Indonesian Journal of Veterinary Sciences
Vol. 3. No. 1 March 2022, Page. 38-40
DOI:10.22146/ijvs.v3i1.88586, ISSN 2722-421X (Online)
Available online at https://jurnal.ugm.ac.id/ijvs

Comparison of Progesterone Hormone in Feces and Milk of Friesian Holstein Cattle at Lactating Period

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Received: January 18, 2022, Accepted: February 19, 2022, Published: March 1, 2022

Abstract

Measurement the level of reproductive hormone can be done by invasive or non-invasive methods. The invasive method is carried out by measuring hormone levels obtained from the blood while the non-invasive method is obtained from feces, urine, and milk. The levels of steroid hormone in feces shows similarity to the levels in plasma, but there was a different time lg for each species, about 12 hours to 2 days. This research aims to determine the comparison of progesterone levels from feces and milk sample so it can be used as a reference for physiological data. The research uses feces and milk sample from Friesian Holstein cattle maintained at the Faculty of Veterinary Medicine. Sampling was carried out for 2 weeks. The result showed the range of the levels of progesterone in feces sample was 327,62 ± 53,68 ng/gr dry feces, while in milk sample it was 40,32 ± 29,41 ng/ml. It can be concluded that the levels of the progesterone in faces and milk sample did not show a significant difference.

Key words: progesterone, milk, feces, non-invasive method

Introduction

Development of dairy cattle industry is one alternative to supply the nutritional necessary and reduce national dependence on imports of milk and meat (Putra, et.al., 2019). It is necessary to consider several things to achieve maximum profit in the development of dairy cattle production, one of which is the selection of superior livestock (Subronto and Tjahajati, 2001). Reproduction is an important factor in determining the efficiency of livestock productivity (Ball and Peters, 2004). Reproductive hormones are responsible for determining livestock productivity. The role of progesterone is very important to support the reproductive process especially maintaining pregnancy, and participating in the growth and development of the embryo (Mekonnin, et.al., 2017).

Level of reproductive hormone can be measured invasively or non-invasively. The invasive method is carried out by measuring hormone levels obtained from the blood while the non-invasive method is obtained from feces, urine, and milk (Rahmawati, *, et.al,,* 2021). Progesterone levels can not only be detected in the blood, but can also be detected in the milk and feses. Steroid hormone concentrations in feces showed similarity to plasma concentrations, but there was a different time lag for each species, about 12-24 hours (Schwarzenberger, *, et.al,,* 1996). Steroid hormones excreted through feces can be used as material to be analyzed to monitor reproductive status, because feces contain hormones which are

substances from hormones secreted by endocrine glands, so that the hormone profile in feces remains in accordance with the hormone profile in the blood (Rahmawati, *, et.al,,* 2021). In this research, we would to examine progesterone levels in feces and milk from dairy products during lactation using an enzyme immunoassay linked assay (ELISA).

Materials and Methods

The research used feces and milk sample from the Friesian Holstein cattle maintained at Animal Health Education and Training Unit (UP2KH) of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta. All of samples were collected twice a day during 27th August – 10th September 2021. The other materials would be used in this research includes steril plastic clips, gloves, refrigerator/ freezer, conickel tubes, microtube, yellow and blue tips, vortex, freeze dryer machine, ELISA washer, ELISA reader, dropper, micropipette, progesterone competitive ELISA kit, 80% methanol, and labels.

Sample collection

Feces and milk sample were collected into plastic bag with date and time code for labelling the sample.

Feces extraction and Milk Centrifuge

Feces sampel was moved into plastic tube. The sample was dried in freeze-dryer at 80^{0}C for 72 hours. The dried feces then was pounding into powder. The sample was measured as 0,5 gram in each tube. 5 ml methanol 80% was added in each tube then mixed with vortex for 10 minutes. Supernatant was separated from pellets, taken with micropipets 1000 µl and moved into a microtube. Milk sample was moved in the microtube and the sample was centrifuge for 10 minutes. Fat in the supernatant was thrown. The feces and milk without fat samples were stored in the freezer until assayed.

Progesterone measurement using ELISA

Sample and standard of progesterone 50 µl was prepared in microwell. Progesterone enzyme and biotin conjugates added in each microwell and then incubated in the room temperature for 60 minutes. Samples were washed three times with 300 µl of wash buffer and 100 µl of TMB was added to each well. Incubated the plate for 20 minutes in the dark room. The reaction was stopped by adding 50 µl stop solution. Finally, samples were read on ELISA reader at 450 nm.

Result

The results of progesterone measurement using ELISA showed that the average value of milk progesterone levels in all samples was 40.32 ± 29.42 ng/ml, while the progesterone in feces was 32.76 ± 53.68 ng/ml (Table 1)

Table 1. The Progesteron Level of Samples

Sample	Progesterone level (ng/ml)	
	Milk sample	Feces sample
1	9,275	8,151
2	63,328	142,359
3	3,996	8,201
4	49,432	3,496
5	3,996	2,971
6	49,432	110,114
7	60,874	5,119
8	87,004	11,509
9	35,545	2,935
Mean	40,321	32,762
standard deviation	29,417	53,681

The level of progesterone in milk were detected higher due to lipophilic steroids (Simersky, *, et.al,,* 2007). Progesterone is very fat soluble so it prefers to diffuse into milk fat (Ball and Peters, 2004). Milk contains about 3.4% total fat, depending on the lactation phase. Progesterone, as a lipophilic molecule, can affect detection accuracy in milk samples (Shrivastav, et. Al, 2014). Fecal and plasma progesterone concentrations during the ovarian cycle were detected equally. The lowest fecal progesterone concentrations were detected in cattle in the follicular phase with levels less than 40 ng/g. while the highest concentration of fecal progesterone was found in the luteal phase, which was 74 ng/g l This difference could be used to determine the follicular or luteal phase (Isobe, *, et.al,,* 2009). Hormones in the blood take time to form hormone metabolites in the stool. According to Nugraha, et.al (2016), the time interval between actual hormone levels in blood plasma and hormone levels in feces is determined by gut passage time (GPT), which is the time

of hormone excretion by the liver through the gallbladder and digestive tract until excreted with feces and urine. Lactational dairy cows have a progesterone concentration below normal due to higher hepatic blood flow, which will increase the rate of progesterone catabolism. This subnormal concentration of progesterone allows an increase in the frequency of LH secretion that interferes with follicles and oocytes, and embryonic viability (Antanaitis, et.al., 2020).

Conclusion

The results of research, different progesterone levels were detected in plasma, milk and feces during the estrus cycle and milk production had no effect on fecal progesterone concentrations

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