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การศึกษาเปรียบเทียบการสกัดฮอร์โมนคอร์ติซอลในน้ำนมโคด้วยสารสกัดชนิดต่างๆ

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[1]ศูนย์วิจัยและพัฒนาเทคโนโลยีการผลิตปศุสัตว์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพมหานคร 10330

บทคัดย่อ: การวัดค่าฮอร์โมนคอร์ติซอล (Cortisol) ในน้ำนมโคโดยตรงนั้นจะถูกรบกวนด้วยไขมันที่อยู่ในน้ำนม ดังนั้น การสกัดฮอร์โมนออกมาก่อนการตรวจวัดโดยใช้เทคนิคเรดิโออิมมิวโนเอสเสย์ (Radioimmunoassay, RIA) จึงเป็นสิ่งสำคัญ งานวิจัยนี้จัดทำขึ้นเพื่อศึกษาสารสกัดชนิดต่าง ๆ ได้แก่ ไดคลอโรมีเทน (Dichloromethane) ไดเอทิลอีเทอร์ (Diethyl ether) และปิโตรเลียมเบนซีน (Petroleum benzene) ต่อการสกัดฮอร์โมนคอร์ติซอลในน้ำนมโค โดยใช้ปริมาณน้ำนมและการเจือจางของสารสกัดที่แตกต่างกันออกไป จากผลการทดลองพบว่า ปริมาณของน้ำนมที่แตกต่างกันในการทดสอบโดยเทคนิค RIA นั้นมีผลต่อการสกัดฮอร์โมน โดยสารสกัดDichloromethane และ Diethyl ether ให้เปอร์เซ็นต์การวิเคราะห์ (% Recovery) ต่ำในการสกัดน้ำนมที่ปริมาณ 500 µl นอกจากนั้น ปริมาณน้ำนมต่อสารสกัดก็มีผลต่อการสกัดฮอร์โมนด้วยเช่นกัน ซึ่งสารสกัด Dichloromethane ให้เปอร์เซ็นต์การวิเคราะห์ได้สูงสุดด้วยการเจือจางที่ 1: 24 ส่วนสารสกัด Diethyl ether ให้เปอร์เซ็นต์การวิเคราะห์ได้ดีด้วยการเจือจางที่ 1: 26 อย่างไรก็ตาม สารสกัด Petroleum benzene สำหรับการสกัดฮอร์โมนคอร์ติซอลนั้นให้เปอร์เซ็นต์การวิเคราะห์ต่ำกว่า 50 เปอร์เซ็นต์

คำสำคัญ: การสกัดฮอร์โมนคอร์ติซอลในน้ำนม สารสกัด เรดิโออิมมิวโนเอสเสย์

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The Comparative Study of Cortisol Extraction in Cow's Milk by Different Extract

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Abstract: Direct measurement of cortisol in milk interfered with milk lipids. Hormone extraction is essential prior before the measure by using radioimmunoassay technique. This experiment was designed to study the different extracts: Dichloromethane, Diethyl ether and, Petroleum benzene for cortisol extraction in cow's milk by using different quantities of milk and dilution of the extract. From the results, it was found that the different quantities of milk on radioimmunoassay affect cortisol extraction. The extraction of 500 µl of milk provides a low recovery percentage (% Recovery) in dichloromethane and diethyl ether extract. Also, the quantities of milk per extract affect cortisol extraction. Dichloromethane extract yielded the highest recovery percentage by diluting 1: 24 and 1: 26 in diethyl ether extract. However, petroleum benzene extract for cortisol extraction was disappointing.

Keywords: Milk cortisol extraction, Extract, Radioimmunoassay

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Introduction

Each day, animals were exposed to stress-inducing conditions such as temperature, transportation, and animal density in a corral. The measurement of cortisol in blood or milk can be used as a biomarker of adverse environmental or pathophysiological conditions. Forslund et al. (2010) has reported that the serum cortisol levels were highest in serum from recumbent cows 27.9 ng/ml and cows suffering from parturient paresis 22.5 ng/ml. But plasma cortisol is affected by the sampling technique. So, measuring cortisol in milk can be an appropriate method because milking is a routine and can be used to assess the welfare of dairy cows.

Milk is a complex biofluid consists of macromolecules: proteins, lipids, large amounts of polar substances such as amino acid and vitamins, and hormones. Fat is one of the most important constituents of milk that can interfere with the measurement. So, the researcher designed to study the different extracts: Dichloromethane, Diethyl ether and, Petroleum

benzene for cortisol extraction in cow's milk by using radioimmunoassay technique.

Materials and Methods

Skim milk samples were purchased from shops in Bangkok and stored at -20 °C. Prior to assay, the frozen skim milk samples were thawed at RT (25°C) and mixed.

Recovery cortisol

A 100 - 1000 µl portion of skim milk samples (n = 10) and 100 µl of ^{3}H-Cortisol (1000 CPM) were added into a 10 ml glass tube. The tube was mixed by mixer for 10 secs. Extraction of ^{3}H-Cortisol from skim milk was accomplished with 3 extracts: Dichloromethane (4 ml), Diethyl ether (1:10) and Petroleum benzene (1:10).

The extraction tubes of diethyl ether and petroleum benzene were mixed by mixer for 90 secs. The tubes then were frozen at -70 °C for 15 min. The solvent layer was decanted into vial tubes and evaporated to dryness under lyophilizer in preparation for assay. After complete dying, 100 µl of assay buffer (PBS pH 7.0) was put into the tube, mixed by a mixer for 5 secs, and added 4 ml of scintillator (Toluene, POPOP and p-Terphenyl).

For the extraction with dichloromethane as described by Bulter et al (7), except for the freezing step which frozen the aqueous phase for 15 min at -70 °C. After complete drying, skim milk dichloromethane extract either was assay as above.

^{3}H-Cortisol were assayed by radioimmunoassay (RIA) technique as described by Kamonpatana et al.

Dilution assay

100 µl of ^{3}H-Cortisol was extracted from the right quantities of skim milk (100 - 1000 µl) with three extracts at the different dilution (1:10, 1:12, 1:14, 1:16, 1:18, 1:20, 1:22, 1:24 and 1:26). The next step was assayed the same as above.

Results and Discussion

The investigations of three extracts of 100, 200, and 500 µl of skim milk were added to assay tubes containing 100 µl of ^{3}H-Cortisol. As in Figure 1, the extraction of skim milk was disappointed in petroleum benzene resulted in the lowest graph that less than 30% Recovery in all quantities of skim milk. The quantities of skim milk that provide the highest recovery percentage in dichloromethane (68.4%) and diethyl ether (72.4%) extracts were 200 and 100 µl respectively. However, even with solvent purification of the milk extract, extraction of 500 µl yielded the low recovery percentage in both extracts.

As in Figure 2, Dichloromethane extract yielded the highest recovery percentage at dilution of 1: 24 (71.7%) and 1: 26 (82.9%) in diethyl ether extract. However, the graph showed that the quantities of milk per extract at different dilutions provide not much different recovery percentage.

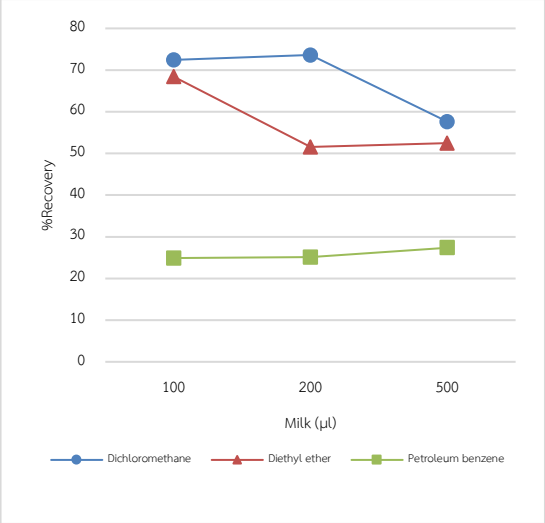

Figure 1 Comparison of three extracts with different quantities of milk on radioimmunoassay of cortisol. Symbols: dichloromethane extract (circles), diethyl ether extract (triangles), and petroleum benzene extract (squares).

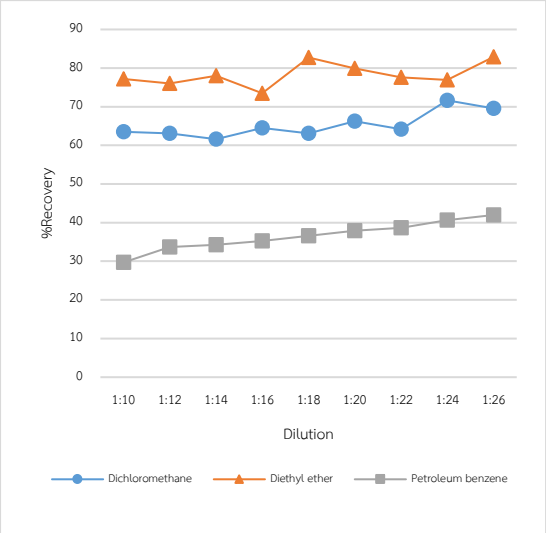

Figure 2 Comparison of quantities of skim milk per extract (dilution) on radioimmunoassay of cortisol by three extracts. Symbols: dichloromethane extract (circles), diethyl ether extract (triangles), and petroleum benzene extract (squares).

All the results of these experiments suggest that the good quantities of milk for the analysis of cortisol were 200 µl in dichloromethane extract at a dilution of 1:24 and 100 µl in diethyl ether at a dilution of 1:26. Petroleum benzene extract was not suitable for milk cortisol extraction and excessive quantities of milk extract on radioimmunoassay of cortisol can interfere with milk lipids as reported by Butler et al.

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