Research Paper

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THE IMPROVEMENT OF ANESTROUS PE GOATS' REPRODUCTION PERFORMANCE WITH MICRO-ALGA SUPPLEMENTATION THROUGH IDENTIFICATION OF OVARIAN ACTIVITIES AND 17B ESTRADIOL LEVELS

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ABSTRACT

This research aims to illustrate the ovary of PE goats by using USG and estrogen levels test. This study consists of 3 treatments for each group consisting of 6 goats. T1 consists of normal PE goat without any treatment, T2 consists of anestrous PE goats treated with PGF2α and GnRH hormones, T3 consists of anestrous PE goats treated with PGF2α and GnRH hormones which was also fed with superantioxidant with dose at 0.5% of body weight. The data which are being observed is estrus onset and duration and also the estrogen levels analyzed with ANOVA post hoc LSD test. The result of this study showed that microalga feeding could raise the 17B estradiol levels (176,7± 13,019c) in the serum of post-partum anestrous goat more than hormonal therapy (110,4 ± 6,112b). Microalga could improve the anestrus condition.

KEYWORDS

PE goats, Ultrasonography, Astaxanthine antioxidant, *Haematococcuspulvialis*

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INTRODUCTION

Etawah crossbred (PE) goat is a farm animal which is sufficiently productive and adaptive to the environmental and territorial condition of Indonesia so that most farmers raised PE goats as their additional income. Etawah Crossbred (Peranakan Ettawa/PE) is a crossbred between Etawah goat (from India) and Kacang goat from Indonesia. In view of the high milk production during lactation period of PE goats, then an ovarian hypofunction is predicted to be the main cause of the failure of dairy cattle's reproduction. The failure of estrous and anestrous in farm animal is the main symptom besides other factors affecting the estrus cycle. Based on Hafez (2000), anestrous was caused by the ovarian hypofunction which was frequently associated with the failure of follicle cells to respond to hormonal stimuli, changes in quantity and quality of hormone secretion, a decline of stimuli related to hypothalamic-pituitary-ovarian function, resulting in the decrease of gonadotropin secretion and ovarian activity after birth.

One of the alternative ways to improve the hormonal function is byimproving the nutrition with additional fodder. *Haematococcuspulvialis* containing astaxanthin which is a carotenoid substance and acts as a source of nutrients for animals and plants. Astaxanthin acts as an important nutritional component for growth and reproduction and acts as an antioxidant (Ciapara et al., 2006).

It became necessary to conduct a study in analyzing the response after super antioxidant administration from microalga *Haematococcu spulvialis* to PE goats which experienced post-partum anestrous with or without hormonal therapy. This study was the basis for applied research in the field which was initiated by observing and comparing the effectiveness of its administration on the response of post-partum estrous. The goats which showed the post-partum estrous response would then get artificially inseminated in order to reveal the conception success rates, and also the ultrasound imaging of ovary as a further evaluation for post administration of the supportive therapy model.

MATERIALS AND METHODS

Population and Sample

This study used a completely randomized design which included 18 PE female goats aged 2-3 years, weighed 35±5 kg, and had experienced giving birth. Those 18 goats would then be divided into 3 groups, which are: (K-), consisted of 6 goats with normal labor and had shown first estrous on day 30-60 after giving birth, (K+), consisted of 6 goats with normal labor and did not show estrous yet until day >60 after giving birth and were administrated with conventional hormonal therapy (double injection of PGF2α), and (KP), consisted of 6 goats with normal labor and did not show estrous yet until day >60 after giving birth and were administrated with fodder containing microalga *Haematococcuspulvialis*.

Postpartum examination of female goats

The reproduction of PE goats is observed after they give birth. If the goats show signs of estrus in 30 days after the delivery, it will be categorized as normal. Meanwhile, the goats which do not show any signs of estrous in 30 days after delivery will get examined using ultrasonography. If there are no any signs of activity of the ovary (no folliculogenesis) or if there is a cyst found, the goats will be categorized as an anestrous group which then each group consists of 6 repeats.

Estrus Response Examination

The estrus response is observed on both two treatment groups every three hours by putting male goats as teasers into the female trial goats' stall after the administration of hormonal therapy. The goats will be considered having a positive estrus (estrus onset) if they let the mating happen. The goats which are already having estrus will be marked with a red marker on the base of their necks. The observation of the goats' estrus response includes:

Estrus Percentage: The number of goats having estrus divided by the number of goats in treatment (%)

Estrus Onset: The amount of time needed since the PGF2α injection or since the second PGF2α hormone administration until the female goat let the mating happen estrus intensity: reddish and swollen vulva

(Good = ++, Fair = +)

Estrus Duration: The amount of time since the estrus onset until the

female goat refuse to continue the mating.

Estrogen Levels Observation using ELISA

Prepare a pipette 50 µl, a standard and put the sample in the microplate. Add 100 µl estrogen enzyme conjugate for each microplate, then put it in the shaker for 30 seconds. Incubate it at 37 °C for 1 hour. After the incubation, throw the liquid on the microplate away then wash it with 250-300 µl of washing solution and put it in the shaker for 3 minutes, repeat the washing for 5 times, after it is done, put it back, press forcefully with blotting paper to dry using tissue paper. Add 100 µl TBM substrate solution for each microplate in the correct sequence. Incubate the tube for 10 minutes at room temperature and cover it with film glass then wrap it up with aluminum foil. In order to stop the reaction, add 50 µl stop solution into the microplate gently, shake the mixture for 5 seconds. Then, put the microplate in the ELISA reader, read and observe. (Aulanni'am, 2005).

RESULT AND DISCUSSION

The Identification of Ovarian Activity

The ultrasonography was used to determine which the postpartum sample goats are anestrus. The result of the ultrasound examination was used to diagnose whether the goats are anestrus or normal (Figure 1).

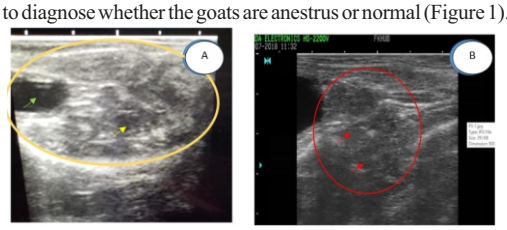

Figure 1. The ultrasound image of the ovary to identify follicles and corpus luteum. The red arrow shows the corpus luteum. The yellow arrow shows the follicle. The green arrow shows the dominant follicle indicated that the goat could have any sexual desire after giving birth.

Follicles which have 5 mm diameter or more in one follicular wave will undergo ovulation (Rubianes and Menchaca, 2003) and follicles which its size does not reach 4 mm are classified as dynamic follicle group. Those mature follicles will synthesize estrogen hormone which then is secreted into the blood circulation that causes the female goat to become estrus. This thing happened because of the decrease in progesterone concentration during the final stages of pregnancy in blood, which is resulting in the absence of negative feedback.

During the postpartum period, the livestock will experience oxidative stress caused by the increase of free radicals in the body (Tanaka et al, 2004), and also by the hormonal changes during the final stage of pregnancy until the delivery. The oxidative stress could induce prolonged anestrus, the free radicals (nitric oxide) may inhibit the estradiol production and androstenedione synthesis in the ovary, which results in the absence of sexual desire during post-partum. The increase of estradiol levels is essentially required to stimulate any positive feedbacks in order to create LH surge and ovulation (Kresno et al, 2007).

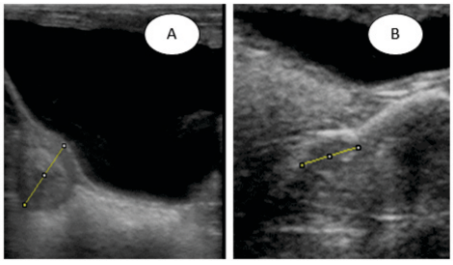

Figure 2. The ultrasound images of the ovary on group K+(A) and KP (B). The yellow arrow shows that there is a follicle development with various sizes.

From those two ultrasound images above stated that in group K+, showed an improvement of its ovarian function marked by follicle development, while the group KP showed a better follicle development than group K+ marked by follicles that grew in greater size than the follicles of group K+. This thing indicated that microalga *Haemotococcuspulvialis* supplementation resulted in a better outcome compared to goats from group K+ marked by active follicle development and better follicle size.

Microalga is a unicellular microorganism, commonly found in large waters like sea, lake, river and brackish water. Microalga contains an essential fatty acid for the body, including linoleate acid, arachidonic acid, linolenic acid, and all the others which are used by the body as a precursor of steroid hormone production which function in the reproduction system. Fatty acid normally undergoes esterification through the cyclooxygenase and lipoxygenase to form a lipid complex which functions as a precursor of some important substances, including hormone-like substances like prostaglandin, thromboxane, and leukotriene.

According to Tanjung (2015), the lack amount of estrogen affects the FSH secretion to function optimally and causes estrogen secretion inhibition in the ovary. This condition is affected by the gonadotropin hormone (GnRH) secretion which is not able to be responded by the anterior pituitary. Senger (1999) reported that lower body weight and high lactation stress or prolactin levels are some of the inhibiting factors of hormonal secretion. The inhibition of follicle development is affected by estrogen levels. Estrogen is found in follicular antrum and produced by internal theca cells. Therefore, estrogen was absorbed and distributed through blood vessels to a target organ. Toelihere (1981) stated that the low level of gonadotropin hormone especially FSH can cause postpartum ovarian hypofunction. The decrease of estrogen secretion in the body can affect the activity ovary making it difficult for the goat to show any signs of estrus like normal goats.

SERUM ESTROGEN LEVELS WITH METHOD

The main function of estrogen hormone is to stimulate sexual desire, stimulate secondary sex characteristic, maintaining the female mammary gland system and its growth (Wodzicka-Tomaszewska et al., 1991). Based on the study conducted by Katongole and Gombe (2006) with Small East African (SEA) goats, the profile of estrogen during estrus is 120-900 pM/L and during mid-pregnancy, the number will drop from 554 pM/l to 424 pM/l. The study conducted by Akusu et al. (2006) with West African Dwarfs (WAD) goats showed the profile of estrogen during estrus was 152,62±31,6 pg/ml, on Day 20 was 131,7±4,3 pg/ml, 24-6 hours before the delivery was 309,9±27,62 pg/ml, during delivery was 191,60±58,90 pg/ml, right after the delivery 150,30±24,30 pg/ml, 1-3 days after the delivery 109,60±34,60 ph/ml, and day 4 after the delivery 92,90±48,40 pg/ml. The estrogen concentration difference in this study with previous study possibly caused by the breed difference of the animals used. Besides the reason possibly caused by the timing of blood serum collection, the examination process, and the amount of samples used. Akusu et al. (2006) reported that the concentration during estrus in Small East African (SEA) goat was higher, which was around 152,62±31,6 pg/ml.

ELISA Table 1 Average Serum Estradiol 17β Levels

Treatment	Average Blood Estradiol 17β (ug/L) ± standard deviation
K-	52,5 ± 6.302[a]
K+	110,4 ± 6,112[b]
KP	176,7± 13,019[c]

K- : goats with normal postpartum estrus cycle
K+ : anestrus goats treated with hormonal therapy
KP : anestrus goats treated with microalga

Table 1 shows the average estradiol 17β concentration obtained using ELISA method. The estradiol 17β concentration in group K- was significantly different with K+, KP ($p<0.05$), with the highest concentration group KP and the lowest was K- ($p>0.05$).

Based on the results above, it can be concluded that the administration of microalga 0,5% as supplementation in P2 could increase the estradiol 17β levels which can be seen on table 5.1 showing an increase in P2 compared to K- and P1 which were not supplemented with microalga. Polyunsaturated fatty acids (PUFAs) contained in microalga take part in the tissue and cellular metabolism, to maintain the fluidity and endurance of cell membrane, electrons, and oxygen transport in blood, and also thermal adaptation. Microalga is one of the largest carotenoid producers which its composition contained in it consisted of β-carotene, lutein, astaxanthin, zeaxanthin, cryptoxanthin, and also fucoxanthin. Some of them are having a better

antioxidant strength than vitamin A, vitamin C, or vitamin E. *Haemotococcuspulvialis* has been developed as the source of astaxanthin antioxidant, and is used as additional ingredients for feeding, and also in cosmetics and food supplements (Capelli et al., 2006; Heuer et al., 2007). The activity of antioxidant could improve nutrition and minerals, and also optimizing the function of the hypothalamus (Eritsland, 2010) so that the reproduction hormone produced will reach the optimal levels. The optimal gonadotropin (FSH and LH) levels in the body affect the ovarian follicle development, therefore it could improve the reproduction performance of the postpartum small ruminant livestock.

CONCLUSION

The administration of microalga could increase the serum estradiol 17 β levels of the goats which were postpartum anestrus better than the goats with hormonal therapy. Furthermore, microalga supplementation could improve the anestrus condition which was observed by the ultrasound image of ovary and the signs of sexual desire of anestrus goats which were treated with hormonal therapy and microalga 0.5%/body weight for 2 estrus cycles.

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