42' N, 4° 2' E) province were chosen randomly. The study region has four distinct seasons: winter (January to March), spring (April to June), summer (July to September) and autumn (October to December) with a seasonal mean of day length of 610.6; 785.88; 851.03 and 677.16 minutes/day, respectively. The mean maximum summer temperature reaches 36.9 °C (September) and the mean minimum winter temperature falls to 9.8 °C (February).

Animals and samples

Blood samples were withdrawn in the morning (8:00 am to 10:00 am) from the jugular vein into a tube containing EDTA (Sarstedt®, Numbrecht, Germany). The samples were immediately centrifuged (15 min at 1500×g), and stored at -20 °C until assayed. A total of 24 mature healthy donkeys were selected randomly under condition of a free stabling period. The age of the animals ranged between 3 and 20 years. Male donkeys had a body corporal score between 2.5 and 3.5. Animals were checked by a veterinarian and presented no signs of disease clinical.

Testosterone and biochemical parameters assay

T concentrations in male donkeys were measured by enzyme-linked immunosorbent assay kit (Human Diagnostics Worldwide: ELISA Testosterone, direct REF: 55010, Germany) according to the kit instruction. Each sample was analyzed in duplicate. The detecting antibody was rabbit anti-T IgG as biotin-conjugate. The enzyme substrate was avidin-horseradish peroxidase (HRP). Intra- and inter-assay coefficients of variation were 7.5% and 11%, respectively.

AST, ALT and ALP concentration (nmol/ml) were measured according to the manufacturer recommendation (Spinreact, S.A./S.A.U. Sant Esteve de Bas, Spain). As regards the concentration of Ch and Tg, the assay was carried out using automatic biochemical analyzer (COBAS INTEGRA 400 plus Analyzer REF: 5179282).

Statistical analyses

Statistical analyses were carried out in Statview, version 4.55 (Abacus concepts Inc., Berkeley, CA, USA). The concentration values were expressed as mean ± SE, and $P<0.05$ was considered significant. The one-way variance analysis (ANOVA) was used to evaluate the obtained data. Statistical analysis was performed using *t*-test to compare different seasons.

RESULTS

The length day and mean temperature during the period of study are presented in Figure 1. The overall mean (±SE) T concentration and biochemical parameters of donkeys were shown in Table 1. Mean value of T concentration is 1.47±0.28 ng/ml, ranged between 0.21 and 4.77 ng/ml. Mean values of Ch and Tg are 0.87±0.11 and 0.81±0.1 mmol/l, respectively. Mean values of ALT, AST and ALP are 21.4±1.66, 199.63±10.08 and 123.6±4.8 U/l, respectively.

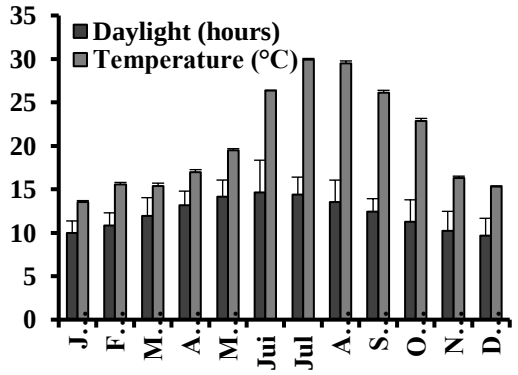

Figure 1. Mean (±SE) values of daylight (hours) and temperature (ºC)of the study area, Northern Algeria.

Table 1. The overall descriptive data (Mean±SE) of plasma testosterone, total cholesterol, triglyceride, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase in Algerian local donkeys (*Equus asinus*).

Parameters	Mean	SE	Minimum	Maximum	CV
Testosterone (ng/ml)	1.74	0.28	0.21	4.77	0.8
Total cholesterol (mmol/l)	0.87	0.11	0.25	1.77	0.62
Triglyceride (mmol/l)	0.81	0.1	0.22	2.2	0.59
Alanineaminotransferase (U/l)	21.4	1.66	5.83	37.91	0.38
Aspartateaminotransferase(U/l)	188.63	10.08	109.66	268.91	0.26
Alkaline phosphatase (U/l)	123.6	4.8	91.3	184.8	0.2

The changes of T concentrations and biochemical parameters expressed by seasons in donkeys (*Equus asinus*) are shown in Figure 1 and 2, respectively. The results indicated that T concentration values were significantly ($P<0.05$) higher in winter and autumn (2.468±0.66 ng/ml and 2.785±.0.49 ng/ml, respectively) seasons than in spring and summer (0.95±0.343 ng/ml and 0.745±0.236 ng/ml, respectively) seasons. Likewise, Ch and Tg values were significantly ($P<0.05$) higher in winter and autumn seasons than in spring and summer seasons.

Also, it is noteworthy that ALT values were significantly (P<0.05) higher in winter and autumn seasons than in spring and summer seasons. Whereas, AST values were significantly (P<0.05) lower in summer than in winter, autumn and spring seasons. As regards ALP, values mean were practically similar in all seasons.

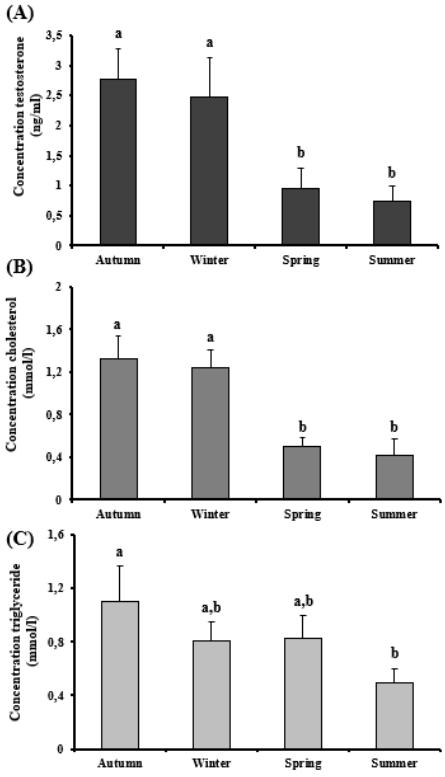

Figure 2. Seasons changes in testosterone concentrations (A), total cholesterol (B), Triglyceride (C) in Algerian local donkeys (*Equus asinus*). [a,b] Means (±SE) with the same superscripts in each groups of different seasons are significantly different (p<0.05).

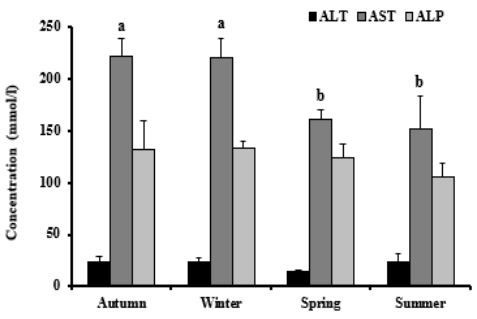

Figure 3. Season changes of transaminase activities (ALT, AST and ALP) in Algerian local donkeys (*Equus asinus*). [a,b] Means (±SE) with the same superscripts in each groups of different seasons are significantly different (p<0.05).

The correlation coefficients between T concentrations and different biochemical parameters are summarized in Table 2. The analysis of the correlation coefficients between T concentration and cholesterol (r=0.894), AST (r=0.537) and ALP (r=0.51) shows high positive correlations (P<0.01). Also, the highest correlation coefficient found between cholesterol and triglyceride (r=0.642), and AST (r=5.28) (P<0.01). In the other hand, there was a high negative correlation (P<0.001) between photoperiod and T concentrations, cholesterol and AST (-0.61, -0.74 and -0.64, respectively) (Table 3).

Table 2. Correlation coefficients (r) between, plasma testosterone (T), total cholesterol (Ch), triglyceride (Tg), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in donkeys (*Equus asinus*).

	T	Ch	Tg	ALT	AST	ALP
T	1					
Ch	0.894***	1				
Tg	0.446*	0.642***	1			
ALT	0.073	**0.105**[s]	-0.175	1		
AST	0.537**	0.528**	0.145	0.252[s]	1	
ALP	0.51**	0.19	0.3	-0.152	0.183	1

*P<0.05, **P<0.01, ***P<0.001

Table 3. Correlation coefficients (r) between photoperiod and testosterone, total cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in donkeys (*Equus asinus*).

Parameters	Correlation coefficient
Photoperiod and testosterone	-0.61*
Photoperiod and cholesterol	-0.74*
Photoperiod and triglyceride	-0.24
Photoperiod and ALT	-0.31
Photoperiod and AST	-0.64*
Photoperiod and ALP	-0.35

*P < 0.05

DISCUSSION AND CONCLUSION

It is known that equines have been considered for a long time as seasonal breeders with sexual activity being related to long days. However, some studies have demonstrated that donkey females (*Equus asinus*) can cycle throughout the year (Contri et al., 2014). Also, the ovarian activity, pregnancy, and parturition appear to be much less seasonal in domestic donkeys than in wild donkeys (Ginther et al., 1987). In another investigation, it has been reported that female donkeys can presented an oestral cycle year-round in the southern United States, while 64% of the females have a cycle in December in Wisconsin (Tibary, 2004). In Morocco, field observations show that the birthing peak season is in March and a few sporadic births in February and from June to September (Tibary et al., 2006). Further, the results obtained by Carluccio et al., (2013) showed that the season has no effect on testicular morphometry and semen characteristics. One study conducted by Canisso et al., (2019) reported an increase of percentage of sperm morphology and decreased of motility in the spring and summer in comparison with the autumn. Another study concluded that there were no apparent seasonal variations in semen quality (Gastal et al., 1997; Canisso et al., 2019). To our knowledge, the present study is the first to assess the influence of season on T concentration and biochemical parameters in Algeria local breed donkeys, and this could help to determine the reproduction season in the northern of Algeria.

Testosterone is known as the male sexual hormone that controls the changes of epididymis and testis, as well as accessory glands. It is essential and responsible for

increasing sexual activity and the testicular size expressed by the proliferation of Leydig cells and germ cells. The results obtained revealed that the T concentrations in the short days were significantly higher than in the long day in Algeria local donkey breeds. According to Aissanouand Ayad, (2020; 2022), the donkey breeds living in northern Algeria express a seasonal influence on biometry and histophotometry of testis, *i.e.* during short days length; this is in full agreement with data registered under same latitudes. This could be explained by coincident with spermatogenesis and peak testosterone levels in winter and autumn seasons. In addition, the increased testosterone may be caused by increased androgen levels paralleling increased sexual activity in winter and autumn. Also, Schuler et al., (2019) recorded plasma T concentration values in donkeys were similar to the results of the present study. However, Jiaha, (1983) revealed that the mean T concentrations in the breeding season (Mar-Sep) were higher than in the non-breeding season (Oct-Feb) in Guan-Zhong donkey. These differences among studies may be due to differences betweenbreeds, feeding level, season, photoperiod, and different latitude, as described in other specie animals (Aldori et al., 2017; Ismaeel 2018; Ait-Amran et al., 2013), it is likely to be due to heterogeneous management in various agro-climatic regions and feed practices (Gupta et al., 2016; Kabukçu et al., 2020).

Lipids have an important position in the organism function and serve as hormones or hormonal precursors such as steroidogenesis. (Welteand Gould, 2017; Maxfield and Tabas, 2005). The results of this study revealed that the serum biochemical parameters of Algerian local donkeys recorded in this study are very similar to those published previously in wild donkeys (*Equus africanus asinus*) (da Silva et al., 2018) and German donkeys (*Equus asinus*) (Schuler et al., 2019). In our study, Ch and Tg levels were significantly higher ($P<0.001$) in winter and autumn, which corresponds to a period of short days. This result is in agreement with previous reports (Roy et al., 2004; Longodor et al., 2020), in which Ch and Tg levels were significantly higher in winter than in summer in Indian and Romanian donkeys. Also, Seri et al., (2010) has reported that the values of (ALT, AST, Ch and Tg) showed significant seasonal changes in Sudanese donkeys and the highest concentrations were detected during the rainy season. Moreover, Hasković and Suljevi, (2011) recorded similar results to the present study in Bosnian mountain horse, whose enzymatic activity of AST and ALT showed significant variation in seasonal changes between summer and autumn season, while ALP shows no significant seasonal changes. Conversely, our findings noticed that Tg, Chol and AST values were lower in Algerian local donkeys compared to England donkeys, mentioned in previously by Burden et al., (2016). Our results could be explains by the existence of a close relationship between AST, located in the spermatozoa mid-piece part, and sperm motility; therefore, membrane defect and loss of AST lead to blockade of ATP production (Pesch et al., 2006). In the literature, the enzyme system (AST, ALT, and ALP) plays an essential role in the sperm metabolic processes, providing energy for sperm survival, motility, and fertility in stallions (Kosiniak et al., 2000; Turner and McDonnell, 2003). Likewise, it has been reported that transaminase activities (AST and ALT) in semen are a good indicator of sperm quality as they measure the stability of the sperm membrane. (Sirat et al., 1996; Corteel et al., 1980).

A significant positive correlation was recorded between the T concentrations and biochemical parameters; this probably due to steroidogenesis increased and the sperm production. Cholesterol is one of the precursors for steroid biosynthesis and is used for the synthesis of testosterone (Amerkhanov et al., 2013). In addition, several enzymes have been identified in the accessory glands in seminal plasma of stallions. These correlations study of enzyme activities with testosterone might be provides information on semen quality and fertility of stallions (Kareskoski, 2011). It was reported that photoperiod is the most important environmental factor that affect reproduction function in donkeys (Cappai et al., 2017; Deikman et al., 2002), this because of its influence on circulating levels of gonadotropins and gonadal hormones. The data of this study showed a correlation of high negative correlation ($P<0.001$) photoperiod with T, Ch, and AST in Algerian donkeys. The findings of this study are also consistent with those published previously, which showed significant correlations between T values with photoperiodic data in the Guan Zhong donkey (Jiaha, 1983). Note that the photoperiod influences seasonal breeders through changes in melatonin secretion that affects the function of the hypothalamic – pituitary – testicular axis (Vivid and Bentley, 2018).

The present study is the first to describe the seasonal variations of T concentrations and biochemical parameters in Algerian local donkeys. Our finding suggests that sexual activity probably occurs during winter and autumn in local donkeys of Northern Algeria. In addition, our results of Ch and Tg levels are correlated with the season, which may be a good indication of the reproductive performance in donkeys. However, other approaches such as sperm analysis are necessary to undertake to strengthen these results. Further the measuring of the melatonin level is needed to confirm the photoperiodic aspect in male donkey.

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Conflict of Interest
The authors declare that they have no competing interests.

Authorship contributions
Concept: A.A., Data Collection or Processing: S.A., A.A., Analysis or Interpretation: S.A., A.A. B.O., B.M., Literature Search: S.A., B.O., B.M., Writing: S.A., A.A.

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