The comparison of creatinine, iron, and blood metabolites in primiparous and multiparous Saanen Etawah crossbred goats in tropical country, Indonesia

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Abstract

The study aimed to explore changes in serum creatinine, iron, and blood metabolites in different parity statuses in traditionally managed Saanen Etawah crossbred goats. Mature lactating goats (n = 130) were divided into primiparous (n = 45) and multiparous groups (n = 85) (body condition score 3, early-middle stage of lactation). Blood samples were collected from the jugular vein and centrifuged to collect plasma; blood metabolites were measured using UV Vis methods. The result showed creatinine concentrations in multiparous goats were significantly ($P < 0.05$) higher (0.87 ± 0.21 mg/dl) than in the primiparous group (0.79 ± 0.15 mg/dl). Meanwhile, the albumin concentration in primiparous goats (3.99 ± 0.20 mg/dl) was significantly higher than in multiparous goats (3.82 ± 0.16 mg/dl); also the total cholesterol level of primiparous goats (122.28 ± 29.20 mg/dl) was significantly higher than in multiparous goats (107.37 ± 24.40 mg/dl). The urea-creatinine ratio was higher in primiparous goats (27.07 ± 11.90) than in multiparous goats (22.37 ± 8.12). It was concluded that distinct blood metabolites between primiparous and multiparous goats were due to different physiological needs which led to different mobilization of stored nutrients inside the body. Different feeding strategies were suggested for each parity group following their nutritional needs, such as a high-protein diet to optimize primiparous body development, and a high-energy diet to enhance multiparous milk production before and after giving birth. Further research is needed to understand the exact optimal ratio of feed energy and protein.

Adapted tropical Saanen, blood profile, parity statuses

The Saanen Etawah crossbreed is a dairy goat locally adapted to tropical environments as a result of crossing between Saanen goats with Etawah crossbred goats. The Saanen Etawah crossbred goats have a great potential as new Indonesian national livestock with high milk production abilities in tropical environments. Therefore, information on this goat breed is an interesting topic to be reviewed and studied further since there is scant data on this commodity, especially for the tropical-adapted crossbreed of Saanen goat (Sitaresmi et al. 2017).

Parturition is a crucial period for the subsequent performance of dairy goats. The gestation period is an important time when the nutritional requirements of dairy goats need to be met for the growth and development of the foetus. After the gestation period, lactation period immediately occurs which causes further imbalance and discrepancy between nutrient utilisation and intake. With the increased parity status of tropical dairy goats, it is possible to see changes in blood metabolites due to the accumulation of nutritional needs throughout the repeated gestation and lactation period, but this aspect has rarely been studied (Kumala et al. 2022a; Kumala et al. 2022b). Therefore, it was speculated that primiparous and multiparous goats would have different blood metabolite profiles due to differences in the physiological status that may have occurred.

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The association between creatinine (Cre) and iron with the parity status and other blood metabolite indicators (total cholesterol, blood urea nitrogen, albumin, and total protein) has rarely been studied. A decent amount of nutrients is required to achieve sufficient and continuous reproduction, therefore blood metabolites are needed to illustrate their nutritive availability since deficiency might disrupt the reproductive cycle and efficiency. Cre is an essential product of the catabolism process, and changes in its concentration are based on the feed but also muscle metabolism (Kurpińska et al. 2020) which is associated with renal function (Widiyono et al. 2020) and which changes following the gestation stage in goats (Donia et al. 2014; El-Hamid et al. 2017) and dairy cows (Kurpińska et al. 2020) to support foetus development (Donia et al 2014). Iron is classified as a micromineral nutrient with many physiological functions. One of them is considered essential for binding and transporting oxygen in its key functions as a major protein of haemoglobin and myoglobin (Joerling and Doll 2019). Iron is needed at optimal levels and its deficiencies have been reported to cause an increase in the number of repeat breeding cases in dairy goats (Widiyono et al. 2020).

Good reproductive management is needed to achieve continuous lactation in goats (Kumala et al. 2022b) since milk production efficiency reflects continuity as a dairy business (Suranindyah et al. 2018). This study aimed to understand the changes in serum Cre, iron, and blood metabolites with different parity statuses to provide a new breakthrough to enhance reproduction efficiency. The Saanen Etawah crossbred goats used in this study represent a new alternative for dairy goats with high milk yield and high adaptability in tropical climates. Therefore, the biochemical status related to their reproductive performance is of utmost importance.

Materials and Methods

Ethical statement

The use of animals and blood collection procedures in this study were approved by the Research Ethics Committee of the Faculty of Veterinary Medicine at Universitas Gadjah Mada, Indonesia, Yogyakarta, Indonesia (00070/ECFKH/Eks./2021). The data collection process was conducted according to the given suggestions.

General methodology and treatments

This research was conducted on traditional farms in Girikerto, Turi, Sleman, Yogyakarta, Indonesia (7.37°LS and 110.22°LE) from early July 2021 until January 2022. This study attempted to identify uniform external factors such as feeding, housing, body condition score (BCS 3), and lactation stage (mid-lactation). Healthy mature lactating goats (n = 130) were classified into a group of primiparous goats (n = 45) and a group of multiparous goats (n = 85). The given feed consisted of ground *Ipomoea reptants*, dried mung bean skin, and concentrates. The proximate feed value was analysed at the Laboratory of Feed Technology, Faculty of Animal Science, Universitas Gadjah Mada, Indonesia to observe the contents of dry matter, inorganic residue, crude protein, extract ether, crude fibre, nitrogen-free extract, and total digestible nutrient. The given feed was then calculated and compared to the standards of the NRC for dairy goats. The details of nutrients from the final feed are shown in Table 1, and the goats' feed intake is shown in Table 2. All animals had free access to water.

Blood collection and serum preparation

Blood collection was conducted before feeding and after milking time, from around 07.00 to 08.00 a.m. Blood samples were then collected from each doe (n = 130) from the jugular vein using a BD 22G vacutainer (New Jersey, USA) and kept in vaculab with EDTA (New Jersey, USA) (Darmawan et al. 2020) for no longer than 5 h. The segregation of blood into serum was carried out in the Laboratory of Animal Physiology and Reproduction, Faculty of Animal Science, Universitas Gadjah Mada, Indonesia. The obtained blood was centrifuged (Dlab Scientific, US) at 3020 *g* for 15 min, then stored inside the freezer at −20 °C (Darmawan et al. 2019; Widayati et al. 2019; Darmawan et al. 2020; Kumala et al. 2021).

Biochemical blood analysis

The obtained serum was analysed at the Integrated Research and Testing Laboratory, Universitas Gadjah Mada, Indonesia. The GOD-PAP method, CHOD-PAP method, urease-GLDH, bromcresol-blue method, and biored method were used to determine the blood concentrations of glucose, total cholesterol, blood urea nitrogen (BUN),

albumin, and total protein, respectively (Widiyono et al. 2020; Kumala et al. 2022a). Serum iron concentrations were analysed by atomic absorption spectrophotometry (Thermo Fisher, Massachusetts, USA) (Omidi et al. 2018) using ContrAA300 (Analytik, Jena, Germany). The Cre concentrations were analysed by the Jaffe method (Kumala et al. 2022a) using a Microlab 300 (Merck, Darmstadt, Germany) with a wavelength of 492 nm. The ratio of BUN to Cre data is required to understand their comparisons.

Statistical analysis

The Cre, iron, and blood metabolite (glucose, albumin, total cholesterol, BUN, and total protein) concentrations were analysed using the independent sample *t*-test method ($\alpha = 5\%$). The results are shown as mean ± SD. The correlation between the obtained data was analysed using Pearson Correlation. All statistical analyses were conducted using the Statistical Program for Social Sciences (SPSS) 25.0.

Results

The results of Cre, iron, and blood metabolites concentration on primiparous and multiparous goat are given in Table 3. The result showed that the Cre concentration in multiparous goats was significantly ($P < 0.05$) higher (0.87 ± 0.21 mg/dl) compared to the primiparous group (0.79 ± 0.15 mg/dl). Meanwhile, the albumin concentration

Table 1. Nutritional feed composition of primiparous and multiparous Saanen Etawah crossbred goats (kg/goat/d).

	DM (kg)	Inorganic residue (kg)	CP (kg)	EE (kg)	CF (kg)	NFE (kg)	TDN (kg)
Primiparous	2.04	0.27	0.29	0.11	0.58	1.08	1.12
Multiparous	1.89	0.25	0.33	0.11	0.57	1.03	1.24

DM: dry matter; CP: crude protein; EE: ether extract; CF: crude fibre; NFE: nitrogen free extract; TDN: total digestible nutrient

Table 2. Feed consumption in primiparous and multiparous Saanen Etawah crossbred goats (mean ± SD).

	DM (kg)	Inorganic residue (kg)	CP (kg)	EE (kg)	CF (kg)	NFE (kg)	TDN (kg)
Primiparous	2.04 ± 0.23	0.27 ± 0.03	0.29 ± 0.01	0.10 ± 0.01	0.58 ± 0.13	1.09 ± 0.12	1.17 ± 0.05
Multiparous	1.87 ± 0.24	0.25 ± 0.04	0.33 ± 0.05	0.11 ± 0.01	0.56 ± 0.10	1.03 ± 0.11	1.23 ± 0.10

DM: dry matter; CP: crude protein; EE: ether extract; CF: crude fibre; NFE: nitrogen free extract; TDN: total digestible nutrient

Table 3. Creatinine, iron, and blood metabolite concentrations of primiparous and multiparous lactating Saanen Etawah crossbred goats (mean ± SD).

Indicator	Primiparous group (n = 45)	Multiparous group (n = 85)	F values	*P* values
Cre (mg/dl)	0.79 ± 0.15*	0.87 ± 0.21*	0.79	0.029*
Iron (mg/dl)	0.02 ± 0.07	0.02 ± 0.01	0.881	0.512
Glucose (mg/dl)	61.73 ± 5.83	60.06 ± 7.60	2.030	0.202
Total cholesterol (mg/dl)	122.28 ± 29.40*	107.37 ± 24.40*	3.377	0.154
BUN (mg/dl)	20.02 ± 6.11	18.52 ± 5.46	1.985	0.154
Albumin (g/dl)	4.00 ± 0.20*	3.82 ± 0.16*	3.632	0.001**
Total protein (g/dl)	7.05 ± 1.41	7.09 ± 1.88	0.250	0.746
BUN/Cre	27.07 ± 11.90*	22.37 ± 8.12*	9.564	0.009**

Cre: creatinine; BUN: blood urea nitrogen; TP: total protein; BUN/Cre: ratio of blood urea nitrogen concentration and blood creatinine concentration
*Significant difference between parity groups ($P < 0.05$) for each indicator
**Significant difference between parity groups ($P < 0.01$) for each indicator

Table 4. Correlation between Cre, iron, and blood metabolite concentrations in primiparous lactating Saanen Etawah crossbred goats.

		Cre	Iron	Glucose	BUN	TP	Albumin	Cholesterol	BUN/Cre
Cre	P. Corr	1	−0.024	−0.294**	0.067	−0.081	0.135	0.115	−0.503**
	Sig *P*V		0.827	0.006	0.543	0.463	0.217	0.297	0.001
Iron	P. Corr	−0.024	1	0.046	0.140	−0.007	−0.273*	0.110	0.192
	Sig *P*V	0.827		0.678	0.201	0.947	0.011	0.318	0.079
Glucose	P. Corr	−0.294**	0.046	1	0.268*	0.056	−0.153	0.316**	0.363**
	Sig *P*V	0.006	0.678		0.013	0.612	0.612	0.003	0.001
BUN	P. Corr	0.067	0.140	0.268*	1	−0.069	0.063	0.346**	0.791**
	Sig *P*V	0.543	0.201	0.013		0.528	0.565	0.001	0.001
TP	P. Corr	−0.081	−0.007	0.056	−0.069	1	0.152	−0.069	−0.052
	Sig *P*V	0.463	0.947	0.612	0.528		0.166	0.513	0.535
Albumin	P. Corr	0.135	−0.27*	−0.153	0.063	0.152	1	0.51	−0.091
	Sig *P*V	0.217	0.011	0.162	0.565	0.166		0.640	0.408
Cholesterol	P. Corr	0.115	0.110	0.316**	0.346**	−0.069	0.051	1	0.210
	Sig *P*V	0.297	0.318	0.003	0.001	0.531	0.640		0.054
BUN/Cre	P. Corr	−0.503**	0.192	0.363**	0.791**	−0.052	−0.091	0.210	1
	Sig *P*V	0.001	0.079	0.001	0.001	0.635	0.408	0.054	

Cre: creatinine; BUN: blood urea nitrogen; TP: total protein; BUN/Cre: ratio of blood urea nitrogen concentration and blood creatinine concentration; P. Corr: Pearson correlation; Sig *P*V: significant *P* values.
**Significant correlation at $P < 0.01$; *significant correlation at $P < 0.05$ for each indicator

Table 5. Correlation between creatinine, iron, and blood metabolites concentrations in multiparous lactating Saanen Etawah crossbred goats.

		Cre	Iron	Glucose	BUN	TP	Albumin	Cholesterol	BUN/Cre
Cre	P. Corr	1	0.004	−0.148	−0.373*	0.292	−0.054	0.195	−0.723*
	Sig *P*V		0.918	0.379	0.010	0.041	0.761	0.192	0.000
Iron	P. Corr	0.004	1	−0.204	0.096	−0.27	−0.101	−0.279	0.020
	Sig *P*V	0.918		0.194	0.541	0.082	0.523	0.063	0.931
Glucose	P. Corr	−0.148	−0.204	1	0.085	0.136	0.062	−0.055	0.097
	Sig *P*V	0.379	0.194		0.592	0.337	0.662	0.727	0.560
BUN	P. Corr	−0.373*	0.096	0.085	1	−0.08	0.081	−0.023	0.879**
	Sig *P*V	0.010	0.541	0.592		0.562	0.601	0.877	0.000
TP	P. Corr	0.292*	−0.272	0.136	−0.084	1	0.273	0.209	−0.233
	Sig *P*V	0.041	0.082	0.337	0.562		0.063	0.162	0.108
Albumin	P. Corr	−0.054	−0.101	0.602	0.081	0.273	1	-0.104	0.078
	Sig *P*V	0.761	0.523	0.662	0.601	0.063		0.497	0.628
Cholesterol	P. Corr	−0.195	−0.279	−0.055	−0.023	0.209	−0.104	1	−0.022
	Sig *P*V	0.192	0.063	0.727	0.877	0.162	0.497		0.878
BUN/Cre	P. Corr	−0.723**	0.020	0.097	0.87**	−0.23	0.078	−0.022	1
	Sig *P*V	0.000	0.931	0.560	0.000	0.108	0.628	0.878	

Cre: creatinine; BUN: blood urea nitrogen; TP: total protein; BUN/Cre: ratio of blood urea nitrogen concentration and blood creatinine concentration; P. Corr: Pearson correlation; Sig *P*V: Significant *P* values.
**Significant correlation at $P < 0.01$; *significant correlation at $P < 0.05$ for each indicator

in primiparous goats (3.99 ± 0.20 mg/dl) was significantly higher ($P < 0.05$) compared to multiparous goats (3.82 ± 0.16 mg/dl) and also the total cholesterol level of primiparous goats (122.28 ± 29.20 mg/dl) was significantly ($P < 0.05$) higher than in multiparous goats (107.37 ± 24.40 mg/dl). The ratio of BUN to Cre (BUN/Cre) was also higher ($P < 0.05$) in primiparous goats (27.07 ± 11.90) compared to multiparous goats (22.37 ± 8.12). Other indicators did not differ significantly between the groups ($P > 0.05$). This study also found a negative correlation between glucose-Cre ($P < 0.01$), BUN/Cre-Cre ($P < 0.01$), and iron-albumin ($P < 0.05$), and a positive correlation between BUN-glucose, glucose-cholesterol ($P < 0.01$), glucose-BUN/Cre ($P < 0.01$), BUN-cholesterol ($P < 0.01$), and BUN-BUN/Cre ($P < 0.01$) in primiparous goats (Table 4). A negative correlation was found between Cre-BUN ($P < 0.05$) and Cre-BUN/Cre ($P < 0.01$), and a positive correlation was found between BUN-BUN/Cre ($P < 0.01$) in multiparous goats (Table 5).

Discussion

In general, the feed was given equally to primiparous and multiparous groups. Therefore, the consumption rate was considered the same even though the nutritional needs between the two tend to be different because of the association with their physiological status. The association between blood metabolites and feed metabolism has been known since the function of blood is to transport metabolite products, nutrition, and hormones (Murray et al. 2017; Sitaresmi et al. 2023). Cre and some blood metabolite concentrations differed significantly between the groups of goats in spite of their relatively similar nutrient intakes. Available nutrients were used to maintain the immune system, to support growth, basal metabolism, and reproduction (Cônsolo et al. 2018). Energy for the body, which was reserved inside the body fat and muscle, was considered important in determining the efficiency of reproductive performance (Sitaresmi et al. 2020). However, parturition and lactation processes tend to impose metabolic stress on dairy ruminants, leading to malnutrition (Goff and Horst 1997). Previous findings showed changes in some blood metabolites and oestrus characteristics following with the augmentation of parity status (Kumala et al. 2022a; Kumala et al. 2022b), but the study needs to be expanded on.

Even so, the dairy goats used in this experiment were still in a healthy condition and did not develop malnutrition which was proven by their non-significant results in the iron, glucose, BUN, and total protein. All results, including those for Cre (Al-Suwaiegh 2016), iron and blood metabolite contents (Al-Suwaiegh 2016; El-Tarabany et al. 2018; Widiyono et al. 2020; Kumala et al. 2022a), were still within a favourable range. The differences in blood metabolite concentrations despite similar nutrition intake were due to the enhancement of nutrient mobilization inside the primiparous and multiparous goat's body.

The results showed lower blood metabolite indicators in multiparous dairy goats compared to primiparous ones, much the same as previous studies (Mohebbi 2019; Kumala et al. 2022a, b). According to Sammad et al. (2022), the blood metabolites were affected by the excessive accumulation of blood during pregnancy and the high milk-producing lactating period, primarily to support foetal growth and its development.

Multiparous goats may have enhanced mammary cell proliferation due to high growth hormone released from the mammary glands since they have already attained their maximal body development and conditions for high milk production compared to primiparous goats (Lang et al. 2012). This was proven by lower total cholesterol concentrations in multiparous dairy goats, indicating that high utilisation of nutrients stored inside the body might be due to its high performance, especially during gestation and lactation (Sitaresmi et al. 2020). Lower cholesterol in multiparous goats seems due to the utilisation of blood cholesterol to lactation energy (Ferreira et al. 2021). This finding was supported by the

correlation results (Table 4), which positively correlated ($P < 0.01$) between glucose and cholesterol in the primiparous group, indicating that some of the glucose was stored as cholesterol. In the multiparous group (Table 5) the correlation was negative even though no significant correlations were observed, and this strengthens the argument that cholesterol in the blood of the multiparous was used as an energy source (glucose) due to insufficient energy in the body to support a high enough production. A previous study from Pascottini et al. (2020) also reported that severe body condition loss was related to low cholesterol serum concentrations in dairy cows, which indicated a disruption in their energy balance. A study from Mohebbi (2019) found more than 60% fat loss inside the multiparous dairy cow's body to match the nutrient needs after the parity period and resulted in higher fat metabolism which was twice compared to primiparous dairy cows.

The present findings showed high utilisation of protein, which was proven by higher albumin and a higher BUN/Cre in primiparous goats. We suspected primiparous goats to be still in the process of growing, so their cellular mobilization tended to be higher which resulted in higher albumin and higher BUN/Cre. Despite non-significant BUN concentration, significantly higher BUN/Cre were found in the primiparous group due to significantly higher Cre in the multiparous groups. The obtained results were interpreted as lower body protein mobilization in multiparous goats.

It was concluded that distinctions in blood metabolites between primiparous and multiparous goats were due to different physiological needs which led to different mobilizations of nutrients stored inside the body. Even though blood indicators from both primiparous and multiparous goats in this study were still within favourable range, we suggested different feeding strategies for each parity group following their needs for better reproduction continuity, such as a high-protein diet to optimize primiparous body development, and a high-energy diet to enhance multiparous milk production especially before and after giving birth. The results of this study can be utilised to support the reproductive performance in primiparous and multiparous goats. However, further research is needed to understand the exact optimal ratio of feed energy and protein.

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