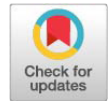


Research Article



Response of Blood Metabolite Levels of Saanen-Etawah Crossbred Does to Ovarian Cycle

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Abstract | The physiological state during the estrus cycles has the potential to affect the blood biochemical composition of livestock and serve as a reference for livestock reproductive nutrition and maintenance management. This study aims to determine the blood metabolite levels of Saanen-Etawah Crossbred does during the follicular and luteal phases. Fourteen non-pregnant-lactating Saanen-Etawah Crossbred does were used in this study (age 2-4 years, BCS 3, and multiparous). The same management was given throughout this research and the feed intake was observed daily. The blood was taken during the follicular and luteal phase which was validated from data of vaginal smear. The obtained serum from whole blood was immediately for total protein, cholesterol, glucose, and blood urea nitrogen (BUN) levels assay. The spectrophotometry method used for biochemical data using specific enzymatic were used. Feed samples were analysed using proximate method to determine dry matter (DM), crude protein (CP), and total digestible nutrient (TDN) intake. The data results were analysed using Independent Sample T-Test and the correlation between blood metabolite profiles and nutrients was analysed using bivariate correlations by Pearson. The data showed that the cholesterol level was higher ($p < 0.05$) on follicular phase (134.04 ± 25.11 mg/dL) than on luteal phase (96.74 ± 22.67 mg/dL), while no differences were found in other parameters despite slight discrepancies between the two phases. During the follicular phase, there was a strong correlation ($p < 0.01$) between respective parameters such as BUN with glucose and DM with TDN intake. However, the correlation data did not show any differences in the luteal phase.

Keywords | Blood metabolites, Dairy goats, Follicular phase, Luteal phase, Nutrients, Ovarian cycle

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INTRODUCTION

Global milk demand has increased over the years, and is always in a deficit situation relative to availability. One alternative for milk producers is by utilising dairy goats, especially Saanen-Etawah dairy goats, which produce quite high levels of milk around 0.8-1.2 litres/

head/day in tropical countries such in Indonesia (Setiati *et al.*, 2023; Suranindyah *et al.*, 2018; Sitaresmi *et al.*, 2023). The most common problem found in the Saanen-Etawah rearing development program is the dairy goat population, especially in developed countries held by traditional farmers who reared the animals extensively, which has led to stagnation of the goat population due to unachieved

reproductive efficiency and lower milk production in general (Sitaresmi *et al.*, 2017).

Regarding this matter, good management must be implemented to optimize the dairy goat's reproduction ability. According to a previous study, goat milk production capacity is influenced by genetic factors, feed, and body size (Santoso *et al.*, 2020). Body nutrition can be obtained from the feed but dairy goats were known to go through the lactation period immediately after the parturition process which makes feed management very important for dairy animals. In the dairy goat industry, nutritional adequacy and good management will affect livestock productivity including reproductive success. For example, the continuous lactation on dairy goats obtained from the implementation of good reproductive management (Kumala *et al.*, 2022a). This will also have an impact on the efficiency of milk production and determine the sustainability of dairy farming business (Suranindyah *et al.*, 2018).

Biochemical blood levels are essential for gauging an animal's health status and are an important indicator of metabolic activity or fertility status in dairy animals (Widayati *et al.*, 2017a; Sitaresmi *et al.*, 2017). According to Hudaya *et al.* (2020) blood biochemical profile in livestock varies at different physiological stages, for example, during pregnancy, after giving birth, and during dry periods. Several components of blood metabolites have important functions, including reproductive functions. Abnormal changes in some blood metabolite components can adversely affect the reproductive performance (Widayati *et al.*, 2018).

Additionally, the patterns and levels of blood biochemistry are strongly related to the nutritional status of the feed given (Mpendulo *et al.*, 2020). Therefore, efforts to monitor blood biochemical data with the aim of evaluating whether there are changes in blood biochemistry are important as a further reference for livestock maintenance management plans, especially for the preparation of diets to improve livestock reproduction which has an impact on increasing livestock populations in general. However, the effect of different estrus phases on blood metabolites was still questionable. Besides that, the correlation between feed intake and blood metabolites during the follicular and luteal phases has not been observed thoroughly, especially in Saanen-Etawah Crossbred does in traditional farmers. The purpose of this study was to determine the blood metabolite levels of Saanen-Etawah Crossbred does in tropical climates, especially on total protein, cholesterol, glucose, and blood urea nitrogen (BUN) during the follicular and luteal phases, which can be used as indicators of the physiological and reproductive status of the Saanen-Etawah Crossbred does. The findings are also expected to be used as basic information for maintenance management

decisions and as a starting point to develop detector kits in future studies for the selecting process, in order to increase the productivity of Saanen-Etawah Crossbred does.

MATERIALS AND METHODS

RESEARCH LOCATION

This study was conducted from August 2021 to February 2022 on a traditional dairy goat farm in Turi, Sleman Regency, Yogyakarta, Indonesia (7.37° LS and 110.22° LE). The proximate feed analysis and serum preparation was carried out at the Laboratory of Feed Technology and Laboratory of Animal Physiology and Reproduction, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta. The blood serum levels was analysed in the Integrated Research and Testing Laboratory, Universitas Gadjah Mada, Yogyakarta.

EXPERIMENTAL ANIMALS

This study utilized 14 non-pregnant and lactating Saanen-Etawah Crossbred does, which were selected based on the age of the doe (2-4 years), had been given birth twice (parturition of 2) and had a body condition score (BCS) of 3 from a scale of 1 to 5 based on the study by (Ghosh *et al.*, 2019). Maintenance management is homogenized without special treatment with the feed given in accordance with the feed given by smallholder farmers, but tended to be the same throughout the research process. The feed consisted of commercial concentrate, dried water spinach (*Ipomoea aquatica*), and soybean husk (*Glycine max*), given twice a day in the morning (7:00 am) and afternoon (2:00 pm) with nutrient composition showed in Table 1. Drinking water is provided *ad libitum*.

Table 1: Feed nutrient composition.

Feed ingredients	Nutritional contents (%)		
	DM	CP	TDN
Commercial concentrate	89.95	19.23	77.66
Dried water spinach (<i>Ipomoea aquatica</i>)	86.71	14.46	41.35
Soybean husk (<i>Glycine max</i>)	26.02	16.74	51.87

DM: dry matter; CP: crude protein; TDN: total digestible nutrient.

EXPERIMENTAL DESIGN

The blood samples were collected approximately three hours after morning feeding and bleed from the jugular vein (Sitaresmi *et al.*, 2017). The samples that have been collected then grouped into two phases of the estrus cycle the follicular and the luteal phases, based on observations of the vaginal cytology (Sitaresmi *et al.*, 2019). The blood was taken from a jugular vein with approximately 8 to 10 mL inside the collecting tube with ethylenediaminetetraacetic acid (EDTA). Blood was centrifuged at 3000 rpm for 15

minutes to separate serum and plasma. The obtained serums are then stored at 20°C (Kumala *et al.*, 2022b). All serums were analysed using reagents for the parameters of blood serum profiles (DiaSys, Germany) and a spectrophotometer Microlab 300 (Merck, Germany). Total protein levels were determined by the biuret method. Cholesterol levels were determined by the cholesterol oxidase-peroxidase aminoantypirin (CHOD-PAP) method. Glucose levels were determined with the glucose oxidase-peroxidase aminoantypirin (GOD-PAP) method. Blood urea nitrogen (BUN) levels were determined with urease-GLDH test method. The feed intake was calculated weighing every day for a week before the blood collection (Kumala *et al.*, 2022b), and was analysed using the proximate method to determine the crude protein (CP), total digestible nutrient (TDN) and dry matter (DM) value (AOAC, 2005).

STATISTICAL ANALYSIS

Analysis of data on blood metabolite levels of Saanen-Etawah Crossbred does was carried out using the Independent sample T-Test from the statistical package for the social sciences (SPSS) 26.0 software. The grouping which is based on the follicular and luteal phases, was considered as a treatment. And the variables of both phases are compared to know the impact of different estrus phase on blood metabolite profiles. The α value used is $p < 0.05$ (5%), and the correlation between blood metabolite profiles and nutrients was analysed using bivariate correlations by Pearson with 2-tail analysis. All the analysis data was presented as mean \pm SD.

RESULTS AND DISCUSSION

BLOOD METABOLITE PROFILES AND FEED INTAKE

The result of total protein, cholesterol, glucose, and blood urea nitrogen on follicular and luteal phases were showed in Table 2. The cholesterol concentration was differed ($p < 0.05$) which was higher in the follicular phase if compared to the luteal phase. Whereas total protein, glucose, and blood urea nitrogen remains in the same range. The blood metabolite profiles still within a normal ranged and healthy condition to support the reproductive performance of Saanen-Etawah Crossbred does (Kaneko *et al.*, 2008; Kumala *et al.*, 2022b). According to Sitaresmi *et al.* (2020) does with BCS score 3 has the most ideal concentration of blood metabolites compared to other variations of BCS scores. Does with parturition 2 have best nutrient availability to support growth and reproductive performance (Kumala *et al.*, 2022b; Safdar and Kor, 2014) and already have an optimal level of physical maturity (Hadisutanto *et al.*, 2012).

According to Khan *et al.* (2013) it has been known that cholesterol is an energy source and acts as a precursor to

steroid hormones, bile acids, and it's also necessary for normal cell function. Based on the results (Table 2), it is known that the levels of cholesterol in a previous study by Sitaresmi *et al.* (2017) were comparable. Higher cholesterol levels on follicular phase are in line with Sitaresmi *et al.* (2020) who stated that cholesterol is necessary for the formation of cell membranes, the synthesis of vitamin D, estrogen, testosterone, and other steroid hormones and naturally changed to support those mechanism. The results of cholesterol levels that are higher in the follicular phase than the luteal phase can be due to does that are at the end of the estrus phase. Blood cholesterol levels will increased at the end of the follicular phase and will decrease in the luteal phase (Tarumi *et al.*, 1988; Sitaresmi *et al.*, 2020). Cholesterol deficiency in the blood can result in silent heat, short heat, a prolonged anestrus period, and a lower pregnancy rate (Sitaresmi *et al.*, 2017). Although further study might still be needed to understand the cholesterol level in each different estrus phases.

Table 2: Blood metabolite levels of Saanen-Etawah crossbred does (mean \pm SD) during ovarian cycle.

Blood metabolite	Follicular phase (n=7)	Luteal phase (n=7)
Total Protein (g/dL)	5.28 \pm 1.98	6.66 \pm 3.58
Cholesterol (mg/dL)	134.04 \pm 25.11*	96.74 \pm 22.67*
Glucose (mg/dL)	61.92 \pm 7.34	60.88 \pm 3.09
Blood Urea Nitrogen (mg/dL)	21.21 \pm 3.99	19.42 \pm 5.48

*Means in the same row are different between groups ($p < 0.05$).

Glucose levels in the blood indicated energy consumption (Kumala *et al.*, 2022b). Widiyono *et al.* (2020) also stated that glucose is an important source of energy for the ovaries. Based on the results (Table 2), it is known that the levels of glucose are comparable and consistent with a previous study (Ginting *et al.*, 2011). The phase factors in each phase of the estrus cycle had no effect on variations in glucose levels in the blood, and more significantly influenced by onset of puberty, gestation period, lactation period, and the age of the animal (Sitaresmi *et al.*, 2020). Dairy goats must have optimal glucose levels in their blood because glucose is one of the factors that can influence ovarian function. Low glucose can result in elevated levels of non-esterified fatty acids (NEFA) which can be toxic to follicles and oocytes and inhibit gonadotropin-releasing hormone (GnRH) secretion (Purwitasari *et al.*, 2020).

Blood urea is the final result of protein metabolism in the body of ruminants (Widayati *et al.*, 2019). Urea circulating in the blood vessels can be measured as urea nitrogen in the blood plasma or serum fraction and can be expressed as the blood urea nitrogen (BUN) value. The results (Table 2) revealed that BUN levels between follicular and luteal phases in does with body condition score of 3 was still

within the normal range and comparable. The result of this study also in similar with previous study (Kumala *et al.*, 2022b; Sitaresmi *et al.*, 2017) in dairy goats. Blood urea nitrogen level indicated great milk production and also can disrupt the reproductive ability of goats (Kumala *et al.*, 2022b). The negative impact of high levels of BUN on reproductive performance can reduced the pH of the uterus and adversely affected the implantation process and interferes with embryonic development (Tshuma *et al.*, 2014; Widayati *et al.*, 2017b). High levels of urea in the blood indicated low availability of energy in the body, and can increase the occurrence of repeat breeding (Widayati *et al.*, 2017b).

Protein is a substance that regulate the metabolic system in the body and as the main source of cell regeneration processes in living animal (Fachiroh *et al.*, 2012). The analysis results (Table 2) showed that total protein levels are similar and in accordance with a previous study (Sitaresmi *et al.*, 2020). Total protein levels were not differed between the follicular and luteal phases, and albumin synthesizes certain specific amino acids used in the synthesis of gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) during the early phases of follicle formation and initiation of ovulation, because they have an important role in the synthesis of reproductive hormones, amino acid components must be available in optimum quantities (Sitaresmi *et al.*, 2020). The results of total protein levels is different with the analysis of crude protein (CP) that has significant different between follicular and luteal phases, it is suspected that protein in blood serum has been used for the formation of hormones and other physiological functions. Levels of protein that are too low in the blood lead to amino acids deficiency and result in a decreased in reproductive performance (Sitaresmi *et al.*, 2020; Widayati *et al.*, 2017a). Lack of blood protein levels in female livestock, can cause weak heat, silent heat, and repeated mating (Ramandani and Nururrozi, 2015).

Table 3: Feed intake of Saanen-Etawah crossbred does (mean±SD) during ovarian cycle.

Nutrient content (g/head/day)	Follicular phase (n=7)	Luteal phase (n=7)
Crude protein (CP)	287.10±1.42*	264.73±12.76*
Total digestible nutrient (TDN)	1032.38±18.89	1009.33±32.49
Dry matter (DM)	1754.39±203.65	1804.09±187.48

*Means in the same row are different between groups ($p < 0.05$).

The feed intake needs in this study were adjusted according to the needs of lactating dairy goats and in accordance with the previous study (Ramadhan *et al.*, 2013). Despite being given the same amount of feed and management, the results of feed intake on follicular and luteal phases

(Table 3) showed that the crude protein (CP) intake differed ($p < 0.05$) which was higher in the follicular phase if compared to the luteal phase, although insignificant DM and TDN intake were found. The increased of CP intake in the follicular phase is explained by Hussein *et al.* (2021) who stated that increased levels of protein in the diet are associated with increased rates of ovulation and increased levels of circulating FSH during the second half of the estrus cycle.

CORRELATION BETWEEN BLOOD METABOLITES AND FEED INTAKE

In order to support the feed consumption data, this study also found a negative correlations between BUN and Glucose in the follicular phase ($p < 0.01$) showed in Table 4. The correlation analysis (Table 4) also showed a positive correlation between TDN and DM intake found in the follicular phase ($p < 0.01$). Meanwhile, based on the result (Table 5) there is no correlation between blood metabolite profiles and feed intake in the luteal phase.

Table 4: Correlations between blood metabolite profiles and feed intake in follicular phase.

Pearson correlation	TP	Choles-terol	Glu-cose	BUN	CP	TDN	DM
TP	1	-.156	-.022	.102	-.125	.624	.622
Cholesterol	-.156	1	.590	-.326	-.049	-.583	-.546
Glucose	-.022	.590	1	-.881**	.132	-.636	-.635
BUN	.102	-.326	-.881**	1	-.368	.514	.569
CP	-.125	-.049	.132	-.368	1	-.108	-.317
TDN	.624	-.583	-.636	.514	-.108	1	.977**
DM	.622	-.546	-.635	.569	-.317	.977**	1

TP: Total Protein; CP: Crude Protein; TDN: Total Digestible Nutrient; DM: Dry Matter; **: Correlation showed a different at 0.01 level (2-tailed).

Table 5: Correlations between blood metabolite profiles and feed intake in luteal phase.

Pearson correlation	TP	Cho-lesterol	Glu-cose	BUN	CP	TDN	DM
TP	1	-.039	.132	.206	-.099	-.144	-.197
Cholesterol	-.039	1	-.308	-.047	.482	.072	-.583
Glucose	.132	-.308	1	-.124	-.150	.334	.438
BUN	.206	.047	-.124	1	.689	-.187	-.383
CP	-.099	.482	-.150	.689	1	-.231	-.657
TDN	-.144	.072	.334	-.187	-.231	1	.729
DM	-.197	-.583	.438	-.383	-.657	.729	1

TP: Total Protein; CP: Crude Protein; TDN: Total Digestible Nutrient; DM: Dry Matter

The interesting founding in recent study was some blood metabolite parameters were correlated each other in follicular phase but did not correlated on luteal phase.

This may be due to the fact that in the follicular phase the physiological conditions of the reproductive organs, especially the hypothalamic-pituitary-gonadal (HPG) axis, the ovaries operated quite intensely under the influence of reproductive hormones to facilitate fertilisation rather than on luteal phase (Kandiel *et al.*, 2010), which will indirectly affect feed intake and the interaction of each blood metabolite parameter in follicular phase. The negative correlations between BUN and glucose were found in the follicular phase (Table 4) was in accordance with the previous study. The negative correlation between glucose and BUN suggested that there was just a little or maybe none of the conversion of protein intake to glucose and also affected by enhanced CP intake on follicular phase. The negative correlation between glucose and plasma urea seems related to the protein content in plasma, because it is known, there was a link between protein storage in the body with glucose levels and high protein content decreased blood glucose (Chikwanda and Muchenje, 2017). The positive correlation between TDN and DM intake in the follicular phase (Table 4) was in accordance and matched with the previous study by Suwignyo *et al.* (2016) which stated that DM consumption is directly proportional to TDN, if DM consumption increased, the TDN consumption also increases and vice versa, this is influenced by feed factors. The increased TDN levels in concentrate also has an effect on increasing DM degradability (Ahn *et al.*, 2019). The positive correlation between TDN and DM in the follicular phase obtained in this study is related to the adequacy of energy sources (Khan *et al.*, 2013). Especially cholesterol which plays an important role for the formation of steroid reproductive hormones (Sitaresmi *et al.*, 2020). Increased cholesterol levels during the follicular phase (Table 2) have an impact on increasing estrogen levels. Furthermore, increasing estrogen levels will provide positive feedback on luteinizing hormone (LH) which plays an important role in the process of follicle development and ovulation (Widayati, 2023; Sitasiwi, 2008).

CONCLUSIONS AND RECOMMENDATIONS

The blood metabolites were required to optimize and support the doe's physiological needs. Therefore, there were slight changes in blood metabolites following the reproduction cycle of Saanen-Etawah Crossbred does, which can be used as an indicator of livestock health and fertility. High cholesterol levels during the follicular phase are associated with follicular development and the occurrence of estrus expression. It is necessary to conduct further study related to the blood metabolites of the Saanen-Etawah Crossbred does by increasing the number of samples also significantly different feed treatments in each phase of reproduction based on the results of current

research, so that more in-depth data are obtained to support the reproductive efficiency of small-scale dairy goat farms.

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NOVELTY STATEMENT

Determination of blood metabolite levels of Saanen-Etawah Crossbred does in traditional farmers in Indonesia has not been widely studied under field condition, especially on total protein, cholesterol, glucose, and blood urea nitrogen (BUN) during the ovarian cycle.

AUTHOR'S CONTRIBUTION

All authors designed and supervised the study, DFFD, SK, PIS, SWP, MA, YYS, and DTW drafted the manuscript and conducted the literature search. DFFD, SK, SWP, MA, and DTW conceived, performed the fieldwork, administered, and helped with the manuscript. DFFD interpreted the data and edited the manuscript. DFFD, SK and PIS performed the statistical analysed and reviewed the manuscript. DTW and YYS supervised the study. In the final stage, all authors have read and agreed to submit the final manuscript.

ETHICAL APPROVAL

All experimental procedures including dairy goat using and blood collection method were approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia (00070/EC-FKH/Eks./2021). This decision was finalized on July 2nd, 2021 and valid throughout the research period. The maintenance carried out prioritizes animal comfort. The pens used in this study were individual pens without animal tied, so that animals could move freely, this aims to reduced stress in animals.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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