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Research Article
Evaluation of Etawah Grade Doe Fertility Based on Milk Urea Nitrogen Levels

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Abstract

Background and Objective: A favorable characteristic of goats is their prolific breeding; however, non-optimal husbandry practices can hamper the reproductive performance of goats. This study aimed to determine the fertility of Etawah grade does based on Milk Urea Nitrogen (MUN) levels and explored the factors affecting MUN levels. **Materials and Methods:** Forty does with a body condition score of 2.0-3.0, in their second lactation, milked once a day were used in this study. They had *ad libitum* access to concentrates and forage (legumes and jackfruit leaves). Milk was collected every morning during two estrous cycles. The MUN was measured by using urea analysis kit (urea FS). Feed composition was analyzed to determine the Crude Protein (CP) level and fertility was evaluated using postpartum estrus (PPE), Services per Conception (S/C) and Days Open (DO). The relationship between reproduction data and MUN was analyzed using correlation analysis. **Results:** The average MUN was 33.75±3.42 mg dL^{-1} and the average PPE, DO and S/C were 64.80±6.34 days, 120.10±11.4 days and 1.68±0.34, respectively. The MUN was negatively correlated with PPE, S/C and DO. Etawah grade does with a high MUN (37.73 mg dL^{-1}) exhibited long PPE (72 days) and DO (133 days) periods, as well as high S/C (2.0). In contrast, Etawah grade does with low MUN levels had low PPE periods, DO and S/C. **Conclusion:** It was concluded that the MUN level was inversely related to the fertility of Etawah grade does.

Key words: Body score condition, days open, Etawah grade does, fertility evaluation, lactation, milk urea nitrogen, postpartum estrus, service per conception

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Goats provide an important source of income for farmers[1] and animal protein to support the national program for meat self-sufficiency. A favorable characteristic of goats is their prolific breeding, as they produce more than one offsprings from each breeding event and can give birth three times in 2 years. Unfortunately, non-optimal husbandry practices can hamper their reproductive performance[2]. Nutritional deficiencies and imbalances in energy, protein, minerals and vitamins are the main problems associated with Etawah grade husbandry. Livestock productivity can be improved by providing a balanced source of carbohydrates, protein and non-protein nitrogen[3].

The protein consumed by ruminants is digested by rumen microbes to form ammonia, which is a major source of nitrogen and essential for rumen microbial protein synthesis. Ammonia in the rumen is not entirely consumed by microbes and some is absorbed through the rumen wall, while a small part goes to other organs of the intestinal tract. The absorbed ammonia is transported through the blood to the liver where it is converted to urea, which is mostly returned to the rumen through the saliva and partly removed from the body through the urine[4]. In cows, elevated plasma urea and ammonia levels negatively affect the quality of uterine fluid by increasing the concentration of ammonia, which, along with urea, is directly toxic to the endometrium[5]. A previous study examined the effect of ammonia and urea on the expression of mRNA of endometrial fertility-related genes. The circulating urea concentration was correlated with changed expression levels of numerous genes in the endometrium shortly after calving. These genes were predominantly associated with tissue repair, innate immunity and lipid metabolism[6].

The present study will be an important reference source for the maintenance of Etawah grade lactation for optimum production and reproduction and for lower rates of death, toxicity and reproductive failure. The main hypothesis of the study was that the fertility of Etawah grade does is low when the Milk Urea Nitrogen (MUN) level is high.

MATERIALS AND METHODS

Study area: Goat milk samples and reproductive data were obtained from smallholder farmers in Rejodani area, Sleman District, Yogyakarta, Indonesia. The MUN analysis was carried out in the Integrated Research Laboratory, University of Gadjah Mada and the entire study spanned form October 1, 2015 to May 31, 2016.

Materials: The research subjects were 40 does with a body condition score of 2.0-3.0[7], in their second lactation period, milked once a day. The does were fed *ad libitum* with wheat pollard and fresh cut forage (legumes and jackfruit leaves) and received *ad libitum* access to drinking water.

Milk samples were stored in 1.6 mL Eppendorf tubes (Brand, Darmstadt, Germany) that were centrifuged (Eppendorf 5417C, Hamburg, Germany). Other equipment included a urea analysis kit (urea FS, DiaSys Diagnostic Systems, Holzheim, Germany), Microlab 300 system spectrophotometer (ELITech, Logan, UT, USA), micropipette, absorbent paper, timer and distilled water.

Data collection and sampling: Milk samples were collected every morning during two estrous cycles and feed samples were collected the day before milk sampling. Reproductive performance includes postpartum estrus (PPE), Services per Conception (S/C) and Days Open (DO). Postpartum estrue (PPE) refers to the length of time starting when a doe gives birth and lasting until the first time she shows estrus signs. Services per Conception (S/C) refers to the average number of times the doe mates before becoming pregnant. Days Open (DO) refers to the length of time starting when a doe gives birth and lasting until she conceives again. The PPE, S/C and DO data were recorded.

Chemical analysis: A 2 mL milk sample was collected from each animal and centrifuged at 12,000 rpm (15294 g) for 10 min at -4°C. Next, 0.1 mL of the supernatant was collected using a micropipette, placed in a 1.5 mL tube and analyzed for MUN. The MUN levels were measured by using a urea analysis kit and a Microlab 300 system at a wavelength of 570-600 nm. The final MUN concentration was calculated using the following Eq. 1:

$$\text{Concentration} = \frac{\text{Sample absorbance} \times \text{standard concentration}}{\text{Standard absorbance}} \quad (1)$$

Feed samples were dried for 48 h at 60°C, weighed and then ground using a hammer mill with a 1 mm pore size screen. The Crude Protein (CP) content of feed samples was analyzed according to the Kjeldahl method[8]. Fertility indexes were measured based on the postpartum estrous (PPE), Services per Conception (S/C) and Days Open (DO) values.

Statistical analysis: Correlation analysis was used to determine the relationship between the reproductive data and MUN levels using Statistical Program for Social Science (SPSS) version 16.0. Probability at $p \leq 0.01$ was considered statistically significant.

Table 1: Milk urea nitrogen level and fertility in Etawah grade does

		Fertility		
Does	MUN (mg dL^{-1})	PPE (days)	DO (days)	S/C
1	37.98	70	133	2.0
2	36.80	68	130	1.5
3	37.73	72	133	2.0
4	37.17	70	130	2.0
5	38.12	74	128	2.0
6	31.00	55	100	1.0
7	32.36	60	115	1.2
8	34.57	61	111	1.5
9	30.11	58	109	1.0
10	32.63	63	113	1.2
11	36.39	68	120	2.0
12	37.88	71	130	2.2
13	38.73	73	135	2.5
14	37.26	74	134	2.4
15	36.23	70	122	2.0
16	31.21	58	100	1.2
17	31.26	61	113	1.2
18	30.75	61	112	1.4
19	30.36	63	113	1.2
20	35.92	71	133	2.0
21	30.09	57	112	1.2
22	39.68	77	136	2.5
23	36.38	74	132	2.0
24	36.78	75	132	2.0
25	33.23	65	119	1.2
26	36.12	74	128	1.5
27	30.00	58	110	1.0
28	36.98	77	122	2.0
29	35.43	73	114	1.5
30	31.00	64	112	1.2
31	38.92	77	138	2.5
32	37.27	76	130	2.0
33	32.26	61	114	1.2
34	32.35	63	116	1.5
35	32.66	65	112	1.2
36	35.76	75	118	1.5
37	38.13	77	132	2.0
38	39.98	80	140	2.5
39	38,88	76	135	2.0
40	37.73	65	135	2.0
Average	35.01±3.11	68.25±6.98	122.53±10.97	1.70±0.48

MUN: Milk urea nitrogen, PPE: Postpartum estrus, DO: Days open, S/C: Services per conception

Table 2: Correlation and significance of fertility index with milk urea nitrogen in Etawah grade does

Variables	R	R^2
Postpartum estrus	0.892	0.7957
Services per conception	0.919	0.8445
Days open	0.904	0.8172

R: Correlation, R^2: Coefficient of determination, *p<0.01 values are significant at p<0.01

RESULTS

The reproductive performance of the Etawah grade does based on their MUN levels is presented in Table 1. The results of the correlation analysis and significance of the fertility index related to MUN in the does are presented in Table 2. The correlation analysis showed that the MUN differed significantly (p<0.01) for different values of PPE, S/C and DO. The Etawah grade does with the highest MUN level (39.98 vs. 30.00 mg dL^{-1}, the lowest level) had the longest PPE period (80 vs. 58 days, the shortest PPE). The coefficient of determination (R^2) indicates that the PPE had a 79.57% effect (positive effect) on fertility (Table 2).

The results showed that a significantly strong correlation was observed between the S/C and MUN (p<0.01). Furthermore, the analysis of the reproductive performance and MUN revealed that Etawah grade does with the highest

Table 3: Crude protein content of diets of Etawah grade does

Types of feed	Crude protein (%)
Forages	
Legumes	16.09
Jackfruit leaves	13.68
Concentrates	
Wheat Pollard	12.10
Average	13.98

S/C (2.5 times the lowest S/C) had the highest MUN (39.98 mg dL^{-1}), while goats with the lowest S/C (1.0) also had the lowest MUN (30.00 mg dL^{-1}). The R^2 value indicates that the S/C had a 84.54% effect on fertility.

The analysis indicated that the correlation between the DO and MUN was strong ($p<0.01$). The results showed that the longest DO period occurred in Etawah grade does with a high MUN level, while the R^2 indicated that the DO had an 81.72% effect and the remaining 18.28% effect was due to external factors. The average MUN was 35.01±3.11 mg dL^{-1} (Table 1). The proximate analysis showed that the CP content of the diet of the Etawah grade does was 13.98% (Table 3).

DISCUSSION

The results showed a significantly strong correlation between PPE and MUN. The Etawah grade does with high MUN levels had longer PPE periods. The PPE had a considerable effect on fertility, while external factors such as environment (feeding and lactating), physiological factors (milk production, uterine involution and follicle growth) and metabolic factors (hormonal levels of gonadotropins, estrogen and progesterone; weight gain and energy intake)[9] had much lower effects on fertility.

The results showed a significantly strong correlation between S/C and MUN. The Etawah grade does with high S/C also had high MUN levels, while those with low S/C also showed low MUN values. The R^2 indicates that the S/C had a considerable effect, while the effects of the external factors, such as environment (climate and location), feed intake of each animal, quality and quantity of feed and animal health, were much lower. The high S/C value was generally due to the late detection of animals in heat and abnormalities in the reproductive organs of the does[10].

The results also showed that the average DO varied between the different study locations. The data showed that the DO value was 122.53±10.97 days but previous studies reported DO values of 110.09 days[11] in another location and 115.5±21, 124.5±21 and 122.4±18.3 days in lowland areas in Lumajang, Malang and Trenggalek Regency, respectively[12]. The length of the DO greatly affects the reproductive efficiency of livestock and is correlated with body condition score. The correlation between the DO and MUN was strong, with the longest DO period occurring in Etawah grade does with the highest MUN values. Moreover, the R^2 indicated that the DO had a considerably higher percentage effect than that of the external factors, such as environment (climate, location and pen layout), feed intake of each animal, quality and quantity of feed and animal health.

The accumulation of ammonia in the rumen increases urea formation in the liver, which is then circulated by the blood throughout the body, thereby disrupting the reproductive activity of livestock[13]. Increased blood urea nitrogen levels tend to alter the ovarian and uterine physiology and changes in the uterine environment, such as decreased pH during the luteal phase, may play a role in reducing fertility. In addition, high dietary protein and sulfur intake could decrease the uterine pH during the luteal phase and interfere with embryonic development[14].

The DO, which is an indicator of reproductive efficiency, directly affected the length of the kidding interval and milk production. Factors that cause delay in the first breeding are related to management issues such as a low body weight, silent heat, inaccuracy of estrus detection, health and the environment[15]. Good quality feed can enhance the appearance of estrus and nutritional supplementation has been shown to improve ovarian physiology, time to onset of estrus, duration of estrus and pregnancy rates in livestock[16,17]. In contrast, nutritional deficiencies reduce health status, which negatively affects the body condition and milk production.

Furthermore, reproductive conditions can also affect the S/C value[18]. Cystic follicles obstruct ovulation by inhibiting the Luteinizing Hormone (LH) surge. The LH facilitates the release of eggs from the follicle and its deficiency causes ovulation failure, leading to a larger than normal follicle or the development of a follicular cyst. The results showed average MUN levels of 35.01±3.11 mg dL^{-1} (Table 1). When the dietary amount of soluble carbohydrates was low in lactating goats, their urinary allantoin level was low but their milk urea content was high. However, when the dietary amount of rumen-degradable N was low, the milk urea content was also low[19].

The concentration of urea nitrogen in milk indicates how goats utilize the CP they consume. High levels of dietary protein enhance milk production; however, they also interfere with reproductive performance[20]. Some products of protein metabolism, such as ammonia, urea and other toxic substances, might be responsible for the interference in

reproductive efficiency. The MUN level can reveal the status of the protein intake and energy output balance. An understanding of an animal's nutritional status can optimize the benefits of the MUN without exerting potential adverse effects on reproductive performance[21].

Protein is required for normal physiological functions of the body. In addition, proteins are decomposed by proteolytic enzymes in the rumen, producing peptides and amino acids. The peptides and amino acids then undergo deamination, which produce ammonia (NH_3), a vital substance for rumen microbial protein synthesis and the proliferation of microbes[4]. The proximate analysis of diet of Etawah grade does showed that the CP content was 13.98%. The CP requirement for lactating goats varies depending on body weight and milk production[22] and lactating goats that weigh 50 kg require 14.5% CP day^{-1}. Thus, the diet used in the present study was below the standard requirement.

In lactating cows, high MUN values indicate that the energy was used to convert ammonia into urea and therefore, was diverted from milk production. The urea nitrogen concentration of cow milk provides an overview of the utilization of consumed CP. The MUN value increased in the presence of an excess level of Rumen Degradable Protein (RDP) and Rumen Undegradable Protein (RUP), which was not balanced by the Non-Fiber Carbohydrate (NFC). The unused CP is converted into urea by the liver and subsequently transported to the blood, urine and milk[23,24]. High MUN levels may be correlated with excessive RDP and/or RUP, as well as a low rumen fermentation rate of NFC, excess dietary CP and an increased ratio of CP to nonstructural carbohydrates[25,26].

CONCLUSION

The present study demonstrated that the fertility of Etawah grade does was low when the MUN was high, indicating that the MUN could be a useful parameter for evaluating the fertility of goats and other livestock.

SIGNIFICANT STATEMENT

The current study demonstrated that the MUN level is significantly correlated with postpartum estrus (PPE), Services per Conception (S/C) and Days Open (DO), while low MUN levels are associated with low PPE periods, DO and S/C in goats. In other words, the fertility of Etawah grade does is low when the MUN level is high; hence, MUN is a potentially useful index for determining the fertility of goats and possibly other livestock.

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