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Reproductive Performance, Cholesterol and Progesterone Status of Garut Ewes Fed Ration Containing Different Levels of Sun Flower Oil

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Abstract: This research was conducted to evaluate reproductive performance, cholesterol, glucose and progesterone of Garut ewes fed rations containing different levels of sun flower oil. Using completely randomized design, four ration treatments were given to thirty two ewes (BW 22.12±1.69 kg) with eight animals for each treatment. Four iso-protein rations were used i.e., control (SFO0) without sun flower oil; SFO2, SFO4 and SFO6 were the same as SFO0 but containing 2, 4 and 6% of sun flower oil, respectively. Estrous synchronization was conducted by double injection of Prostaglandin ($PGF_{2\alpha}$) 11 days apart before mating. Reproductive performance (litter size, ratio of male to female, mortality), cholesterol and glucose plasma were measured two weeks before mating and at the first month of pregnancy. Progesterone serum concentration were measured on day 25th, 28th and 31st of pregnancy, using radioimmunoassay. Number of embryo was detected using Ultrasonograph detector at the day 18-20th after mating. Result showed that plasma cholesterol concentration of ewes fed control ration was lower than ewes fed rations containing sun flower oil, while glucose concentration two weeks before mating was lower than those at the first month of pregnancy. Progesterone concentration was the same in all treatments on day 25th, 28th and 31st of pregnancy. Pregnancy rate and number of embryo in SFO2 and SFO6 was 75% and lower than in SFO4 (100%). The highest litter size was in SFO4 (225%) similar to the number of embryo detected by USG, with the male to female ratio of 3:1. However, the percentage of embryo mortality was still high (>20%). It is concluded that inclusion of 4% sun flower oil in the ration could improve reproductive performance (litter size and male sex ratio) and cholesterol concentration.

Key words: Garut ewes, reproductive performance, sun flower oil, cholesterol, progesterone

INTRODUCTION

Garut ewes are prolific animals, producing two or more lambs every birth. However, in the small farmers the litter size is still low (±120%), high embryonic mortality (30%) and pre-weaning periode (20-70%). Essential fatty acids are important in the ration because it functions as membrane compound and precursor of prostaglandin synthesis which is required for growth, lactation and reproduction (Cheng *et al*., 2001; Encinias *et al*., 2004; Yaqoob and Calde, 2007; Palmquist, 2010). Fatty acid supplementation in ruminant ration can improve fertility and embryo development (Cerri *et al*., 2009) and accelerate lambs ability to suckling milk after birth (Capper *et al*., 2006). Zachut *et al*. (2008) states that in dairy cows, levels of unsaturated fatty acids increase the steroid hormones, size in the follicular phase preovulatory, beneficial to ovarian function. Integration of nutrition programs by providing bypass fat can improve the function of the uterus and fertility (Thatcher *et al*., 1994). Sunflower oil is a high source of linoleic acid, potential to be developed in Indonesia. Linoleic acid (n-6) is essential poly unsaturated fatty acid (PUFAs) that important to improve animal reproduction, because supplementation of n-6 PUFA on ration can influence the process of prostaglandin biosynthesis and steroidogenesis that play double roles in the regulation of reproductive function (Wathches *et al*., 2007).

The use of sunflower oil as a source of linoleic acid combined with coconut meal as a source of glucogenic amino acids and cassava as a source of glucose is expected to support the reproductive performance of prolific Garut sheep. The objective of this experiment was to evaluate reproductive performance, blood cholesterol, glucose and progesterone concentration of Garut ewes fed ration containing different levels of sun flower oil as source of essential fatty acids.

MATERIALS AND METHODS

Experimental animals, feeding and design: Thirty two Garut ewes (BW 22.12±1.69 kg, aged 12-14 months) were assigned into a completely randomized design with 4 treatment rations containing different levels of sun

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flower oil namely: SFO0 (0%), SFO2 (2%), SFO4 (4%) and SFO6 (6%). Rations consisted of *Brachiaria humidicola* grass and concentrate in the ratio of 30:70. Concentrate was formulated iso-nitrogenous (Table 1). The concentrate consist of cassava meal, coconut meal, soybean meal, sun flower oil and minerals.

Oestrus synchronization and reproductive performance: Oestrus synchronization was conducted by injecting $PGF_{2\alpha}$ (Noroprost®, Norbrook Laboratories Limited) hormone intramuscularly twice Estrous synchronization was conducted by double injection of Prostaglandin ($PGF_{2\alpha}$) with 11 days apart before mating. Pregnancy rate was determined as the percentage of ewes pregnant per ewes mated. Prolificacy or litter size was calculated as the number of lambs born per number of ewes lambing. Sex ratio of lambs was calculated as ratio of male lambs per number of female lambs.

Collecting blood sample: Blood sampling were carried out before mating and early pregnancy. Blood samples for serum progesterone levels were taken on day 25th, 28th and 31st after mating. Blood samples were collected at the jugular vein using 5 ml disposable sterile syringe (Jung Rim Medical Indutrial CO, LTD, Korea) into glass tube with EDTA. Blood samples were kept at 5°C and then centrifuged at 3634 x g for 15 min to separate plasma or serum. Plasma and serum were separated and stored at -20°C.

Glucose, cholesterol and progesterone assay: The glucose and cholesterol concentrations were analyzed from blood plasma using Glucose kit (Manufactured in Germany for PT Rajawali Nusindo) Cat No. 112191 and Reg. No. AKL 20101803460). Analysis of serum progesterone using progesterone (I^{125}) RIA KIT (Institut of Isotopes Co.Ltd. Konkoly Thage Miklos ut 29-33 H-1121 Budapest, Hungary, Ref:RK-460M) at the Radioactive Isotope Laboratory, Livestock Research Institute, Ciawi, Bogor (2012).

Ultrasound scanning: Ultrasound scanning was conducted at day 18-20th after mating to determine pregnancy rate and number of embryos. It used detector transrectal ultrasound with frequency of 7.5 MHZ (Aloka SSD-500, SN M07265, Aloka Co., Ltd., Tokyo Japan), using the methodology described by Vinoles (2003).

Statistical analysis: Data were analyzed using IBM SPSS Statistics version 20.0. (2011). Some reproductive performances were Chi Square Test.

RESULTS AND DISCUSSION

Reproductive performance: Reproductive performance includes the percentage of pregnancy, litter size, sex ratio and lamb mortality at birth is presented in Table 2. The ewes which consumed rations containing 4% sun flower oil had pregnancy rate 12.5% higher than control. In addition, ewes fed rations containing sun flower oil had higher litter size compared to that of control. Staples *et al.* (1998) state that supplemental dietary fat can improves fertility; increased progesterone to maintain the pregnancy (Pour, 2011). Zachut *et al.* (2008), stated that in dairy cows, levels of unsaturated fatty acids increased the steroid hormones, size in the follicular preovulatory phase, favorable for ovarian function. Bellows *et al.* (2001) reported that supplementation of various fat sources rich in linoleic acid at the end of 65 days of pregnancy in cows produced percentage of pregnancy above 90 percent. Hayat *et al.* (2012) concluded that supplementation of fatty acid soaps can maintain reproductive performance of sheep in the breeding season to produce normal ovarian function, increased number of ovulated oocyetes and an increased percentage of pregnancy.

The ratio of male to female lambs produced by ewes which consumed rations containing 4% sunflower oil was higher (75:25%) or about 3:1 than the other treatments (Table 2), statistically was significant

Table 1: Chemical composition of the concentrate containing different level of sun flower oil and grass (dry matter basis)

	Concentrate				
	SFO0	SFO2	SFO4	SFO6	B.H
Nutrients	%				
Dry matter	86.99	85.63	87.00	87.16	39.70
Ash	6.41	6.42	6.40	5.63	7.29
Crude potein	21.40	20.81	19.95	20.41	12.88
Ether extract	3.79	4.65	7.49	8.05	0.76
Crude fiber	21.74	26.51	21.00	24.75	33.20
Linoleic acid	0.096	0.65	1.62	2.12	0.17
Calcium	0.97	1.17	1.07	0.98	0.63
Phosphor	1.07	0.94	0.89	0.88	0.35

SFO0 (0% sun flower oil), SFO2 (2% sun flower oil), SFO4 (4% sun flower oil) and SFO6 (6% sun flower oil). B.H: *Brachiaria humidicola*

Table 2: Reproductive performance of Garut sheep fed rations containing different levels of sun flower oil

	Treatments			
Parameters	SFO0	SFO2	SFO4	SFO6
Pregnancy rate (%)	87.5	75	100	75
Litter size (%)	133	225	225	200
Sex ratio (Male:Female)	58:42	37:63	75:25	56:44
Lamb mortality at birth (%)	0.0	55.6	22.2	16.7

SFO0 (0% sun flower oil), SFO2 (2% sun flower oil), SFO4 (4% sun flower oil) and SFO6 (6% sun flower oil)

Table 3: Concentration of plasma glucose and number of embryo

Treatments	Pre-mating glucose levels (mg/dl)	Early gestation glucose levels (mg/dl)	No. of embryos*
SFO0	13.12±10.41	71.65±8.86	1.80±0.41
SFO2	21.16±10.91	58.53±23.94	1.67±0.56
SFO4	15.51±11.70	75.44±10.64	2.25±0.46
SFO6	18.78±16.22	79.67±7.06	2.14±0.38

*Ultrasound results; SFO0 (0% sun flower oil), SFO2 (2% sun flower oil), SFO4 (4% sun flower oil) and SFO6 (6% sun flower oil)

Table 4: Cholesterol and progesterone concentrations of Garut sheep fed rations containing different levels of sunflower oil

	Treatments			
Parameters	SFO0	SFO2	SFO4	SFO6
Pre-mating cholesterol (mg/dl)	78.92±17.70	95.38±22.76	96.79±14.25	88.93±20.68
Cholesterol early pregnant (mg/dl)	68.24±23.82	106.98±12.82	94.18±20.99	96.36±24.14
Progesterone day 25th (ng/ml)	33.28±2.94	35.32±3.33	36.37±6.75	33.95±6.05
Progesterone day 28th (ng/ml)	31.15±7.13	30.07±7.94	31.50±4.55	29.30±3.48
Progesterone day 31st (ng/ml)	24.617±4.30	29.317±6.24	28.56±9.98	30.08±3.02

($p<0.05$). Green *et al.* (2008) stated that an increase in polyunsaturated fatty acids in the ration of ewes could increase the ratio of males to females at day 13 after mating. However lamb mortality at birth was still quite high (>20%), that may be due to the difficulty of ewes giving birth for the first time, related to the ability of immature reproductive organs and limited mothering ability.

Plasma glucose levels and number of embryos: Statistically plasma glucose levels did not differ between treatments either at the time before mating, or in the early of pregnancy, but there was an increasing trend with the addition of sunflower oil (Table 3).

Glucose is the main energy needed by the nerve to regulate hormone of LH and FSH at the beginning the follicles formation before ovulation (Hess *et al.*, 2005). There was a relation between the glucose levels and the number of embryos produced. These results supported the finding of Vinoles (2003), that the injection of glucose intravenously for 5 days in estrus phase was positively correlated with the ovulation rate. Poli (1998) stated that plasma glucose of ewes with two child was higher than single child ewes.

Levels of cholesterol and progesterone: The addition of sunflower oil up to 6% into the rations did not give significant effect on cholesterol and the progesterone levels in pregnant Garut sheep (Table 4). However, there is a tendency that the increase of cholesterol levels is closely related to the progesterone concentration, because cholesterol is a precursor to the synthesis of steroid hormones such as progesterone, cortisol, corticosterone and estradiol (Fall, 2008). Another study resulted that supplementation of fatty acid soaps significantly increased serum cholesterol, triglycerides, low-density lipoprotein cholesterol, glucose and progesterone (Hayat *et al.*, 2012).

The addition of sunflower oil in the rations of ewes can provide long chain unsaturated fatty acids such as linoleic acid, which is useful for the synthesis of the progesterone to maintain pregnancy. The results showed that ewes consumed rations SFO4 (containing 4% sunflower oil) produced number of lambs as many as the number of embryos detected by ultrasound. This suggests that the addition of 4% sunflower oil into the ration can maintain embryo until birth.

Pour (2011) described that fatty acids are important to stimulate follicles (elevated $PGF_{2\alpha}$) to bring about pregnancy but later decrease $PGF_{2\alpha}$, which would result in greater progesterone production to maintain pregnancy. Lopez *et al.* (2010) reported that beef cows supplemented with protected fatty acids (PUFA) had improve reproductive performance, visible from the elevated levels of serum progesterone and increased in follicle size preovulatory. This can be caused by an increase in plasma concentrations of LH that stimulates the growth of the second phase of the follicle, the ovulated follicle more lutea cell may result in the formation of the large corporations with increased steroidogenic capacity and yield greater production of progesterone, that is indicated by a higher conception rate (Funston, 2004). The results of this study are also consistent with the research of Akbarinejad *et al.* (2012), who stated that supplementation with polyunsaturated fatty acids omega 6 and omega 3 in the mating phase ration, especially to increase cholesterol, a precursor steroidogenesis and LDL, a carrier to delivering cholesterol to ovarian tissue, but did not significantly affect the level of fertility, prolificacy, sex ratio and progesterone in Zel sheep.

Conclusion: The addition of sunflower oil by 4% in sheep rations can improve reproductive performance (litter size and male female ratio) and cholesterol concentration.

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