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Reproductive Success Rate and Blood Urea Nitrogen Status of Garut Ewes Fed Sorghum-Indigofera in Majalengka District - West Java

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ABSTRACT

The body's Blood Urea Nitrogen (BUN) concentration could be used as diagnostic information for interpreting animal nutritional status, improving nutritional management, and preventing metabolic disorders in ewes during the reproductive phase. This study aimed to evaluate the reproductive success rate and changes in the BUN status of Garut ewes fed sorghum-Indigofera in Majalengka District – West Java, Indonesia. Twenty-eight multiparous Garut ewes with an average body weight of 30 kg, aged 2-3 years or parity from 3rd to 4th, and non-pregnant were separated into two feed treatment groups; divided field grass (FG) and sorghum-Indigofera (SI) mixed feed. This study was designed using a Complete Randomized Design (CRD). After one month of feed acclimatization, all ewes were served an estrus synchronization protocol and mated naturally. A pregnancy test was carried out on the 28th day after mating using ultrasonography (USG). Seven blood samples were taken (1 time in the pre-mating phase and three times each in the pregnancy and lactation phases), and the BUN parameter was analyzed. The results showed that SI feed increased the number of ewes pregnant and giving birth compared to FG (10 vs. 9 and 9 vs. 6, respectively). Meanwhile, the average value of the BUN content of ewes in both FG and SI treatments during the premating, pregnancy, and lactation phases did not show any differences ($p>0.05$). The concentration of BUN is still in normal condition even though it has decreased from pre-mating until the lactation phase. It can be concluded that Sorghum-Indigofera mixed feed could increase reproductive success. However, the average value of BUN in the two types of feed treatment experienced the same status change in all reproductive phases. Both can overcome the energy deficit problem if they have sufficient feed.

Keywords: Energy balance, Indonesian sheep, Renewable forage

Introduction

The mortality rate of the lambs before and after birth was high, and Dixon *et al.* (2007) state that fetal sheep mortality is 19.9%. Meanwhile, HolmØy *et al.* (2017) reported that the mortality rate of neonatal lambs in Norway was 27% occurring 3 hours after birth and 41% within the first day of birth. The lamb's death was caused by infection and starvation. Furthermore, Somanjaya *et al*. (2016) said sheep grazed in several regions of the West Java Province of Indonesia and had a mortality rate of pre-weaned lambs reaching 13.24%.

Many factors can lead to a high mortality rate of lambs before and after birth. The causal factors of loss can occur due to congenital diseases of the ewes, such as fetal malformations, mineral deficiencies, and infections in the womb (Haughey, 1991). Meanwhile, the factors causing post-natal death are more commonly caused by birth weight, age, parity, milk production, litter size, mothering ability, infections, and mineral deficiencies (Tiesnamurti *et al.*, 2002; Somanjaya *et al.*, 2016).

Manalu and Sumaryadi (1998) stated that one of the factors of low reproductive efficiency in ewes is nonlinear between the growth and development of the mammary glands and the number of fetuses. Hayden *et al*. (1979) also state that milk yield is not linear with increased offspring. The low milk yield in the ewes is thought to be due to the insufficient energy content in the feed. Blood Urea Nitrogen (BUN) positively correlates with Milk Urea Nitrogen (MUN) content. High MUN can be caused by insufficient energy to utilize non-protein nitrogen in the body, thus hormonal imbalances inhibit the reproductive process (Rizal *et al.*, 2020). The body's energy adequacy status can be known through high or low levels of BUN. Elevated BUN indicates insufficient energy to synthesize proteins sourced from non-protein nitrogen (NPN) (Tahuk *et al.*, 2008).

Feeding with energy balance is only possible in some sheep-rearing methods in Indonesia since they still utilize a traditional system. The feed is mainly field grass, the nutritional content of which is highly influenced by the season. Several studies have reported that pregnant and nursing ewes suffer from energy deficits as indicated by reduced body conditioning scores (Haughey, 1991). The selection of plant vegetation that can solve these problems is a solution to meet the quantity and quality of feed throughout the year. Sorghum-Indigofera mixed feed is designed so that its energy and protein content meet ewes' pregnancy and lactating standards. The mixed feeding is expected to maintain the condition of blood urea nitrogen in a normal condition and be able to increase the reproductive efficiency of ewes. The concentration of BUN is closely related to the energy balance in the ewe's body in the optimum use of non-protein nitrogen during the reproductive phase. Therefore, this study aimed to evaluate the status of the blood urea nitrogen content of ewes fed sorghum-Indigofera mixed feed during the reproductive phase.

Materials and Methods

Location, animals, and experimental feed

The research was conducted experimentally for nine months (February-October 2022) and was located at Garut sheep farming center in Majalengka Regency, West Java, Indonesia. The location is a coordinate point of 6°48'50.0"S 108°13'35.8"E, with average temperature and humidity at 21-35°C and 66-86%, respectively, and an altitude of 141 m above sea level (Bappeda Kabupaten Majalengka, 2014).

The experimental feed used is Sorghum-Indigofera (SI) mixed feed and field grass (FG) as a control. This research used the sweet sorghum of the Samurai variety one strain Zh-30 irradiated gamma-ray at 30 Gy (Batan, 2014) and Indigofera Zollingeriana as a legume plant (Abdullah, 2014). The first harvest of sorghum was carried out 70 days after planting, and then the stems, leaves, and seeds were processed into silage. Meanwhile, the *Indigofera zollingeriana* was used 50 days after the first harvest, and the leaves and addible stems were processed into Hay. The composition of the experimental feed used was a combination of 50% sorghum silage, 40% Indigofera hay, and 10% rice bran to increase palatability. This combination of forage feed had been tested for nutrient content close to the National Research Council (NRC) recommendations for ewes during pregnancy and lactation (Table 1).

Two types of experimental feed (FG and SI) were served as much as 4% of body weight on dry matter per day (Oqla *et al.*, 2004) to 28 multiparous Garut ewes randomly placed into individual cages, respectively. The criteria for garut ewes include being healthy, aged 2-3 years or parity from 3rd to 4th, having a minimum body weight of 30 kg, and not pregnant (ultrasound testing). Meanwhile, three Garut rams were prepared to mate the ewes. Garut rams criteria were being healthy, having a high libido, having produced offspring, being 2-3 years old, and having an average body weight of 60 kg.

Estrus synchronization, mating, and pregnancy detection

The estrus synchronization protocol was carried out by two intramuscular (i.m.) injections of $PGF_{2\alpha}$ (Lutalyse®, Zoetis, USA) 11 days apart. The dose of $PGF_{2\alpha}$ was 2.50 ml, equivalent to 125 mcg of dinoprostrometamine, served by the recommendations of Mekuriaw *et al*. (2016). After signs of estrus showed, the ewes were naturally mated with a ram. Furthermore, checking and recording of pregnancy were carried out on the 28th day after mating using Ultrasonography (ALOKA type SSD500 Co.LTD, Japan) to see the status of embryos in the uterus in a transrectal (Andriyanto *et al*., 2015).

Blood urea nitrogen test

Blood samples were taken 4 hours after being fed seven times during the reproductive phase (1 time during the acclimatization phase and three times each during the pregnancy and lactation phase). Blood sampling was done with a venoject needle on the jugular vein. The venoject needle was connected to the vacutainer tube without anticoagulants. The required blood was about 3 mL and stored at -40°C for further analysis. Subsequently, the blood was centrifuged at 2000 rpm speed to separate the serum from the blood

Table 1. Nutrient content between the experimental feed (FG and SI mixed feed) with NRC requirement for ewes during the reproductive phase

Description	NRC recommendations	FG	SI
Body weight (kg)	70	30	30
ADG(g d^{-1})	180	-	-
DM (%)	-	30.88	26.45
Ash(%)	-	11.89	10.34
TDN (%)	59	51	59.35
ME (Mcal/kg)	2.1	2.34	2.1
GE (cal/g)	-	3396	4423
Crude protein (%)	10.7	12.60	19.82
Crude fiber (%)	-	22.90	30.57
Ca (%)	0.35	0.83	1.48
P (%)	0.23	0.45	0.56

NRC (National Research Council, 1985); SI = 50% sorghum silage + 40% hay indigofera + 10% rice bran; FG = field grass; ADG = average daily gain; DM = dry matter; TDN: total digestible nutrients; ME: metabolic energy; GE: gross energy; Ca: calcium; P: phosphor; Laboratory tests are conducted in the Lab. Animal Nutrition Science IPB University.

and stored in Eppendorf tubes. Blood urea levels were analyzed using a spectrophotometer at a wavelength of 365 nm by the Berthelot Reaction method (Roseler *et al*., 1993).

Statistical analysis

The data obtained were analyzed using a two-sample t-test. The type of t-test used was an independent sample t-test since the two groups of treatment samples were not paired or not interrelated (Sugiyono, 2010). Furthermore, the data were presented as average values ± standard errors mean and processed using SPSS Program version 25 (SPSS Inc., Chicago, IL) at a 95% confidence level (α=0.05).

Results and Discussion

The conception rate of ewes

Ultrasound examination results on the 28th day after mating detected 9 and 10 pregnant ewes in the FG and SI treatments, respectively. From the number of pregnancies, 90% of the ewes fed SI mixed feed succeeded in parturition and nursing, compared to the FG treatment, which only leads to 66.67% (Table 2). Pregnancy failure is more caused by losing the embryo or fetus in the reproductive tract. Interestingly, this study showed that SI feed could maintain pregnancy and increase fetal survival in the uterus compared to FG. The nutrient content of SI mixed feed is very favourable to the ewe's reproduction process. It was found that the SI mixed feed metabolic energy concentration was consistent with the NRC concentration for ewes during the reproductive phase. Meanwhile, the protein content of the SI feed is higher than FG (Table 1). Both nutrients are thought to have a role in improving ewe's reproduction.

McDonald *et al.* (2010) state that nutrition plays a crucial role in the reproductive process; it can affect fertility, fecundity, puberty, the production of ovum, as well as the survival and growth of embryos and fetuses. Meanwhile, Ayuningsih *et al.* (2018) state that the value of the energy balance and protein of the feed must be considered to support the reproductive process of the ewes. Overall, the nutrient content of SI feed is better than FG (Table 1). Good feed nutrient content can stimulate the growth and development of dominant follicles and oocyte quality, ultimately improving reproductive performance (Gallet *et al*., 2011; Senosy *et al*., 2013; Vlčková *et al*., 2014; Chavarro and Schlaff, 2018). Conversely, the negative energy balance can inhibit the growth of follicles which may be due to a decrease in the production of reproductive hormones through a reduction of the expression of intra-follicular steroidogenic genes, including for the enzyme cytochrome (Ying *et al.*, 2013). In addition, a lack of protein and energy in the feed can result in body weight loss and reproductive failure (Olson *et al*., 1999).

Besides energy and protein content, other chemical compounds in SI feed may affect the reproductive performance of ewes as well. It is known that Indigofera has essential amino acids and antioxidants that are pretty high, namely Arginine and β-carotene. As reported in the study by Palupi *et al*. (2015), Indigofera leaf meal has the highest Arginine (1.67%) compared to other amino acids. It has β-carotene (507.6 mg/kg), one of the components in vitamin A. Wu *et al.* (2013) convey that the amino acid arginine plays a role in the development of conception (embryonic membranes). Furthermore, Arginine's role in sheep's reproductive system is to stimulate the development of brown adipose tissue in the fetus. Arginine supplementation in feed is an effective solution to improve embryonic survival and fetal growth.

Meanwhile, antioxidants (β-carotene) contained in Indigofera forage also play a role in the reproductive process. Agarwal *et al*. (2006) state that antioxidant supplementation can better maintain fetal survival during pregnancy until parturition. Fassah *et al*. (2015) added that antioxidant supplementation (Vitamin E and black tea extract) on sheep rations increased fetal maintenance, the number of twins, and the birth weight of lambs. These antioxidants reduce the oxidative stress level of the ewes and can protect feed with a high polyunsaturated fatty acid (PUFA) content. Gulliver *et al*. (2012) state that PUFA functions to decrease PGF2α synthesis and is continued with progesterone secretion which can improve embryo survival and reduce embryo loss.

Blood urea nitrogen (BUN) status of ewes during the reproductive phase

The high or low concentration of BUN in the animal's body can be attributed to the energy and protein content of a ration served (Boland *et al*., 2001). Changes in BUN status during the

Table 2. Blood urea nitrogen content of ewes during the reproductive phase

Reproductive phase	Sampling time	Number of ewes/n (tails)		Blood urea nitrogen (mg/dl)	
		FG	SI	FG	SI
Pre-mating	7 days before mating	14	14	36.18±0.93	35.27±1.13
Pregnancy	7 days after the ultrasound	9	10	21.86±1.04[a]	18.61±1.10[b]
	middle period	9	10	25.91±1.22	27.14±1.48
	late period	9	10	19.20±1.67	17.88±0.69
Average		9	10	22.49±0.90	21.21±1.00
Lactation	The beginning period	6	9	14.68±1.15	18.07±1.49
	middle period	6	9	13.25±0.60[a]	15.86±0.82[b]
	late period	6	9	15.18±0.67[a]	12.43±0.56[b]
Avarage		6	9	14.37±0.50	15.45±0.73

FG = Field grass; SI = 50% sorghum silage + 40% hay Indigofera + 10% rice bran.
[a,b] Different superscripts on the same line showed a difference (p<0.05).

reproduction phase can be used as a parameter for the appearance of an energy deficit in ewes. The concentration of BUN increases during a negative energy balance which accumulates in the fluid of the dominant follicles and adversely affects oocyte maturation which could reduce fertilization rate and early embryo development (Farman *et al*., 2016). Tahuk *et al*. (2008) state this feeding with high crude protein could cause an increase in BUN in the animal's body. BUN is a product that is formed in the liver as the final product of protein metabolism. Ammonia in the liver formed together with other small molecules will produce urea. Almost the entire urea is included in the liver from the catabolism of amino acids and is the main product of the excretion of protein metabolism.

Interestingly, the results of this study differ from previous studies in that there is often an energy deficit during the reproductive phase. The content of BUN during the reproductive phase, whether fed FG or SI mixed feed, did not increase (Table 2). BUN concentration has gradually decreased from the premating to the lactation period (Figure 2). BUN content at all reproduction stages is under normal conditions. Mitruka and Rawnsley (1981) state that the expected average BUN content in sheep's bodies is around 15-36 mg/dl. This condition showed that FG and SI mixed feed have a good energy balance, thus protein and NPN can be appropriately utilized.

At the beginning of the pregnancy phase, BUN content of ewes with FG treatment was higher ($p<0.05$) than SI treatment, with a difference of 3.52 mg/dl. However, in the middle and late phases of pregnancy, BUN content of the two treatments showed no differences ($p>0.05$). The content of BUN in the middle phases of pregnancy has increased relatively high compared to the beginning and late of pregnancy. Tahuk *et al*. (2008) argue that an increase in BUN content can be related to high levels of protein consumption or stress. Compared to the results by Manalu and Sumaryadi (1998), the content of BUN in FG and SI treatments at the beginning and late phases of the pregnancy has a lower figure. The BUN content of ewes with SI treatment is lower than FG treatment; this could be because SI energy content is higher than FG's.

The ideal ratio between energy and protein feed content causes the protein to be synthesized and appropriately digested by the body. Elrod and Butler (1993) stated that the high protein content in the feed, without an appropriate energy content, could cause the retained nitrogen in the rumen to increase, causing a high level of urea in the rumen and the blood of livestock. During the gestation period, the energy and protein balance of the feed must be strictly observed. Tur *et al*. (2017) state that urea is essential to the rumen's protein and nitrogen metabolism cycle. However, Boakari *et al*. (2020) reported that the high Blood Urea Nitrogen (BUN) concentration in cows and ewes harms embryo development. In addition, McEvoy *et al*. (1997) reported that ewes with high BUN concentrations resulted in a low prolification rate and embryo survival. Meanwhile, Ferreira *et al*. (2011) added that blastocyst hatching was lower in vitro for embryos collected from cows fed a high BUN-producing diet. Therefore, the concentration of BUN must be maintained under normal conditions (15-36 mg dl^{-1}) since high BUN content can reduce fertility and increase the death rate or embryos loss.

The content of BUN in lactation phase is at lower level than during pre-mating and pregnancy. The lowest BUN content in FG treatment was 13.25±0.06 mg/dl, and the highest was 15.18±0.67 mg/dl. Meanwhile, in SI treatment, the BUN content was 12.43±0.56 – 18.07±1.49 mg/dl during lactation. The BUN concentration between FG and

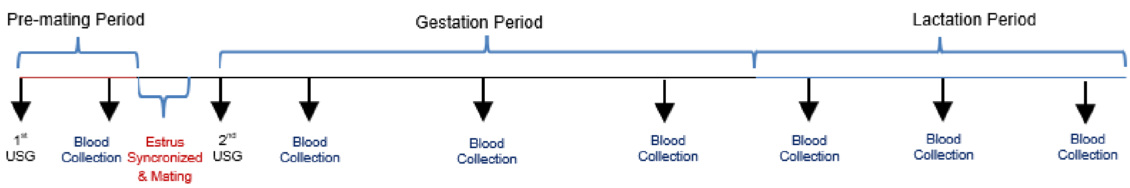

Figure 1. Research flow scheme (the process of measurement of blood urea nitrogen during the reproduction phase of ewes).

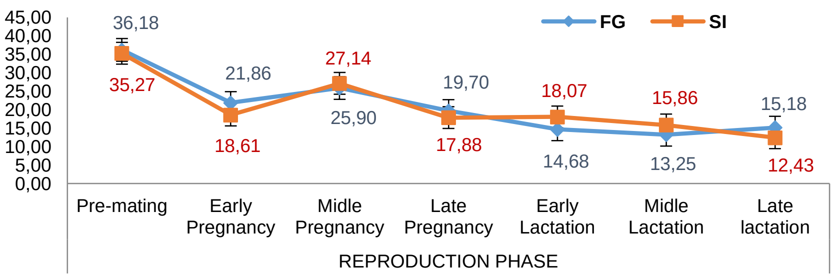

Figure 2. Blood urea nitrogen (BUN) status of ewes fed sorghum-Indigofera during the reproductive phase. FG = field grass; SI = sorghum-indigofera.

SI treatments showed a difference ($p<0.05$) in middle and late lactation. In contrast, at the beginning of the lactation and average BUN in all lactation periods, it did not show a difference ($p>0.05$).

The lower BUN concentration in lactation phase compared to the previous two phases may be related to better utilization of non-protein nitrogen in the animal's body. Even though the energy requirement is higher than in the previous phases, this condition can be overcome with endogenous energy content in body fat. Manalu and Sumaryadi (1997) states that normal BUN conditions indicate sufficient energy in the body to synthesize Non-Protein Nitrogen (NPN) into protein. Meanwhile, energy adequacy, as reported by Chowdhury and Orskov (1997), Ballard *et al*. (2001), and Lien *et al*. (2010), could be found in the form of body fat. If there is much fat in the body, then the endogenous fat can be an energy source to trigger protein gain. It means that the strengthening of normal tissue could be lead without exogenous energy. Caldeira *et al*. (2007) concluded that the condition of BUN in the body could be used as diagnostic information for interpreting animal nutritional status, improving nutritional management, and preventing metabolic disorders.

Overall, BUN content in both treatments in this study was much lower than the results by Manalu and Sumaryadi (1998) and Ginting *et al*. (2020) studies, with BUN content in the range of 26.53±1.21 – 32.52±0.96 mg/dl (Thin-tailed sheep) and 34.48±2.11 – 43.22±2.16 mg/dl (Boerka goat), respectively. Compared to this study's results, field grass (FG) and mixed feed based on Sorghum-Indigofera (SI) have been proven to maintain energy balance in the animal's body as long as they get sufficient feed. The concentration of BUN in each treatment is 13.25-36.18 mg dl^{-1} for FG and 12.43-35.27 mg dl^{-1} for SI mixed feed.

Conclusions

Sorghum-Indigofera mixed feed could increase reproductive success. However, the average value of BUN in the two types of feed treatment experienced the same status change in all reproductive phases. Both can overcome the energy deficit problem if they have sufficient feed.

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