


(W.A.)

ABSTRACT

The objectives of this study were to modify and develop technique for determination of 1,25-(OH)2D3 concentrations in sera and milk samples. The developed technique, used non-HPLC for extraction and purification of the samples and involved radio receptor assay which used calf thyriod as 1,25-(OH)2D3 receptor for quantification of 1,25-(OH)2D3. Results of this study were compared to that of the technique developed by Horst et al. (1981) which used HPLC for samples extraction and purification. The correlation between the two techniques was r=0.95. Using the modified technique the 1,25-(OH)2D3 concentrations in cow and sow sera were 57.42±5.3 pg/ml (n=20) and 75.22±6.05 pg/ml (n=20) respectively, whereas the 1,25-(OH)2D3 concentrations in cow and sow milk were 16.62±2.2 pg/ml (n=20) and 21.52±3.125 pg/ml (n=20). Based on the results could be concluded that the technique that had been developed in this study was found satisfactory for determination the 1,25-(OH)2D3 concentration either in serum or milk samples.

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ABSTRAK

Penelitian ini bertujuan memodifikasi dan mengembangkan teknik pengukuran konsentrasi 1,25-(OH)\textsubscript{2}D\textsubscript{3} di dalam serum dan susu. Teknik yang dikembangkan tanpa menggunakan HPLC untuk ekstraksi maupun penskalaan sampel dan melibatkan radio receptor assay menggunakan kelejenar thymus anak sapi sebagai reseptor 1,25-(OH)\textsubscript{2}D\textsubscript{3}. Hasil dari penelitian ini dibandingkan dengan teknik yang telah dikembangkan oleh Horst et al. (1981) yang menggunakan HPLC untuk ekstraksi dan penentuan konsentrasi sampel. Korelasi antara kedua teknik (r=0.98), Menunjukkan tersebut teknik, konsentrasi 1,25-(OH)\textsubscript{2}D\textsubscript{3} di dalam serum sapi dan babi meningkat 57.42 \pm 5.3 pg/ml (n=20) dan 75.22 \pm 6.05 pg/ml (n=20) sedangkan konsentrasi 1,25-(OH)\textsubscript{2}D\textsubscript{3} di dalam susu sapi dan babi meningkat 16.62 \pm 2.2 pg/ml (n=20) dan 21.52 \pm 3.125 pg/ml (n=20). Dari hasil ini dapat disimpulkan bahwa teknik yang dikembangkan dalam penelitian ini cukup memuaskan untuk penentuan konsentrasi 1,25-(OH)\textsubscript{2}D\textsubscript{3} baik di dalam serum maupun susu.

INTRODUCTION

1,25-dihydroxycholecaliferol (1,25(OH)\textsubscript{2}D\textsubscript{3}) is the most active metabolite of vitamin D\textsubscript{3} (DeLaca, 1981). It is considered as a steroid hormone produced by the kidney. Measurement of (1,25(OH)\textsubscript{2}D\textsubscript{3}) in serum is helpful in the diagnosis and management of various diseases associated with vitamin D and mineral metabolism disorders such as: parathyroid gland disorders; renal failure; certain bone diseases; sarcoidosis; parturient hypocalcaemia etc. Because changes in circulating 1,25(OH)\textsubscript{2}D\textsubscript{3} are physiopathological importance in many diseases, the development of an assay that is specific for and sensitive to 1,25(OH)\textsubscript{2}D\textsubscript{3} is really required. Several different assay techniques namely: high performance liquid chromatography (HPLC) (Jones, 1978); bioassay (Stern et al. 1978) and radioimmunoassay (RIA) (Clemen et al. 1980; Bouillon et al., 1980) have been reported previously. However, the methods frequently involve difficult extraction's, very laborious procedures and require relatively large amount of sample. The objective of this study was to find techniques which could reduce the high and instrumental cost and the total amount of samples requirement. A new assay was developed by Reinhardt et al. (1984) combined with

MATERIAL AND METHODS

Sample extraction. Sera and milk collected from 20 cows and 20 sows were used as samples. Half to four millilitres of each sample was pipetted into a 12 x 75 mm borosilicate glass tube. Fifty microliters of ethanol buffer containing 1700 disintegrations per minute (DPM) of \textsuperscript{3}H 1,25(OH)\textsubscript{2}D\textsubscript{3} were added to tube and also to a scintillation vial containing 5 ml scintillation liquid for calculating recoveries. One volume of acetonitrile was added to each sample and each tube was then vortexed for 5 seconds and centrifuged for 10 minutes at 760 x g. While the samples were being centrifuged, Sep-Pak C\textsubscript{18} columns were prepared by washing with 5 ml acetonitrile followed by two 5 ml washed distilled water.

After centrifugation, the supernatant was poured off into another 12 x 75 mm glass tube containing 0.5 volume of 0.4 M potassium phosphate (pH 10.5) and vortexed for 5 seconds. This extract was then applied with a pasteur pipette into the washed Sep-Pak C\textsubscript{18} columns. Excess salt and pigments were removed from the columns by washing twice with distilled water and the interfering polar lipids were removed by washing with methanol: distilled water (70:30). The purified vitamin D metabolite was then eluted with 5 ml acetonitrile and the eluate was evaporated to dryness using a vacuum evaporator.

After the eluates had dried, each samples was reconstituted with 5 ml of hexane/isopropanol (98:2), mixed well by vortexing and applied to a Sep-Pak Silica column. The column was prepared before use by washing with 5 ml of isopropanol followed by 2 washes of 5 ml of hexane/isopropanol (98:2). Each eluate tube was rinsed with an additional 5 ml of hexane/isopropanol (98:2), vortexed for 5 seconds, and the rinse was applied to the Sep-Pak Silica column. The 25(OH)\textsubscript{2}D\textsubscript{3} and 24,25(OH)\textsubscript{2}D\textsubscript{3} were removed from the Sep-Pak Silica column by washing with 5 ml of hexane/isopropanol (4:6). The purified 24,25(OH)\textsubscript{2}D\textsubscript{3} was eluted from the silica column with 5 ml of hexane/isopropanol (70:30) and dried in a vacuum evaporator.

The dried samples containing the 1,25(OH)\textsubscript{2}D\textsubscript{3} fraction were immediately reconstituted with 200 \mu l ethanol buffer. From this volume, 50
RESULTS AND DISCUSSION

The importance of 1,25(OH)₂D₃ in calcium homeostasis requires the development of improved procedures for the measurement of this metabolite. Previous assay procedures generally involve lengthy and cumbersome extraction procedures and require large volumes of samples and solvent. To the assay we modified the extraction procedures developed by Turnbull et al. (1981). Serum and/or milk samples (8.5-8.4 ml) were mixed with 1 vol. of acetonitrile to remove denatured protein and insoluble lipids. The addition of 0.4 M K₂HPO₄ buffer to the acetonitrile extract in additional removal of lipids. Purification of the acetonitrile-K₂HPO₄ buffer-treated sample on a C₁₈ Sep-Pak column extracted and partially purified 1,25(OH)₂D₃. Washing the C₁₈ Sep-Pak column before elution of the vitamin D metabolite removes most of the plasma lipids and pigments and reduces the recovery of greater than 85% of the vitamin D metabolites. The results obtained were then compared with standard assay which involved HPLC for samples extraction and purification. There was a good correlation (r=0.95) between the two techniques (Fig. 1) Therefore, this step eliminated the need for the time-consuming and large solvent demands of Sephadex LH-20 bath column which are used in HPLC procedures.

After solid phase extraction, 1,25(OH)₂D₃ was further purified on a silica Sep-Pak. This final purification is very simple and inexpensive, because requires only silica Sep-Pak and hexane/isopropanol solvent systems to effect elution and purification of the most active metabolite form of vitamin D. Recovery during the extraction stage of the procedures was done to validate the assay and as determined by comparing radioactivity present in lipid extracts to radioactivity added to serum or milk samples. Recoveries were 94.9% for 1,25(OH)₂D₃ in serum (n=40) and 93.6% for 1,25(OH)₂D₃ in milk (n=40).

Assay sensitivity was optimized by using a non-equilibrium protein binding assay adapted from Radio immune Assays. Maximum specific binding and sensitivity were obtained by preincubation of standard and samples with bovine thymus cytosol a specific receptor for 1,25(OH)₂D₃. The boiphilized receptor was very stable for up to 1 year and could supplies more than 5000 assays. However, after reconstitution of the receptor using distilled or deionized water, the stability would be reduced by repeatedly freezing and thawing. Separation of bound and free 1,25(OH)₂D₃ by dextran coated charcoal was optimal at 20 min. and decreased thereafter. Assay sensitivity
was also tested by using different amount of samples from the same pool (Table 1.) The results showed linearity over a wide range of volumes.

CONCLUSION

From the results can be concluded that radio receptor assay has several advantages: high sensitivity; small sample requirements; inexpensive, eliminate the need for HPLC and found satisfactory for determination the 1,25(OH)2D3 concentrations either in serum or milk samples.

REFERENCES


Table 1. The 1,25-dihydroxycholecalciferol concentration in varying volumes of samples from the same pool

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Sample volume (ml)</th>
<th>Measured value (u=20)</th>
<th>Concentration (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow Serum</td>
<td>0.5</td>
<td>29.1 ± 2.0</td>
<td>58.2</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>56.3 ± 2.9</td>
<td>56.3</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>158.6 ± 6.1</td>
<td>57.9</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>229.5 ± 10.2</td>
<td>57.3</td>
</tr>
<tr>
<td>Cow Milk</td>
<td>0.5</td>
<td>8.7 ± 1.0</td>
<td>17.4</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>16.9 ± 1.9</td>
<td>16.4</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>32.8 ± 3.1</td>
<td>16.4</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>63.4 ± 2.8</td>
<td>15.8</td>
</tr>
<tr>
<td>Sow Serum</td>
<td>0.5</td>
<td>35.6 ± 3.2</td>
<td>71.2</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>77.2 ± 1.8</td>
<td>77.2</td>
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<tr>
<td></td>
<td>2.0</td>
<td>152.8 ± 3.8</td>
<td>76.1</td>
</tr>
<tr>
<td>Sow Milk</td>
<td>0.5</td>
<td>304.4 ± 15.4</td>
<td>22.0</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>11.0 ± 1.9</td>
<td>22.8</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>22.8 ± 2.6</td>
<td>19.9</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>39.9 ± 5.4</td>
<td>21.4</td>
</tr>
</tbody>
</table>
DINAMIKA JUMLAH LARVA NEMATODA PADA RUMPUT DI PADANG PENGEMBALAAN

Joko Prastowo dan Sumartono *

ABSTRAK

Adanya larva nematoda pada rumput di padang pengembalaan dapat menjadi salah satu indikator terjadinya infeksi atau reinfeksi pada ternak yang seramput di padangan tersebut. Penelitian ini bertujuan untuk mengetahui dinamika jumlah larva pada rumput di padang pengembalaan di Unit Pendidikan dan Pelatihan Kebersihan Hewan (UPKKH) Universitas Gadjah Mada.

Tiga lokasi padang pengembalaan milik Unit Pendidikan dan Pelatihan Kebersihan Hewan Fakultas Kedokteran Hewan Universitas Gadjah Mada digunakan sebagai lokasi pengambilan sampel. Sebagian sampel adalah rumput yang tumbuh di padangan tersebut. Sekali pengumpulan, di potong 12 batang rumput tepat pada permukaan tanah. Penometakan rumput dilakukan pada pukul 05.00, 06.00-09.00, 10.00-13.00, 14.00-17.00 dan terakhir pada pukul 18.00. Secara tetap, 12 sampel didipotong, setiap rumput dimasukkan ke dalam tabung reaksi. Isolasi larva nematoda dari rumput dilakukan dengan cara mencari larva dalam larutan deterjen. Larutan cancaan rumput diperkirakan dari hasil mikroskop dan larva nematoda yang ada dibuang. Pengumpulan datar larva nematoda pada tiap rumput tersebut dilakukan selama 10 hari. Data yang diperoleh dianalisis secara statistik.

Hasil penelitian menunjukkan bahwa rumput yang tumbuh di tiga lokasi padang pengembalaan tampak rumput dinihilai mengandung larva nematoda dengan jumlah berkisar antara 8,3 ± 0,67 - 8,5 ± 2,35 larva per rumput asuhan jumlah tersebut secara statistik tidak berbeda secara nyata (P > 0,05). Perbedaan jumlah larva tersebut sangat nyata (P < 0,05) pada berbagai waktu jam pengambilan sampel. Jumlah nematoda paling banyak ditemukan pada rumput yang dinihilai pukul 05.00 dan 06.00-09.00, sedangkan paling sedikit pada rumput yang dinihilai pukul 18.00.

Kata kunci: dinamika, larva, nematoda, rumput, padang pengembalaan.

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