*European Bison Conservation Newsletter Vol 2 (2009) pp: 1–10*

# Difficulties with the choice of proper method for determination of serum levels of total testosterone (TT) in European bison males

Elżbieta Czykier

Department of Histology and Embryology, Medical University in Białystok

**Abstract:** The objective of the study was to compare two methods used to determine serum levels of total testosterone (TT) in European bison *Bison bonasus* (Linnaeus, 1758) males: the radioimmunological method (RIA) and the immunoenzymatic method (ELISA). The study material included sera from 83 European bison males aged 2 months – 19 years, which were culled in the autumn and winter periods (in the post-rutting season), in the years 1993–2002, in the Białowieża Primeval Forest. TT levels were determined using the two methods (RIA and ELISA) in the same animals. The following age groups were distinguished: class I – calves under the age of 1 year, class II –juveniles under the age of 2 years, class III –juveniles under the age of 3, class IV – adult males aged 4–5 years, class V – adult males aged 6–12 years, class VI-old bulls over the age of 12. In our study, serum TT levels measured using the RIA method were lower, higher or similar in comparison with those determined by ELISA in the same animals, although higher concentrations were more frequent when the ELISA method was used. With the RIA method, we obtained 26 results (31.30%) below the bottom limit for this method, i.e. 0.14ng/ml. Included in this number there were 18 animals in class I (56.25%), 6 in class II (37.50%), 1 in class III (14.28%) and 1 in class V (11.11%). The mean serum levels of TT determined by ELISA were higher than those obtained using RIA in the same age classes, the difference being statistically significant in class I and II ($p < 0.001$), and on the border of statistical significance in class IV and VI ($p < 0.088$ and $p < 0.047$, respectively).

**Key words:** European bison, testosterone, ELISA, RIA

## Introduction

Total testosterone (TT), which belongs to androgens representing steroid hormones, originates via synthesis from cholesterol. In males, Leydig cells forming the interstitial gland of the testis are the main site of TT production, with minor function of the reticular layer of suprarenalis. TT promotes spermatogenesis in seminiferous tubules of the testes, is responsible for the maintenance of the structure and function of the outlet pathways and accessory glands, affects the development and maintenance of secondary sexual characteristics. Moreover, as an anabolic hormone TT causes proliferation of cells, including skeletal muscle cells. In the circulatory system, TT combines with sex hormone-binding globulins (SHBG).

In a monograph on European bison *Bison bonasus* (Linnaeus, 1758), the author compares serum levels of TT in young bulls and adult males (Gill

1999). Other authors describe serum levels of free testosterone (FT) in male European bison in postnatal development (Czykier, Krasińska 2006) and compare serum FT in young animal specimens with and without spermiogenesis (Czykier 2008).

In mammals, serum levels of TT have also been determined in other wild ungulates (*Axis axis*, *Capreolus capreolus*, *Cervus elaphus*, *Cervus nippon, Odocoileus virginianus*) (Lincoln, Kay 1979; Haigh *et al.* 1984; Loudon, Curlewis 1988; Suzuki *et al.* 1992; Blottner *et al.* 1996; Bubenik *et al.* 2005) and in domestic animals: cattle (Weathersbee, Lodge 1976; Earl Gray *et al.* 2006), horses (Ganjam, Kenney 1975; Roser, Hughes 1992; Inoue *et al.* 1993; Hoffman, Landeck 1999; Lemazurier *et al.* 2002) and pigs (Dubiel *et al.* 1985).

The aim of the current study is comparison of two methods used to determine serum levels of total testosterone in European bison males, i.e. the radioimmunological method (RIA) and the immunoenzymatic method (ELISA).

## Materials and Methods

The material used for analysis included serum samples obtained from 83 European bison males aged 2 months – 19 years, culled in autumn and winter seasons (in the post-rutting period) (Krasiński, Raczyński 1967), in the years 1993–2002, within the Białowieża Primeval Forest. The animals, which were culled to reduce the population number, were chosen for elimination for various reason, including diseases, especially necrotic *posthitis*, poor condition, exterior defects, injuries of various origin, aggression towards people or senile age (Krasińska, Krasiński 2007). The age of bison from the free-ranging population was determined by Krasiński (BNP) according to a sequence of deciduous tooth eruption and exchange from deciduous into permanent dentition (Węgrzyn, Serwatka 1984). The bison were shot in the morning hours. Whole blood was collected from the femoral artery (post mortem) and then sera were centrifuged in the Mammal Research Institute, PAS in Białowieża. The serum samples were frozen and stored at –20°C.

The study animals were divided into age classes: class I – calves up to the age of 1 year, class II –juveniles under the age of 2 years, class III –juveniles under the age of 3, class IV – adult males aged 4–5 years, class V – adult males aged 6–12 years, class VI-old bulls over the age of 12 (Table 1). The division into 6 age classes resulted from our earlier study (Czykier *et al.* 1999, Czykier, Krasińska 2004), which revealed morphological differences in the course of spermatogenesis in seminiferous tubules of the bison testes between these classes.

The serum level of TT in European bison males were determined using two methods: the radioimmunological method (RIA) No 68628 (Orion Diagnostica, Espoo, Finland) with a sensitivity of 0.14 ng/ml and the immunoenzymatic

**Table 1.** The number of European bison in the respective age classes having serum level of testosterone <0.14ng/ml (RIA).

| Age class | n | <0.14 ng/ml | % |
|---|---|---|---|
| Calves (< 1 yr) | 32 | 18 | 56.25 |
| Juveniles (2 yrs) | 16 | 6 | 37.50 |
| Juveniles (3 yrs) | 6 | 1 | 14.28 |
| Adult males (4–5 yrs) | 12 | 0 | 0 |
| Adult males (6–12 yrs) | 9 | 1 | 11.11 |
| Old bulls (13–19 yrs) | 8 | 0 | 0 |
| Total | 83 | 26 | 31.30 |

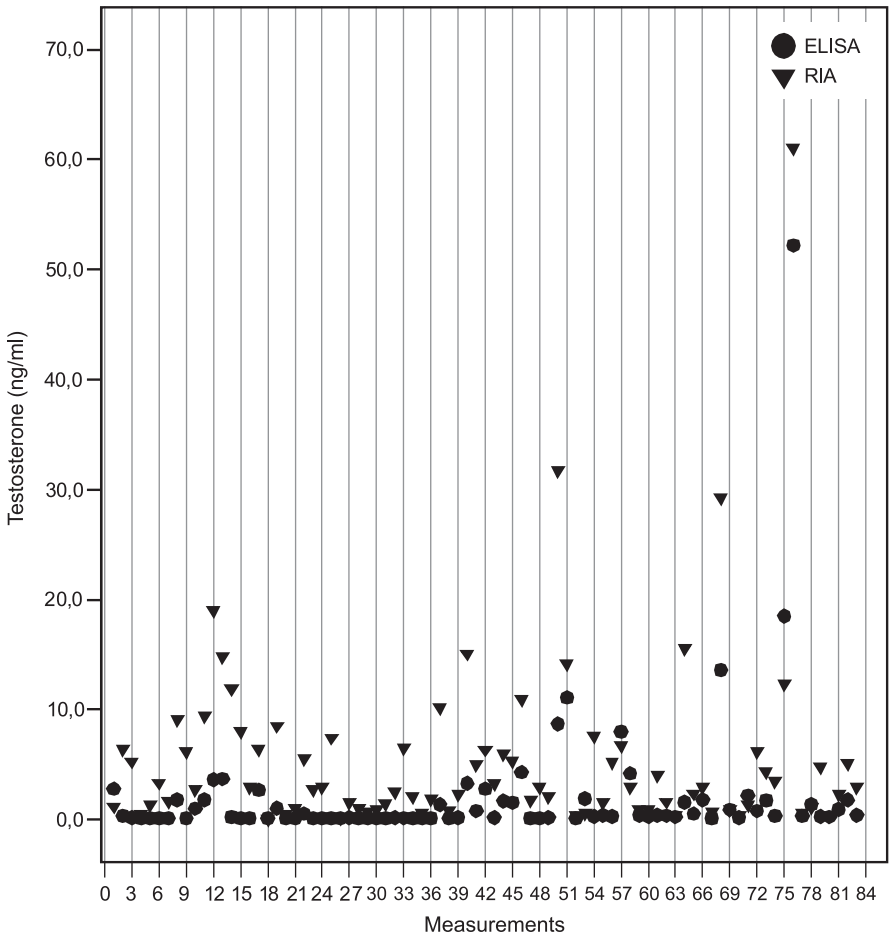


**Figure 1.** Serum levels of testosterone in the same 83 European bison specimens measured both by RIA and ELISA.

**Table 2.** The mean serum levels of testosterone in European bison in 6 age classes measured by RIA and ELISA. Class I-calves up to one year old, class II-juveniles up to 2 years old, class III-juveniles up to 3 years old, class IV-adult males 4 and 5 years old, class V-adult males 6 and 12 years old, class VI-old bulls above 12 years old.

| Age class | n | Testosterone [ng/ml] | | | | | | | | p |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | | SD | | Min | | Max | | |
| | | RIA | ELISA | RIA | ELISA | RIA | ELISA | RIA | ELISA | |
| I | 32 | 0.71 | 4.61 | 1.07 | 4.58 | 0.14 | 0.07 | 3.7 | 19.04 | 0.001 |
| II | 16 | 1.08 | 5.08 | 1.34 | 4.06 | 0.14 | 0.59 | 4.3 | 15.09 | 0.001 |
| III | 6 | 3.72 | 9.43 | 4.89 | 12.14 | 0.14 | 0.42 | 11.1 | 31.77 | 0.18 |
| IV | 12 | 1.55 | 3.77 | 2.33 | 4.16 | 0.26 | 0.35 | 8 | 15.57 | 0.088 |
| V | 9 | 4.27 | 6.57 | 6.82 | 9.33 | 0.14 | 0.42 | 18.5 | 29.29 | 0.28 |
| VI | 8 | 7.20 | 9.79 | 18.19 | 20.79 | 0.25 | 0.38 | 52.2 | 61.06 | 0.047 |
| Total | 83 | 2.13 | 5.64 | 6.32 | 8.46 | 0.14 | 0.07 | 52.2 | 61.06 | 0.001 |

method (ELISA) No DSL–10–4000 (Diagnostic Systems Laboratories, Webster, USA) with a sensitivity of 0.04 ng/ml. Serum testosterone levels in the same European bison males were determined by RIA in the Laboratory of the Department of Gynecological Endocrinology, University Hospital in Białystok, and by ELISA in the Laboratory of the Department of Endocrinology and Internal Diseases, University Hospital in Białystok. The t-Student test for pairs was used to make a comparison between the two methods.

## Results

In our study, serum levels of TT measured in 83 European bison males using the RIA method were lower, higher or similar as compared to those determined by ELISA in the same animals, although higher concentrations were more frequent when the ELISA method was used (Fig. 1).

Serum TT concentrations in the same European bison specimens measured by ELISA and RIA were most similar in age classes V and VI, being most different between class I and class II.

With RIA, we obtained 26 results (31.30%) below the bottom limit for this method (0.14ng/ml). In this number there were 18 animals in class I (56.25%), 6 in class II (37.50%), 1 in class III (14.28%) and 1 in class V (11.11%) (Table 1).

The mean serum TT levels determined using the ELISA method were higher in all age classes as compared to those obtained with RIA in the same age classes, the difference being statistically significant in class I and II ($p < 0.001$), and on the border of statistical significance in class IV and VI ($p < 0.088$ and $p < 0.047$, respectively) (Table 2). The mean serum level of TT

in all the 83 European bison males determined by the ELISA method was found to be 5.64 ± 8.46 ng/ml (range 0.07–61.06 ng/ml), whereas using the RIA method 2.13 ± 6.32 ng/ml (range 0.14–52.2 ng/ml). The mean difference between the measurements using the two methods was 3.51 ± 4.47 ng/ml (range –6.13–23.07 ng/ml, $p < 0.001$).

## Discussion

The mean serum TT levels measured by the ELISA method in the current study are higher than those presented by Gill (1999) (also using ELISA), both in young and adult European bison bulls. However, with the use of the RIA method in our study, the mean serum levels of TT in sexually immature animals were lower in age classes I and II, but higher in class III as compared to the concentrations obtained from young bulls by Gill (1999) using the ELISA method. With the RIA method, in sexually mature animals, we obtained lower mean TT levels in age class IV as compared to the findings reported by Gill (1999) from adult bulls, whereas in classes V and VI our RIA measurements were higher than those obtained by Gill (1999) for adult bulls.

The mean serum levels of TT obtained in the current study in age class IV European bison with the RIA method are similar to the data reported by other authors from wild living animals: tropical deer, male wapiti, red deer stag (Loudon, Curlewis 1988; Haigh *et al.* 1984; Lincoln, Kay 1979) using the same method. The mean serum TT concentrations found in our study in age class V and VI European bison (also with the RIA method) resemble those noted by other researchers in wild sika deer and bulls (Suzuki *et al.* 1992, Weathersbee, Lodge 1976). However, the mean serum levels of TT obtained in our study from class V and VI adult European bison specimens (measured with both methods – RIA and ELISA) differ from those reported by other authors using the RIA method. Our results are higher than those found in tropical deer, male wapiti, red deer stag, white tailed deer and stallions (Lincoln, Kay 1979; Haigh *et al.* 1984; Loudon, Curlewis 1988; Roser, Hughes 1992; Hoffman, Landeck 1999; Bubenik *et al.* 2005).

In our study, the mean serum levels of TT in class V and VI adult European bison determined by the ELISA method are by 35.35% and 33.33% higher than those obtained using the RIA method. It seems to us that each of these methods can be used for adult European bison belonging to these two age classes.

Among 48 sexually immature animals, under 2 years of age, in 24 (50%) serum TT level was below the bottom limit for RIA. Moreover, in these animals the mean serum levels of total testosterone determined by RIA account for 15.20% (class I) and 21.20% (class II) of the mean TT levels found when the ELISA method was used in the same animals. Considering these two aspects, we suggest that measurements of serum testosterone in young animals should rather be performed with the ELISA method.

In the current study, the RIA and ELISA methods used to determine TT levels in the serum of European bison yielded different results. However, this may be due to human error, since each of these measurements was performed in another laboratory by a different team of professionals.

## Acknowledgements

I am grateful to Prof M. Krasińska and Dr Z.A. Krasiński for the assistance in collecting the material and making accessible documentation concerning culled animals, as well as to Arkadiusz Arasim and Paweł Kordecki, technical staff of the Mammal Research Institute PAN in Białowieża, for their help with the preparation of the material for further investigations.

## References

Blottner S., Hingst O., Meyer H.H.D. 1996. Seasonal spermatogenesis and testosterone production in roe deer (*Capreolus capreolus*). J Reprod Fert. 108: 299–305.

Bubenik G.A., Miller K.V., Lister A.L., Osborn D.A., Bartos L., Van Der Krak G.J. 2005. Testosterone and estradiol concentrations in serum, velvet skin, and growing antler bone of male white-tailed deer. J Exp Zool. 303A: 186–192.

Czykier E., Sawicki B., Krasińska M. 1999. Postnatal development of the European bison spermatogenesis. Acta Theriol. 44: 77 – 90.

Czykier E., Krasińska M. 2004. Cases of spermiogenesis in young European bison. Acta Theriol. 49: 543 –691.

Czykier E., Krasińska M. 2006. Stężenie wolnego testsoeronu w surowicy samców żubra (*Bison bonasus*) w zależności od wieku, masy ciała i masy jąder-badanie wstępne. Parki nar Rez Przyr. 25: 119–131. [In Polish with English summary].

Czykier E. 2008. Serum concentration of free testosterone (FT) in European bison with early spermiogenesis-a preliminary study. European bison conservation newsletter 1: 65–71.[In Polish with English summary].

Dubiel A., Stańczyk J.F., Barcikowski B., Romanowicz K., Houszka M. 1985. Stężenie testosteronu w osoczu krwi knurów rasy wbp w różnym wieku. Med Wet. 11: 687-691. [In Polish].

Earl Gray L.J.R., Wilson S., Stoker T., Lambright C., Furr J., Noriega N., Howdeshell K., Ankley G.T., Guillette L. 2006. Adverse effects of environmental antiandrogens and androgens on reproductive development in mammals. Int J Androl. 29: 96–104.

Ganjam V.K., Kenney R.M. 1975 Androgens and oestrogens in normal and cryptorchid stallions. J Reprod Fert. Suppl. 23: 67–73.

Gill J. 1999. Zarys fizjologii żubra. Wydawnictwo Severus, Warszawa: 42–60. [In Polish].

Haigh J.C., Cates W.F., Glower G.J., Rawlings N.C. 1984. Relationships between seasonal changes in serum testosterone concentrations, scrotal circumference and sperm morphology of male wapiti (*Cervus elaphus*). J Rep Fert. 70: 413–418.

Hoffmann B., Landeck A. 1999.Testicular endocrine function, seasonality and semen quality of the stallion. Anim Reprod Sci. 57: 89–98.

Inoue J., Cerbito W.A., Oguri N., Matsuzawa T., Sato K. 1993. Serum levels of testosterone and oestrogens in normal and infertile stallions. Int J Androl. 16: 155–158.

Krasińska M., Krasiński Z.A. 2007. European bison. The Nature Monograph. Mammal Research Institute, Białowieża 1–317.

Krasiński Z., Raczyński J. 1967. The reproduction biology of European bison living in reserves and freedom. Acta Theriol. 12: 407–444.

Lemazurier E., Toquet M.P., Fortier G., Seralini G.E. 2002. Sex steroids in serum prepubertal male and female horses and correlation with bone characteristics. Steroids. 67: 361–369.

Lincoln G.A., Kay R.N.B. 1979. Effects of season on the secretion of LH and testosterone in intact and castrated red deer stags (*Cervus elaphus*). J Reprod Fert. 55: 75–80.
Loudon A.S.I., Curlewis J.D. 1988. Cycles of antler and testicular growth in an aseasonal tropical deer (*Axix axis*). J Reprod Fert. 83: 729–738.
Roser J.F., Hughes J.P. 1992. Seasonal effects on seminal quality, plasma hormone concentrations, and GnRH-induced LH response in fertile and subfertile stallions. J Androl. 13: 214–23.
Suzuki M., Kaji K., Nigi H. 1992. Annual changes of testis size, seminiferous tubules and plasma testosterone concentration of wild sika deer (*Cervus nippon yesoensis* Heude, 1884) in Hokkaido. J Vet Med Sci. 54: 551–556.
Weathersbee P.S., Lodge J.R. 1976. Serum testosterone and estrogen concentrations in the Holstein-Friesian bull after successive ejaculations. Am J Vet Res. 37: 465–7.
Węgrzyn M., Serwatka S. 1984. Teeth eruption in the European bison. Acta Theriol. 29: 111–121.

**Trudności z wyborem właściwej metody oznaczania stężeń całkowitego testosteronu (TT) w surowicy samców żubra**

**Streszczenie:** Celem obecnego badania jest porównanie dwóch metod służących do oznaczania stężeń całkowitego testosteronu (TT) w surowicy: metody radioimmunologicznej (RIA) oraz metody immunoenzymatycznej (ELISA). Do badań użyto surowice 83 samców żubrów w wieku od 2 miesięcy do 19 lat, pobrane od zwierząt odstrzelonych w sezonach jesienno-zimowych (po zakończeniu okresu rujowego) w latach 1993–2002 na terenie Puszczy Białowieskiej. Stężenia TT oznaczano w surowicy tych samych zwierząt dwiema metodami: metodą radioimmunologiczną (RIA) oraz metodą immunoenzymatyczną (ELISA). Zwierzęta podzielono na następujące klasy wiekowe: I-cielęta do 1 roku, II-młodzież w wieku do 2 lat, III-młode samce w wieku do 3 lat, IV-dorosłe samce w wieku 4–5 lat, V-dorosłe samce w wieku 6–12 lat, VI-stare samce powyżej 12 roku. W niniejszym badaniu u tych samych osobników wartości stężeń TT w surowicy mierzone metodą RIA były niższe, wyższe lub zbliżone do wartości stężeń testosteronu mierzonych metodą ELISA, przy czym częściej obserwowaliśmy wyższe wartości stężeń testosteronu w surowicy u tych samych zwierząt przy zastosowaniu metody ELISA. Po dokonaniu pomiarów stężeń TT w surowicy tych samych osobników dwiema metodami RIA i ELISA stwierdziliśmy, że po zastosowaniu metody RIA otrzymaliśmy 26 wyników (31.30%) poniżej dolnej granicy normy dla tej metody wynoszącej 0.14ng/ml. Przy czym w I klasie wiekowej było 18 takich zwierząt (56.25%), w II 6 osobników (37.50%) w III i V klasach wiekowych po 1 żubrze (14.28% i 11.11%). Średnie stężeń TT w surowicy zwierząt wykonane metodą ELISA wykazywały wyższe wartości we wszystkich klasach wiekowych w porównaniu do średnich stężeń TT w tych samych klasach wiekowych oznaczonych metodą RIA, przy czym w I i II klasie wiekowej była to różnica istotna statystycznie ($p < 0.001$), natomiast w IV i VI klasie wiekowej były to różnice na granicy istotności statystycznej ($p < 0.088$, $p < 0.047$).