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DEVELOPMENT OF A BOVINE THYROTROPIN (TSH) RADIOIMMUNOASSAY AND ITS APPLICATION IN THYROID FUNCTION STUDIES IN CATTLE

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A specific and relatively simple radioimmunoassay for the determination of bovine thyrotropin (bTSH) in bovine serum samples has been developed in our laboratory. Essential data on the preparation and evaluation of the basic components for the determination of bTSH, the precision and reproducibility of the test and its application in studies of thyroid function in cattle are presented.

Key words: radioimmunoassay, thyrotropin, cattle, thyroid function, iodine supplementation.

INTRODUCTION

Thyroid hormones have a pronounced effect on the efficiency and rate of growth as well as the proportion of lean to fat in the carcass of meat animals. In large, high-productive animals, thyroid hormone deficiency may cause disturbances in growth rates, milk and meat production and reproductive performance (Jovanović, 1990). The regulation of synthesis and secretion of thyroid hormones is dependent on the pituitary secretion of thyroid stimulating hormone, thyrotropin (TSH), by a physiological negative feed-back mechanism. Elevated concentrations of thyroid hormones in the circulation decrease pituitary secretion of TSH while low hormone levels due to iodine deficiency and disturbance in iodine utilization (antithyroid substances) increase TSH secretion leading to increased activity and growth of thyroid follicle cells. Thus, simultaneous follow up of blood levels of thyroid hormones and thyrotropin are necessary for proper investigation of pituitary - thyroid axis function in man and animals.

In order to study thyroid function in cattle we have prepared a specific-double - antibody radioimmunoassay (RIA) of bovine TSH (bTSH). A similar radioimmunological system for determination of human TSH was previously develepod in our laboratories (Kostić et al., 1978). Four basic components are necessary for the radioimmunological determination of bTSH:a) radioiodinated bTSH, b) antiserum against bTSH,c) secondary antibody as immunoadsorbent and d) standard solutions of bTSH. Essential data on the preparation and

evaluation of the components for the determination of bTSH and on the possibility of applying the assay in the study of cattle thyroid function are presented in this paper.

MATERIALS AND METHODS

Radiolabelling of bTSH. A highly purified preparation of bovine TSH (NIAD-DK bTSH-I-1, 21 IU/mg) was labelled with ^{125}I by the slightly modified method of Hunter and Greenwood (1962) using chloramine T. ^{125}I - labelled bTSH was separated from the unreacted, free radioiodine and other components of the reaction mixture by gel filtrationon a Sephadex G-75 column (1,5 x 30 cm) by eluting with 0,05 M phosphate - buffered saline (pH 7.4.)

Testing of antiserum. Antiserum against bovine TSH of high specific titre and avidity was supplied by Dr J. G. Pierce. The optimal antiserum titer for use in the test was determined by incubating serial dilutions of the original anti bTSH antiserum with the radioiodinated bTSH.

Standard solutions. Standard solutions of bTSH (NIAMDD bTSH-9) were prepared in several concentrations (7,8 - 500 mU/l) and standardized against NIH-bTSH reference standard.

Preparation of immunoadsorbent. Purified sheep-anti rabbit IgG (second antibody) was used for the precipitation of bTSH - anti bTSH complex. The second antibody was previously bound to CNBr - activated microcrystalline cellulose as described by Wide (1969).

The assay procedure. To tubes containing 0.2 ml of PBS were added 100 ml of anti bTSH antiserum and 100 ml of labelled bTSH (~40.000 cpm). After incubation for 18 to 20h at room temperature 0.5 ml of sheep anti rabbit gamma globulin in 2-5% microcrystalline cellulose was added. The tubes were further incubated for 2h at room temperature, centrifuged for 10 min at 3000 rpm and the free ^{125}I-bTSH was removed by aspiration of the supernant. The radioactivity of the precipitates was counted in a gamma scintillation counter.

Collecting and storage of blood samples. Blood samples were supplied by the local slaughterhouse and by the "Jedinstvo" - Gaj cattle farm, Kovin. The blood samples were collected by jugular puncture and kept at room temperature until they clotted, then centrifuged at 2000 rpm for 10-15 min. Each serum supernatant was collected and kept at -20^{0}C until use.

Serum thyroxine (T4) and triiodothy-ronine (T_3) measurement. Commercially available radioimmunoassay kits (INEP, Zemun) were used for the determination of T_4 and T_3 concentrations in the serum samples.

RESULTS AND DISCUSSION

In figure 1. the separation of radiolabelled bTSH from unreacted, free radioiodine on a Sephadex G-75 column is shown. The specific activity of the labelled bTSH was from 250-300 mCi/mg. The fractions from the first peak were pooled (as indicated on the figure) and used for the test.

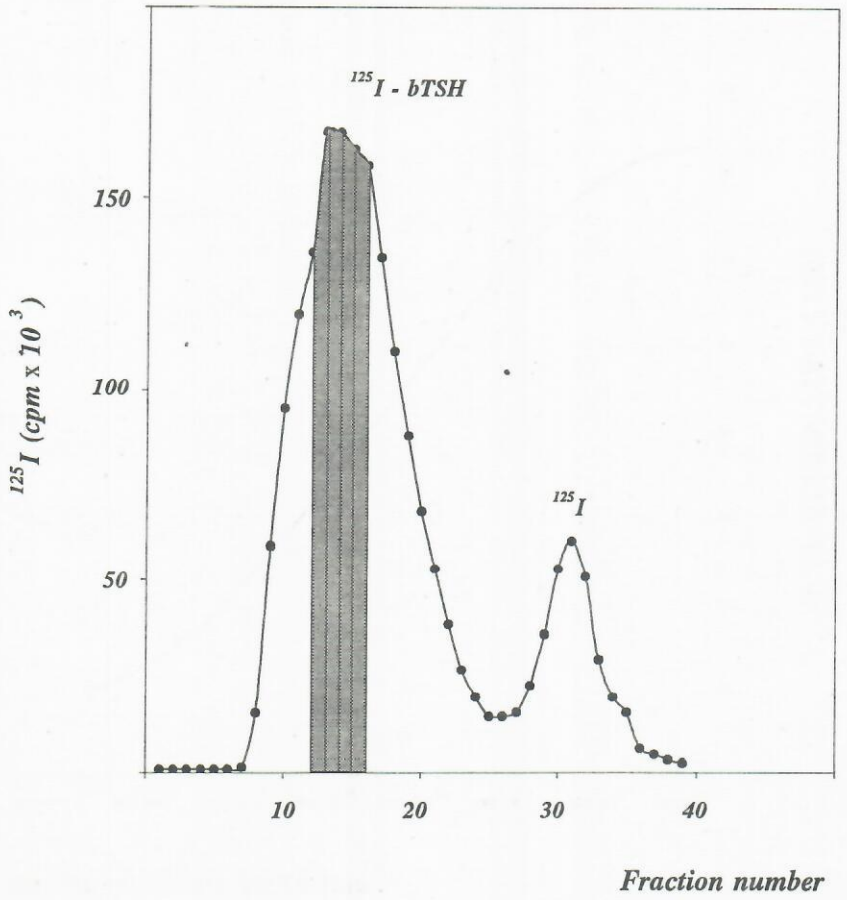

Figure 1. Separation of bTSH labelled with ^{125}I from unreacted (free) ^{125}I on a Sephadex G-75 column (1.5 x 30 cm). The hatched column in the first peak indicates the fractions used for radioimmunoassay.

Using bTSH labelled with radioactive ^{125}I, the binding properties of the original anti bTSH antiserum were determined. The proper choice of antibody dilution is of great importance for accurate determination of the hormone concentration in serum. The optimal antiserum titer was determined by incubating serial dilutions of the original antiserum with radioiodinated bTSH. The binding capacity as a function of the dilution of the antiserum is presented in figure 2.

Maximal binding at excess antibodies was about 75%. The 50% binding point was reached at the antiserum dilution of approximately 1:100.000. For the test, the maximal binding capacity was chosen to be about 25%, corresponding to 1:500.000 for the final dilution of antiserum. This dilution provides maximal

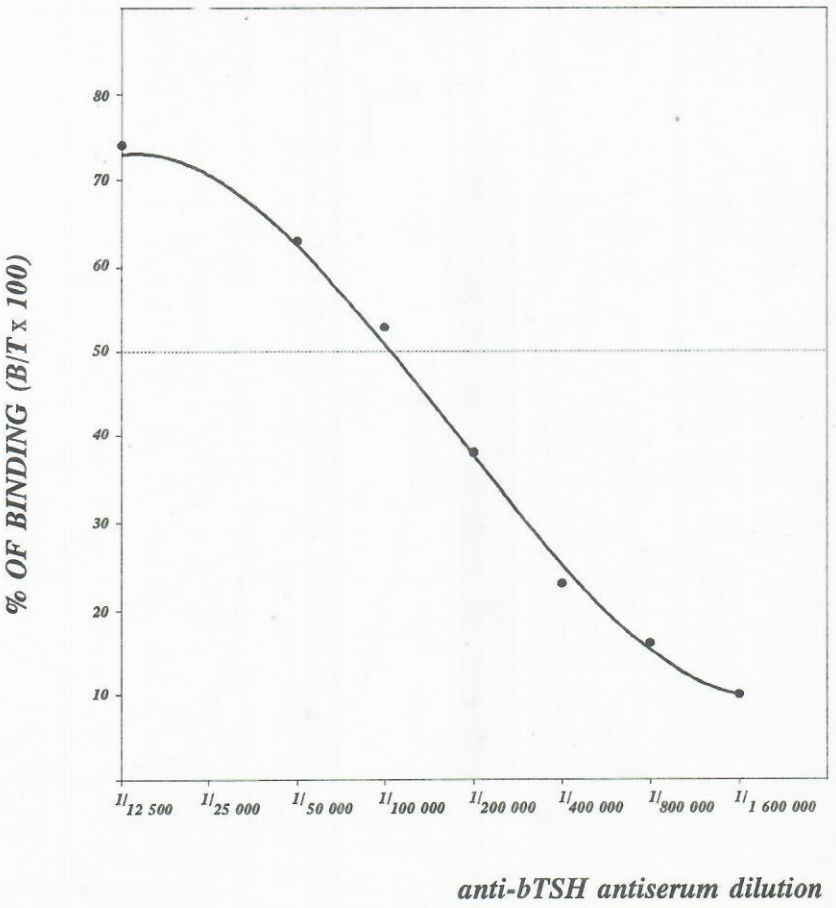

Figure 2. The response curve obtained by plotting dilution of antibovine TSH antiserum against percent binding of ^{125}I-bTSH. B/T x 100=% of total radioactivity.

accuracy for the determination of bTSH in all parts of the standard curve as shown in figure 3.

It can be seen that the addition of increased of unlabelled bTSH (cold) in the presence of excess sheep - anti rabbit IgG decreasd progressively the relative amount of bound ^{125}I - bTSH. At the antiserum dilution of 1:500.000, the amount of ^{125}I-bTSH bound in the tube without cold bTSH was 25-30% (maximal binding). The binding of labelled TSH in the absence of the antibody amounted to about 3-5% (nonspecific binding).

In order to define the range of normal bTSH concentrations, blood samples were collected from cattle of the domestic Simmental breed in the local slaughter-

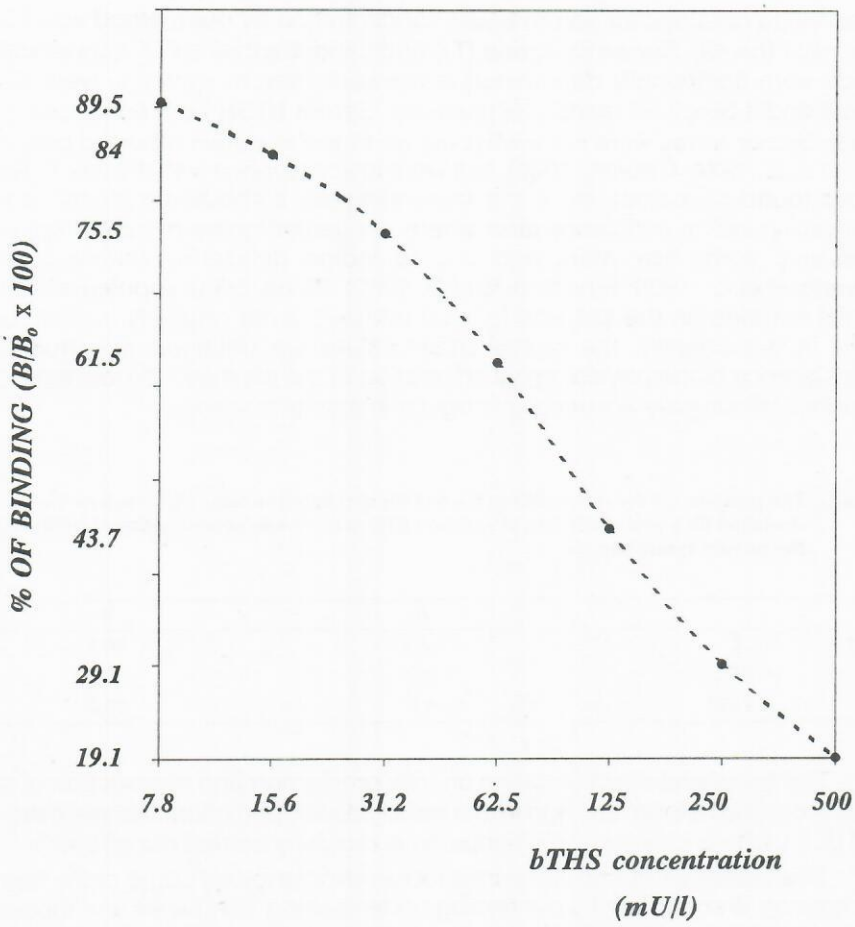

Figure 3. Dose - response curve. The inhibition of binding of ^{125}I-bTSH by increased amounts of unlabelled bTSH is shown on a semilogarithmic plot. B/Bo x 100=% of maximal binding.

house. The bTSH concentrations measured in these serum samples were in the range from 30-50 mU bTSH/1.

A control serum was prepared as a pool of five cattle serum samples and aliquoted into portions. These were assayed during a three month period ten times in order to monitor the reproducibility of the results between tests. Ten aliquots of the control serum were measured in a single test to obtain the within-assay precision. Standard deviations and coefficients of variation were determined and are presented in table 1. It can be seen that the intra-and interassay coefficients of variation (%) were 4.7 and 10.3, respectively.

Serum bTSH concentrations were also determined in 59 nonpregnant Holstein heifers kept on a cattle farm near Kovin ("Jedinstvo" - Gaj, Kovin). The

mean value obtained for serum bTSH concentration by our method was 40.3 ± 10.6 mU/l (n=59). Serum thyroxine (T_4) and triiodothyronine (T_3) concentrations which were additionally determined in the same serum samples, were 47æ14 nmol/l and 1.36æ0.30 nmol/l, respectively. Serum bTSH concentrations determined by our assay were somewhat higher than the values reported previously (Goret et al. 1974; Cabello, 1980), but were in accordance with the low T_4 and T_3 values found concomitantly in the same samples. It should be mentioned that Kovin is an iodine deficiency area where congenital goitre of calves appeared massively some ten years ago due to iodine deficiency during gravidity (Jovanović et la., 1980; Sinadinović et al, 1982). Since iodine supplementation in animal nutrition (in the salt and mineral mixture) is not regularly carried out all times in our country, the higher bTSH values we obtained are probably a consequence of the physiological adaptation of the pituitary - thyroid axis to the presence of naturally accuring goitrogens in animal nutrition.

Table 1. The precision of the results within the test (A) and between tests (B) shown as the standard deviation (SD) and coefficient of variation (CV) of the mean concentration of bTSH found in the pooled serum sample.

	A	B
$\bar{X}$	42.9	46.1
SD	2.0	4.8
CV (%)	4.7	10.3

The beneficial effect of iodine on milk production and reprodution of cattle was recognized a long time ago in industrially developed countries, such the USA and U. K., where iodine supplementation is regularly carried out all times.

The existence of chronic partial iodine deficiency on some cattle farms in this country is confirmed by our finding of decreasing TSH levels and increasing thyroid hormone levels following dietary iodine supplementation on these farms (Sinadinović, 1995). Thus, careful monitoring of thyroid hormone and TSH levels are necessary for timely introduction of iodine supplements to be carried out in order to prevent losses in animal production.

In conclusion, it may be stated that a sensitive, specific and relatively simple double antibody radioimmunoassay for the determination of bovine thyrotropin in serum samples has been developed in anticipation that its application will improve the health and production of cattle.

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PRIPREMA RADIOIMUNOLOŠKOG TESTA ZA ODREĐIVANJE GOVEĐEG TIREOTROPINA I NJEGOVA PRIMENA U IZUČAVANJU FUNKCIJE TIREOIDEJE GOVEDA

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SADRŽAJ

Specifičan i relativno jednostavan radioimunološki test za određivanje goveđeg tireotropina u uzorcima seruma primljen je u našoj laboratoriji. U radu su prikazani bitni podaci o pripremi i evaluaciji osnovnih komponenti potrebnih za određivanje goveđeg tireotropina, preciznost i reproducibilnost test, kao i mogućnosti njegove primene u izučavanje funkcije tireoideje goveda.