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Concentration of Estrogen and Progesterone during Estrus and the 14th Day of Mating in the Javanese Thin-Tailed Ewes

M Socheh, D M Saleh, S W Purbojo and A Setyaningrum

Faculty of Animal Science, Universitas Jenderal Soedirman, Purwokerto, Indonesia

E-mail: msocheh1956@gmail.com

Abstract. The aim of this study was to study the effect of giving different levels of energy feed on the concentrations of estrogen and progesterone during estrus and on the 14th day after mating on thin-tail ewes. The material used in this study was 15 head of thin-tail ewes aged between 2.50-3.00 years had a normal estrus cycle and had once lambing. All ewes were randomly placed into three types of treatment of energy feed with different levels, namely: non-flushing 1.01 Mcal / kg ME (f0), flushing 1.3 Mcal / kg ME (f1) and flushing 2.31 Mcal / kg ME (f2). Each treatment was repeated 5 times. The general linear model of SPSS was used to analyze variables measured. The results showed that the average estrogen concentration in thin-tailed ewes during estrus in the flushing group (f1 and f2) was higher than non-flushing (f0). The average progesterone concentration in the thin-tailed ewes on the 14th day after mating in the flushing group (f1 and f2) was higher respectively than the non-flushing group (f0). The increase in feed energy given to thin-tailed ewes in flushing, during estrus increases estrogen concentration and on the 14th day after mating, increase progesterone concentration.

Keywords: thin-tailed ewes, feed energy, estrus, 14 days after mating, estrogen, progesterone

1. Introduction

In Indonesia, sheep farming is dominated by small farmers in rural management, and runs traditionally. In general, animals never fed concentrates, and as a consequent, the reproductive performance is low. Thin-tailed sheep is one of the local sheep breed in Indonesia in addition to two other indigenous sheep namely Priangan and Javanese Fat-Tailed Sheep. According to [1], nutrition is generally recognized as a significant regulator of reproduction. Flushing has an influence on the improvement of body condition, fertility, ovulation rate of the ewes consumed low quality of feed both before and during mating. Socheh et al. [2] stated flushing can be implemented by providing forage and some additional food rich in energy feed. Flushing is a way to give high-energy feed to ewes 17 days before and 17 days after mating. The flushing is appropriate when combined with the program of estrus synchronization [3].

Socheh [4] studied on two Kejobong females goat, each given a ration with an energy content (1.35 Mcal/kg ME or non-flushing versus 3.06 Mcal/kg ME or flushing) and then injected twice with a sterile solution Lutalyse® (Dinoprost tromethamine) 100ml (1ml dose of 5mg/head). At the time female animals being estrus, concentrations of estrogen in the flushing group were significantly higher than the non-flushing.



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The present study uses 15 thin-tailed ewes fed with three different levels of energy, namely non-flushing 1.01Mcal / kgME (f0), flushing 2.13Mcal / kgME (f1) and flushing 2.31Mcal / kgME (f2). Each treatment was repeated 9 times. The animals injected twice with a sterile solution Lutalyse ® (Dinoprostromethamine) 100ml (1ml dose of 5mg/head). The variables observed were estrogen and progesterone hormone concentrations during estrus, and estrogen and progesterone concentrations on the day 14th after mating.

Khanum et al. [5] reported at estrus period, the concentration of estrogen reaches the highest concentration of 7.70 ± 1.70 pg/ml at this stage of estrus. In contrast, progesterone begins to rise from the average value of basal 0.10 ± 0.03 ng/ml on day 0 up to 3.00 ± 0.90 ng/ml on day 6 of the estrus cycle and reaches a peak value of 7.70 ± 0.60 ng /ml on day 12. Some report stated that during estrus, progesterone concentration did not increase [6] and [7].

At the time of estrus, a group that was injected with PG-600 and accompanied with flushing containing 11.85% crude protein and 73.73% TDN showed a higher level of concentration than control (no treatment) [3]. Some aspects of the reproduction performance have not been reported yet, however, information on estrogen and progesterone concentrations of thin-tailed ewes during estrus and on the 14th day after mating fed with different energy levels based on flushing has not been reported yet.

Habibizad et al. [8] reported in their study, compared two groups which fed a long-term high energy diet (LE) and short-term high energy diet (SE). The LE and SE groups had the highest oestradiol concentration before the estrus time. Results of the present study revealed that change in dietary energy levels for a short-term or long-term period just before ovulation could improve the blood metabolites and reproductive performance of the ewes.

The aim of this study was to study the effect of giving different levels of energy feed on the concentrations of estrogen and progesterone during estrus and on the 14th day after mating on thin-tail ewes.

2. Methodology

The research material used in this experiment was 15 thin-tailed ewes with age 2.50–3.00 years and the average of initial body weight 21.50 ± 3.30 kg. The animals have normal estrus cycles and had once lambing. Each animal was a plot in an individual cage of 75x75x75cm in a typical sheep stage housing.

Ration given to ewes consist of roughage and concentrates. Roughage or field grass was given to each ewes as a basal feed (staple feed) while concentrates were given to each ewes as a supplement feed and as a source of energy. The concentrate given to ewes was a mixture of cassava meal (*onggok*) and rice bran. Fifteen ewes were randomly assigned to three different feeding treatments: non-flushing (f0), flushing (f1 and f2). Each treatment was repeated five times. The composition and nutrient content of the three treatments presented in Table 1.

Table 1. Composition and chemical content of the ration

Feedstuffs	Treatments		
	f0 (n=5)	f1 (n=5)	f2 (n=5)
		----- (% as fed) -----	
Field grass	100	60	60
Concentrate		40	40
Rice bran	—	26.25	24.82
Cassava meal	—	12.50	13.79
Ultra mineral ¹	—	1.25	1.37
CP	2.80	5.76	6.25
TDN	27.90	58.97	63.88

¹ One pack (1 kg) Ultra mineral of the EkaFarma production Semarang, Central Java, Indonesia, containing Calcium carbonate 50.00%; Phosphor 25.00%; Manganese 0.35%; Iodium 0.20%; Potassium 0.10%; Cupprum 0.15%; Sodium Chlorine 23.05%; Iron 0.80%; Zincum 0.20%; Magnesium 0.15%. f0: noflushing = 1.01Mcal/kg ME, f1: flushing = 2.13Mcal/kg ME, f2 : flushing = 2.31Mcal/kg ME.

Roughage given to all ewes in the groups f0, f1, and f2 was 2.5kg/head/day. Concentrated was given to the ewes groups of f1 and f2 as much as 2.5% dry matter (DM) of body weight and was served as flushing. The ratio of roughage and concentrate given to the flushing (f1 and f2) ewes were similar namely 60:40.

Flushing was done referred to the procedure of [2], the concentrate into the basal feed and given to the animals for 34 consecutive days, 17 days before and 17 days after the animals mated. Group f0 were fed only forage (1.01Mcal ME), while group f1 and f2 were 2.13Mcal/kg ME and 2.31 Mcal/kg ME, respectively. On day 34 onwards, both ewe in group f0, f1, and f2 each group only fed basal feed ad libitum.

The ewe in the group f1 and group f2 were fed concentrates at 7:00 and forage at 01.00 p.m to all treatment group. Drinking water is provided ad libitum.

Estrus synchronization was done by injecting a sterile solution Lutalyse® (Dinoprost tromethamine) 100ml © Pharmacia & Upjohn Company, Kalamazoo, MI 49001, USA intravenously to all ewes with a 1ml dose or 5mg per heads. Injecting twice with an interval of 11 days and was done before the ewes fed in the morning.

Estrus detection of the ewes in the morning before the animals was fed, and conducted over two days after the 2nd injection using stud teaser and done for two hours.

Estrus length of ewes was approximately 48 hours. Hormonal measurements were done by taking blood samples about 5 ml through the jugular vein using a 10 ml disposable syringe. Blood collection was done on the hour-0 (estrus, silent when riding for the first time), at the hour-12th, 24th, 36th and at the hour-48th. Blood collection was taken to all treatment group. The blood sample was collected in a test tube and then placed in the ice bucket. Ice bucket brought to the laboratory, the blood allowed to stand for 30 minutes. Blood centrifuged at 2,500 rpm for 15 minutes. Serum taken from the tube with micropipette was then inserted into the Eppendorf. Serum tube stored in a freezer until used for hormonal analysis. ELISA method is used for hormone analysis.

The blood sample from all group was taken on the day 14th post-mating and was treated with the procedure as before. Serum taken from the tube with micropipette was then inserted into the Eppendorf. All Eppendorf were stored in a freezer until used for hormone analysis. ELISA (Enzyme-Linked Immunosorbent Assay) method by using ELISA Reader Dona 3200 for hormone analysis.

Variable concentrations of estrogen and progesterone from ewes expressed descriptively in the form average value \pm standard deviations. Data processed by the method of General Linear Model of Statistical Product and Service Solution or SPSS versi 17.0 tahun 2007.

3. Result and Discussion

Concentration and profile of estrogen of the ewes during estrus

Results of the previous experiment showed that flushing was the highly significant affected ($P < 0.01$) in the improvement of body condition score of the ewe. Ewes given rations with an energy content of equal to 2.13Mcal/kg ME and 2.31 Mcal/kg ME (flushing) have a better body condition score than those that were given rations of the energy content 1.01Mcal/kg ME (non-flushing). The body condition score of ewes that given rations with an energy content 2.31 Mcal/kg ME was similar than those that was fed rations of the energy content 2.13 Mcal/kg ME. This means that increasing the energy content of the feed can improve the body condition score of the animals. This fact is in line with the statement of [1], flushing has an influence on the improvement of animal body condition.

Flushing was highly significant influenced ($P < 0.01$) into the estrogen concentration of ewes during estrus. The estrogen concentration of the ewe that were given rations with an energy content 2.13Mcal/kg ME and 2.31 Mcal/kg ME (f1 and f2) were significantly higher than those by 1.01Mcal/kg ME (f0). The estrogen concentration of the ewes fed flushing (f1) was 0.98pg/ml higher than those of non-flushing (f0). While, the estrogen concentration of the ewe that were given rations with an energy content 2.31 Mcal/kg (f2) was 1.14 pg/ml higher than those of energy content 2.13Mcal/kg ME (f1). Furthermore, the estrogen concentration of the ewes fed flushing (f2) was 2.12pg/ml higher than those of non-flushing (f0) (Table 2).

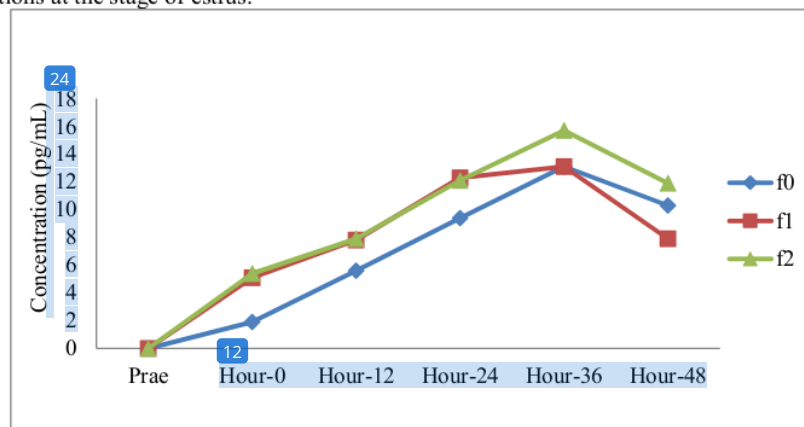
Table 2. Mean of estrogen concentrations in ewes during estrus at different hours

Blood collection	Feed		
	f0	f1	f2
	----- (pg/ml) -----		
Prae	0.00	0.03	0.00
Hour-0	1.97	5.13	5.43
Hour-12	5.60	7.77	7.93
Hour-24	9.37	12.27	12.03
Hour-36	13.03	13.13	15.70
Hour-48	10.33	7.86	11.97
8 Mean	6.72±5.08 ^a	7.70±4.81 ^b	8.84±5.62 ^c

a, b, c: different superscript at the same row indicates a highly significant ($P < 0.01$).

This means that flushing increases the estrogen concentration of the ewes during estrus. This situation is consistent with the statement of [2] flushing is very useful to stimulate the onset of estrus and improve reproductive hormones. The result of the study showed that estrogen concentration of the Javanese thin-tailed ewes was lower than those of Kejobong does during estrus [4]. This is because there are differences in livestock species and maintenance management.

The estrogen concentration profile on h-0, -12, -24, and -36 during estrus is presented in (Table 2). The highest concentration of estrogen achieved in ewes that consumed diets with energy content f2 (15.70pg/ml) at the 36th hour during estrus than those consumers energy f0 and f1 (13.03pg/ml and 13.13pg/ml). The ewes that consume diets with an energy content f0, f1, and f2 at the 48th hour during estrus the profile of estrogen decreased (Figure 1). This shows that high-energy ration increases the concentration of estrogen of ewes during estrus then estrogen concentration tends to decrease at the 48th hour. This result is higher than the findings [5] who reported that estrogen achieves the highest concentrations at the stage of estrus.

**Figure 1.** Estradiol profile of ewes during estrus on the different hour

Concentration and profile of progesterone of the ewes during estrus

The results of the experiment showed that flushing did not significantly affect the progesterone concentration of thin-tailed ewes during estrus. This means that increasing energy levels in the diet does not increase the concentration of progesterone.

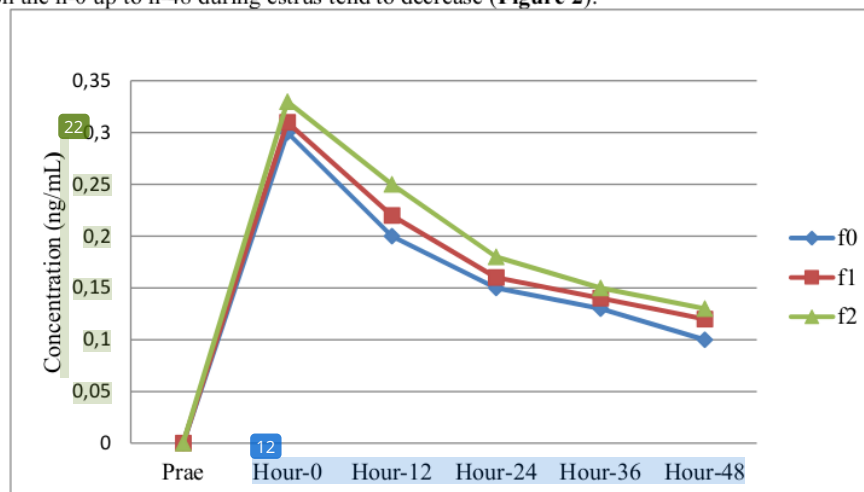
Table 3. Mean of progesterone concentrations of ewes during estrus at different hours

Blood collection	Feed		
	f0	f1	f2
	------(ng/ml)-----		
Prae ^{ns}	0	0	0
Hour-0 ^{ns}	0.30	0.31	0.33
Hour -12 ^{ns}	0.20	0.22	0.25
Hour -24 ^{ns}	0.15	0.16	0.18
Hour -36 ^{ns}	0.13	0.14	0.15
Hour -48 ^{ns}	0.10	0.12	0.13
Mean ^{ns}	0.15±0.10	0.16±0.10	0.17±0.11

^{ns}: not significantly different

Based on **Table 3** that in each of the research feed treatment (f0, f1, and f2) shows the concentration of the progesterone concentration is still very low or close to zero level. This situation was not significantly different from the reports of [12] that ewes exhibiting estrus had decreased concentrations of Progesterone (P₄) at h-24 and h-48 after Controlled Internal Drug Release (CIDR) withdrawal compared with ewes that did not exhibit estrus. This result is different from the result reported by [7] that during estrus the concentration of progesterone did not increase. However, [3] which compared two groups of Barki ewes accompanied by flushing (contains 11.85% crude protein and 73.73% TDN) showed progesterone concentrations higher 0.31 ± 6.4 ng/ml than control (no treatment) which is 0.30 ± 5.88 ng/ml. [8] reported that change in dietary energy levels for a short-term or long-term period just before ovulation could improve the blood metabolites and reproductive performance of the ewes.

The concentration of progesterone during estrus both ewes on the no-flushing (f0) and flushing (f1 and f2) showed at level 0.15 ng/ml, 0.16, and 0.17 ng/ml, respectively. The profile of progesterone of ewes on the h-0 up to h-48 during estrus tend to decrease (**Figure 2**).

**Figure 2.** Progesterone profile of ewes during estrus on the different hours

These conditions not significantly different from the reports of [9] stated that progesterone concentration varies when the animal's estrus with the highest value in the mid-luteal phase by 0.30 ± 0.28 ng/ml. While at the beginning of estrus, progesterone concentrations were high enough to 1.86 ± 0.43 ng/ml at h-35 before the start of estrus. Thereafter, the concentration decreased rapidly,

reaching a minimum at approximately 12 hours before estrus coincides with increasing concentrations of estrogen. The mean concentration of estrogen and progesterone on the day 14th post mating in the ewes presented in Table 4.

Table 4. Mean of estrogen and progesterone concentration on the day 14th post-mating

Concentration	Feed		
	f0 (n=5)	f1(n=5)	f2 (n=5)
Estrogen, pg/ml ^{ns}	0.004	0.008	0.008
Progesterone, ng/ml	5.910 ^a	8.070 ^b	11.760 ^c

^{ns} not significant

^{a, b, c}: different superscript at the same row indicates a highly significant ($P < 0.01$)

The rations (f0 and f1, f2) did not influence the estrogen concentration of ewes at the day 14th post-mating. It meant that increasing the energy ration did not enhance the concentration of estrogen on the day 14th post-mating in the ewe. In this condition, the concentration of estrogen tends constantly on the day 14th post-mating in the ewe. This phenomenon is consistent with the research results of [10] that on the first day after mating the average level of progesterone was 190 pg/ml, and after 2 weeks it increased to more than 300 pg/ml ($P < 0.01$). There was a highly significant positive correlation ($P < 0.01$) between the number of corpora lutea from pregnancy with the progesterone concentration. Furthermore, there was a highly significant negative correlation ($P < 0.01$) between the number of corpora lutea from pregnancy with the litter size and estradiol concentration.

The ration (flushing) was significantly affected ($P < 0.01$) the concentration of progesterone on the day 14th post-mating in the ewe. The present study shows that increasing the ration energy enhances the concentration of progesterone on the day 14th post-mating in the ewe. [2] stated that the function of progesterone is to maintain pregnancy and to avoid the performance of estrus and ovulation. Based on this, the high concentration of progesterone on the day 14th post-mating in the ewe can be used for estimating that of those are pregnant. [11] reported that ewes on the day 17th post-mating, the mean concentration of progesterone at the first sampling (after PG I injection) was 10.26 ng/ml and it turned out that the ewes showed pregnancy, while ewes with the mean concentration of progesterone at 0.39 ng/ml shows no pregnancy. All ewe that mated after second PG injection show pregnant and had a mean concentration of progesterone of 17.37 ng/ml. The minimum value of serum progesterone measured in ewes determined as pregnant was 3.65 ng/ml and the maximum was 34.45 ng/ml.

4. Conclusion

This study on Javanese thin-tailed ewes that feeding a high energy content on the action (flushing) concluded that: during estrus, increased estrogen concentration and decreased progesterone concentration, and on the day 14th post-mating, the concentration of progesterone increased in contrary estrogen concentration decreased. High concentration of progesterone on the day 14th post-mating indicated that the Javanese thin-tailed ewes are pregnant.

Acknowledgment

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