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Improvement of Adaptive Saanen Goat Milk Production and Reproduction Fed Diet Supplemented with *Indigofera zollingeriana* Leaf Meal

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Abstract. The objective of this research was to examine the effect of the addition of *Indigofera zollingeriana* leaf meal (IZLM) in diets on milk production and fermentation products of adaptive Saanen goat. The research was conducted at Balai Besar Pembibitan Ternak Unggul dan Hijauan Pakan Ternak (BBPTUHPT) or or the Center for Superior Animal Breeding and Forage Animal Feed, Baturraden, Central Java. A total of 18 first lactating adaptive Saanen goats with a body weight of 34.83±7.13 and aged 20-24 months were used in this study. The goats were kept in individual pens and grouped into 6 groups based on body weight and each group were randomized to receive three kinds of concentrate substitution with IZLM of 0%, 10% and 20 % of dry matter (DM) concentrates, for A, B and C treatments, respectively. Therefore, this research was designed according to randomized block design. Dry matter intake (DMI) of each goat was 4.5% of live weight with dry matter (DM) ratio of forage and concentrate were 60:40. The variables measured were DMI, partial volatile fatty acid (VFA), energy conversion efficiency of glucose into VFA (ECEVFA), methane gas, total protozoa, milk production and the first estrus after kidding. Analysis of variance showed that the treatment had a significant effect ($P < 0.05$) on propionate, milk production and fat, but no significant effect ($P > 0.05$) on consumption of DM, acetate, butyrate, EKVFA, methane gas, lactose and protein milk. Increasing the IZLM substitution level enhanced linearly ($P<0.05$) on production of milk and milk fat, while the propionate concentrate responded to quadratic ($P<0.05$). Milk production increased at IZLM level of 20%, while goat in this group resulted only 16.70% estrous after birth compared to goat group received 10% IZLM level resulting 50% of estrus goat. The results of this study concluded that the recommended level of using IZLM as a concentrate substitute was only 10%.

Keywords: protozoa, methane, lactose, milk, reproduction

Abstrak. Penelitian ini bertujuan untuk menguji pengaruh penambahan tepung daun *Indigofera zollingeriana* (TDIZ) dalam ransum kambing saanen adaptif terhadap produksi susu dan produk fermentasi rumen. Penelitian dilakukan di Balai Besar Pembibitan Ternak Unggul dan Hijauan Pakan Ternak (BBPTUHPT), Baturaden, Jawa Tengah. Sebanyak 18 ekor kambing Saanen adaptif laktasi pertama dengan bobot badan 34,83±7,13 dan umur 20-24 bulan digunakan dalam penelitian ini. Kambing tersebut ditempatkan dalam kandang individu dan dikelompokkan menjadi 6 kelompok berdasarkan bobot badan dan masing-masing kelompok diacak untuk menerima tiga macam taraf substitusi bahan kering (BK) konsentrat dengan TDIZ yaitu 0%, 10% dan 20%, berturut-turut untuk perlakuan A, B dan C. Penelitian ini dirancang sesuai dengan rancangan acak kelompok. Konsumsi BK masing-masing kambing adalah 4,5% dari bobot hidup dengan imbangan BK hijauan dan konsentrat adalah 60:40. Peubah yang diukur konsumsi BK, *volatile fatty acids* (VFA) parsial, *energy conversion efficiency of glucose into* VFA (EKEVFA), gas metana, total protozoa, produksi susu dan estrus pertama setelah beranak. Analisis variansi menunjukkan bahwa perlakuan berpengaruh nyata ($P<0,05$) terhadap propionat, produksi dan lemak susu, tetapi tidak berpengaruh nyata ($P>0,05$) terhadap konsumsi BK, asetat, butirat, EKEVFA, gas metan, laktosa dan protein susu. Peningkatan taraf substitusi TDIZ dapat meningkatkan secara linier ($P<0,05$) terhadap produksi susu dan lemak susu, sedangkan konsentrasi propionat menghasilkan respon kuadratik ($P<0,05$). Produksi susu meningkat pada sampai kadar TDIZ 20%, akan tetapi estrus setelah melahirkan kambing pada kelompok ini hanya 16,70% dibandingkan dengan kelompok kambing yang mendapat kadar TDIZ 10% yang menghasilkan kambing berahi 50%. Hasil penelitian ini disimpulkan bahwa tingkat penggunaan TDIZ sebagai pengganti konsentrat hanya direkomendasikan 10%.

Kata kunci: protozoa, metan, laktosa, susu, reproduksi

Introduction

Goat farming in Indonesia is a crucial component of livestock, especially for small farmers. Nearly 99% of small livestock such as goats and sheep are found on smallholder farms, indicating the importance of these livestock are important for small farmers (Sodiq et al., 2003). Small ruminant livestock production is a vital source of income for small breeders who play a role in the national program as a contributor to animal protein, both meat and milk (Soedjana, 1993). Dairy goat is one of the alternatives chosen by people in Indonesia. There are consumers who deliberately choose goat's milk despite the high availability of cow's milk because of goat's milk are perceived to have more benefits than cow's milk. In developed countries, dairy goats have received an increased interest economically because of the relatively low investment costs, its efficiency in terms of milk yield per unit of feed intake, and the growing appreciation of goats' cheese by consumers (Rapetti et al., 2020). This condition is an opportunity for goat breeders to further develop their business in Indonesia (Directorate General of Agro and Chemical Industry, 2009).

Saanen goats have been imported by the government several years ago and are kept by The Balai Besar Pembibitan Ternak Unggul dan Hijauan Pakan Ternak (BBPTUHPT) or the Center for Superior Animal Breeding and Forage Animal Feed Batturaden, Banyumas, Central Java as a center for providing superior seeds for breeders. As a tropical country, the temperature and humidity in Indonesia are relatively high compared to the goat's country of origin. This situation forces the goat to adapt, even though adaptation is always in conflict with production, if the livestock adapts, the production will decrease. One strategy to minimize the conflict is to reduce fibrous feed and replace it with concentrate because the fibrous feed produces a high heat increase compared to concentrate (Kleiber, 1975; Sudarman and Ito, 2000). Therefore, the BBPTUHPT still use goat concentrates extensively to feed their animals since the concentrates can be included in the diet at a range of 50%-60%. However, as the main ingredient of concentrates is expensive, the price of commodity fluctuates and increases every year. Under these circumstances, practical supplements may include fodder from trees such as *Indigofera zollingeriana*. The forages of these trees can be incorporated in the diet of milk goats as a protein and roughage supplement to reduce the cost of feeding (Leketa et al., 2019).

Indigofera zollingeriana plant which is one of the forages of leguminosae containing crude protein of 29.16%, crude fiber 14.02%, crude fat 3.62% and TDN 75-78% can be used as green concentrate feed (Abdullah et al., 2016). The use of *indigofera zollingeriana* in forms of pellets at level of 40% is very effective in stabilizing milk production, improving quality and reducing feed costs on Saanen goats and Ettawa cross-bred goats (Abdullah et al., 2012). This is because *Indigofera zollingeriana* can add the nutritional value of ration by increasing the content of crude protein and its digestibility (Suharlina et al., 2016). However, an increase in production was not matched by an improvement in reproductive performance due to the high kidding interval. This may be due to the high level of both protein degradation that results in high NH_3 (Tarigan, 2017) which exceeds the need for microbes, meaning lack of energy availability (Takma et al., 2009). Overfeeding of protein during the early gestation, particularly an inadequate supply of energy may be associated with decreased fertility (Cheng et al., 2015). This decrease in fertility may result from decreased uterine pH during the luteal phase of the oestrous cycle in goat fed high levels of degradable protein. Therefore, the utilization of *Indigofera zollingeriana* in diet of goat needs fermentable carbohydrates as energy source from concentrate (Oguejiofor et al., 2015). Another benefit that may be obtained from the use of this legume is its ability to reduce methane emissions due to its high saponin content as a defaunating agent. According to Herdiawan et al. (2014), *Indigofera zollingeriana* plants contain saponins around 3.13% - 3.68% and tannins only 0.12% - 0.15%. Based on this description, it is necessary to conduct a study on the use of Indigofera zollingeriana leaf meal in adaptive saanen goat feed as an effort to increase milk production, reproduction and metabolic efficiency in the rumen which can reduce methane gas emissions.

Materials and Methods

Experimentals Design and Animals Management

The research used 18 first-lactating Saanen goats aged of 20-24 months and weighed 34.83 ± 7.13, divided into 6 groups according to initial body weight and randomly allocated to individual cage. Each group consisted of 3 goats that randomly received three levels of IZLM substitution as much as 0%, 10% and 20% of total dry matter intake for treatment A, B and C, respectively. In accordance with the treatment, this study was designed according to a randomized block design with three treatments and six groups of live weight goats as blocks. The total dry matter intake for each goat was 4.5% of live weight. The basal diets used were *Mott elephant grass* and concentrate with dry matter ratio of 60:40. The nutrient content of basal diets was presented in Table 1. The concentrate and IZLM were mixed before feeding and offered separately from the grass two times a day at 08.00 and 17.00. The *Mott elephant grass* were fed five times a day at 07.00, 10.00, 13.00, 16.00 and 19.00.

The time required for the study was 72 days that include 4 days of feed adaptation, 14 days of preliminary study and 51 days of feeding trial (data collection). During the data collection, daily feed was offered, and we recorded the feed refusal and weighed daily milk production. Variables measured were dry matter consumption, daily milk production, milk quality of lactose, fat and protein and rumen parameter of individual VFA and methane production.

Samples Collection and Chemical Analyses

Diets and refusals were collected daily during the experimental period and were composited by period prior to chemical analyses. Feeds samples were collected during the last seven days of each period. Composited feed samples were oven-dried at 60°C and ground (1 mm screen using Cyclotech Mill, Tecator, Sweden) and then analyzed for DM, ash, crude protein, crude fiber and crude fat contents (AOAC, 2019). The milk was collected from each goat at milking times in the morning and afternoon, preserved with potassium dichromate ($K_2Cr_2O_7$), and stored at 4°C until further analysis of milk compositions (protein, fat, lactose) by an infrared method using Milko-Scan (Foss Electric, Hillerod, Denmark).

Table 1. Diet composition and nutrient content of treatment diets

Feed ingredients	Treatment Diets		
	A	B	C
Mott elephant grass (%)	60	60	60
Concentrate (%)	40	30	20
Indigofera zollingeriana leaf meal (%)	0	10	20
Total (%)	100	100	100
Nutrient content			
Dry matter (%)	48.68	48.59	48.49
Crude protein (%DM)	15.63	15.81	15.98
Crude fat (%DM)	4.78	4.24	3.69
Crude fiber (%DM)	22.05	23.23	24.42
Ash (%DM)	13.1	12.67	12.24
Calcium (%DM)	0.94	0.9	0.86
Phosphor (%DM)	0.45	0.39	0.33

On the last day of each experimental period, rumen fluid and blood samples were taken at 0 and 4 hours after the morning feeding.

Rumen fluid (200 mL) was collected from the rumen by a stomach tube connected with a vacuum pump. Samples of rumen fluid at a volume of 50 mL were collected and added with 5 mL of 1 M sulfuric acids (H_2SO_4) to stop the fermentation process of microbial activity and then centrifuged at 16,000 x g for 15 minutes. About 20 mL of supernatant were collected and frozen at –20°C until later analyzed in the laboratory for analysis of ammonia (NH_3-N) (Kjeltech Auto 1030 Analyzer, Tecator, Höganäs, Sweden) (AOAC, 2019).

The samples of rumen fluid were used for volatile fatty acids (VFAs) analysis using High-Performance Liquid Chromatography (HPLC; Model Water 600; ultra violet (UV) detector, Millipore Crop; column novapak C18; column size 3.9 × 300 mm; mobile phase10 mM sulfuric acids (H_2PO_4) [pH 2.5]) according to the method of Guan et al. (2008). Estimation of ruminal methane production was conducted based on VFA proportions (CH_4 production= 0.45 (acetate) – 0.275 (propionate) + 0.4 (butyrate) (Moss et al., 2000)).

Estimation of energy conversion efficiency of glucose into VFA (ECEVFA) used formula according to (Ryle and Orskov, 1990). The density of rumen protozoa per mL was obtained using a Sedgewick-Rafter counting chamber (Dehority, 1984) with the modifications proposed by D'Agosto and Carneiro (1999), and the protozoa present in each sample were identified based on the criteria described by Ogimoto and Imai (1981).

Statical Analysis

Data were subjected to analysis of variance according to the general linear model procedure (BLMP) using a model suitable for Randomized Block Design (RBD) and continued with polynomial orthogonal test (Steel and Torrie, 1995).

Results and Discussion

VFA Parsial Production, Methana, Energy Effiiency and Rumen Protozoa

The proportions of partial VFA (acetate, propionate and butyrate), methane gas, and energy efficiency as well as the number of protozoa are presented in Table 3. Analysis of variance showed that the treatment had a significant effect ($P<0.05$) on the propionate concentration but no significant effect ($P>0.05$) on the concentration of propionate, acetate, butyrate, methane gas, energy efficiency and total protozoa. The increase in the substitution level of IZLM caused a quadratic increase in the proportion of propionate ($P<0.05$) with the highest proportion of propionate being achieved at 7.86% of IZLM (Figure 1) and after that the propionate decreased to the level of 20% IZLM. On the other hand, total protozoa and methane production were likely to be lower at 10% *Indigofera zollingeriana* dose compared to control (0%) and 20% IZLM level. It is because IZLM contains saponins as defaunation agents which cause a decrease in protozoa and this will also cause a decrease in methane. This result is also supported by Arving et al. (2018) that lucerne saponin significantly reduced the methane production and total protozoal count in rumen liquor.

Suharlina et al. (2016) reported that inclusion levels of *I. zollingeriana* in rations improved their nutrition value while decreasing methane production in-vitro. Hu et al. (2005) reported that the low number of protozoa caused the production of propionate to increase. This is because the methanogenic bacteria that are in symbiosis with the protozoa are also reduced. Therefore, the formation of methane is disrupted, and hydrogen will be diverted for the formation of propionate. Propionate is formed through two pathways, the acrylate pathway and the succinate pathway which altogether require hydrogen in the process (Syamsi et al., 2018; Ungerfeld, 2020).

Table 2. Average of partial VFA (acetate, propionate, and butyrate), ratio of acetate propionate (C2/C3), methane, efficiency of Energy Conversion (EEC) (%) and protozoa of Saanen adaptive goat fed diet supplemented with *Indigofera zollingeriana*

Level of IZLM in Diets	Variables						
	Acetate (mM)	Propionate (mM)	Butyrate (mM)	C2/C3	CH4 (mM)	EEC (%)	Protozoa (Cell/ml)
0% (A)	66.39±2.5	26.99±2.3	6.63±1	2.46	17.48±1	76.06±1	2735±1826.5
10% (B)	64.65±6.2	28.67±4.9	6.68±1.7	2.25	16.75±2.4	76.79±2.4	2502±2040.9
20% (C)	67.72±2.8	23.83±2.1	8.45±2.1	2.84	18.55±0.9	74.99±0.9	3685.5±980.7

Note: A = *Mott elephant grass* 60% + concentrate 40%; B = *Mott elephant grasst* 60% + concentrate 30% + meal of *Indigofera zollingeriana* leaf 10%; C = *Mott elephant grass* 60% + concentrate 20% + meal of *Indigofera zollingeriana* leaf 20%

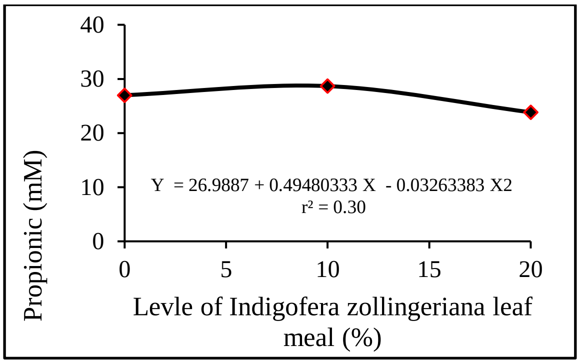

Figure 1. The relationship the level of Indigofera zollingeriana as the concentrate substitution with propionic concentration

The data in Table 2 showed that the increase in IZLM up to 20% in this study actually tends to increase the number of methane and protozoa. The mechanism that causes this phenomenon is not clear, but researchers suspect that in addition to containing saponins, IZLM contains flavonoids. Alagbe and Oluwafemi (2019) reported that the flavonoid content of *Indigofera zollingeriana* extract was 30.11%, which enabled it to act as a buffer by stimulating the growth of *M. elsdenii*, a lactate user bacteri. Accordingly, the rumen pH is relatively more neutral so protozoa can develop properly. Higher rumen pH values were observed in high grain heifer diets supplemented with flavonoids compared to the control group, which is likely due to the beneficial effect of flavonoids in enhancing lactate-consuming microorganisms i.e. M. elsdenii (Seradj et al., 2014; Seradj et al., 2016). This increase in pH causes cellulosic bacteria to develop, thus enabling cellulose degradation to produce acetate with methane gas that tends to be higher than the control and the addition of 10% IZLM in treatment B. Fermentation gas is produced mainly when feedstuffs are fermented to acetate and butyrate, with propionate yielding gas only due to buffering of acid (Getachew et al. 2004).

Milk Production and Reproduction

Milk production and fat, lactose and protein content of milk as well as signs of the first estrus after kidding in adaptive Saanen goats at various levels of IZLM were shown in Table 3. Analysis of variance showed that the treatment had a significant effect ($P<0.05$) on milk production and fat but had no effect ($P>0.05$) on lactose, protein and DMI.

Increasing the substitution of IZLM with concentrate can increase milk production and milk fat content linearly ($P<0.05$). These results are in agreement with Abdullah et al. (2012) on that IZLM pellets to replace commercial concentrates could improve milk production of Ettawah crossbreeds (PE) and Saanen goats. The relationship between milk production and milk fat content with the substitution level of IZLM with concentrate is shown in Figures 2 and 3. Increasing the substitution level of IZLM can improve the quality of feed, especially protein to improve milk and milk fat production. The high degradation rate of IZLM (Tscherning et al., 2005) and the high digestibility (Norton, 1994) could maintain normal ruminal pH, thus increasing the ruminal microbe activity, such as cellulolytic bacteria (Wang et al., 2017).

Table 3. Dry matter intake (DMI), milk production and nutrient content of fat, lactose, protein and estrus percentage (%) of Saanen adaptive goat fed diet supplemented with *Indigofera zollingeriana*

Level of IZLM in the Diets (% DM)	Variable					
	DMI (kg)	Production (ml)	Fat (%)	Lactose (%)	Protein (%)	Estrus (%)
0% (A)	1.56±0.05	836.2± 37.2	4.42±0.6	3.52±0.2	3.39±0.2	16.7
10% (B)	1.51±0,07	905.8 ± 61.2	5.24±0.4	3.72±0.3	3.64±0.3	50
20% (C)	1.54±0,03	948.6± 93.1	5.61±0.9	3.73±0.2	3.61±0.3	16.7

This condition has increased the acetic concentration due to the high digestibility of structural carbohydrates such as cellulose and hemicellulose. Cellulolytic bacteria R. albus produced a large amount of acetate, while *R.flavefaciens* and *F.succinogenes* produced a large amount of succinate, which was eventually converted to propionate (Wang et al., 2016) and propionate was converted to glucose by the process of gluconeogenesis. Acetate and glucose are the precursors of milk fat and milk lactose, respectively; therefore, milk production increases with the level of their precursors.

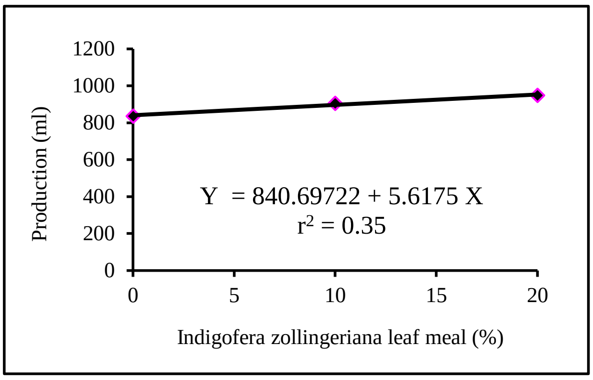

Figure 2. The relationship of the level of Indigofera zollingeriana as the concentrate substitution with milk production

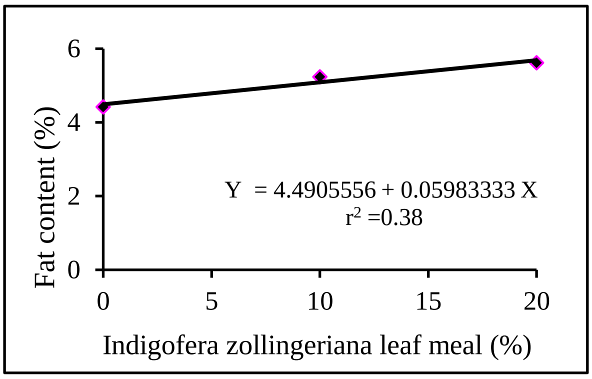

Figure 3. The relationship of the level of *Indigofera zollingeriana* as the concentrate substitution with milk fat content

The increase in milk production (8.26%) was caused by the increase of feed protein content as well as propionate production at the 10% IZLM level (Table 2) and after that level the propionate production decreased in line with the increase in IZLM levels by up to 20%. The increase in propionate at the 10% IZLM level and its decrease in the 20% IZLM level have been discussed previously. Propionate is the basic ingredient for the formation of glucose through the process of gluconeogenesis and glucose is the basic ingredient for the formation of lactose in milk (Dong et al., 2017) and as an energy source for the synthesis of milk fat. Lactose is synthesized in the udder from blood glucose absorbed by the basal membrane of mammary epithelial cells (Osorio et al., 2016). Thus, one of the main factors that determine the amount of milk production is lactose and the lactose concentration in milk is relatively constant at 4.5%. Fox et al. (2015) said that lactose determines the amount of absorbed water in the alveoli, and thus, the volume of produced when lactose production is high.

The decrease in propionate production had a slight impact on production, so the increase in milk production only reached 4.75% at the 20% of IZLM level. This showed that the increase in milk production is not only determined by the amount of lactose but there are other nutrients that play a role. Osorio et al. (2016) mentioned that beside glucose, lactose synthesis is also affected by de novo synthesized glucose and galactose via glycerol and AA precursors in mammary gland. Propionic concentration and lactose level showed different patterns, indicating that propionic concentration is only a basic substance in lactose formation. Besides propionic, the other contributing factor to lactose level is feed protein and fat that plays an important role in forming lactose. Lactate, gluconeogenic amino acids and glycerol are also used as gluconeogenic precursors and the relative contributions are 16 to 26%, 11 to 16% and 0.5 to 3 %, respectively (Aschenbach, 2010). The gluconeogenesis process is crucial in converting amino acid into milk lactose. As a result, it provides an abundant supply of glucose to form lactose so that the level of lactose will keep increasing despite the decrease of propionic concentration. Increased lactose would directly affect milk production. Milk yield greatly depends on mammary lactose synthesis due to its osmoregulation of milk, one that induces mammary uptake of water and concentration of lactose in milk is constant of 4.5%. Therefore, the rate of lactose synthesis in the epithelial cells of the mammary gland serves as a main factor influencing milk volume production (Osorio et al., 2016).

Contributing factors to the increasing lactose are high-quality feed and high degradation of *Indigofera zollingeriana*, which produces neutral ruminal pH (6.8-7) and increase the activity of cellulolytic bacteria in the fermentation (Ginting, 2012), such as forming glucose as the building blocks of milk lactose. Wattiaux and Armentano (2014) reported that milk quality is significantly affected by total glucose that will form lactose as a component of milk and glucose were also used as energy source for fat synthetizes with acetic acid as the precursor. A total of 30% of the carbon atom in milk fat is estimated to have derived from acetic acid, and the rest is from fatty acid (Rounds and Herd, 2012).

The polynomial orthogonal test on milk fat level demonstrated that supplementation of IZLM to substitute concentrate had a linear effect on milk fat level, with an equation of $Y = 4.49 + 0.059\ X$ and coefficient determination (r^2)= 0.38 (Figure 3). The increase in milk fat may be due to IZLM containing flavonoids that can increase lactate user bacteria, not causing a decrease in rumen pH, thus activating fiber-digesting bacteria. High fiber digestion activity will produce acetate, which is the basic ingredient for forming milk fat. Urrutia and HarvatineIn (2017) mentioned that increasing acetate supply improves milk and milk fat synthesis in dairy cows. The improved milk fat due to an increased substitution of IZLM only contributed 38% and the rest was influenced by other factors, such as the availability of energy sources from glucose and glycerol.

Milk fat across treatments in this study have conformed to the standard. According to TAS No. 6006 (2008), premium goat milk contains >4% fat. The contributing factors to fat content are forage feed and concentrate. The increasing portion of forage as the source of fiber would affect fat formation. Besides, the abundant production of acetic acids would affect the amount of fatty acid synthesis and increased milk fat (Zain, 2013; Laryska and Nurhajati, 2013).

This study demonstrated that the treatments did not affect ($P>0.05$) on milk protein. This was because the protein content of the control feed (A) was relatively the same as B and C. There was a tendency to increase milk protein in line with the increase in the level of Indigofera zollingeriana leaf meal. The highest protein content of milk was in treatment B, followed by C and A. This was due to the protein content of feed A being lower than B and C. However, treatment B produced the highest protein milk (3.62±0.6%), followed by treatment C (3.59± 0.6%) and A (3.38±0.6%). Based on the TAS standard No. 6006 (2008), protein content in treatment B and C were classified as optimum, while that in treatment A is standard. Protein milk content is affected by the type of feed offered.

Milk production increased linearly ($P<0.05$) due to an increase in the level of substitution of IZLM, not followed by an increase in reproductive performance which was indicated by the first estrus after kidding. At the level of IzmI of 0% (A), estrus goats were only 1 of 6 heads (16.70%) and increased to 3 heads (50%) at the level of 10% IZLM substitution (B) and after that it decreased to 1 head (16, 70%) at the level of IZLM of 20% (C) (Table 3). In high lactation cattle, an increase in milk production is not always followed by an increase in production performance; on the contrary, it reduces reproductive performance. Low fertility is a major constraint to overall efficiency in high-producing dairy herds (Elrod and Butler, 1993).

The low estrus in goats that received IZLM of 20% in treatment C was due to the high amount and level of degradation of Izlm but not matched by the available energy compared to B. This caused the ammonia from protein degradation not to be utilized by rumen microbes and absorbed to be detoxified into urea in the liver. The process of forming urea from ammonia requires energy, so that the need for goats receiving IZLM of 20% (C) is higher, while the availability of energy through concentrate is lower than treatment B. Thus, the chance of a negative energy balance (NEB) in the long term quite high compared to goats receiving IZLM of 10% (B). Urea is one metabolite of dietary protein that is formed from detoxification of NH_4 by the liver. The level of urea in the plasma or serum (PUN or SUN) is reflective of the quantity and degradability of the protein consumed, of the severity of negative energy balance in fasted animals, or of the combination of protein feeding and negative energy balance. High urea and ammonia in the blood will have a negative impact on the reproduction of lactating goats. Roy et al. (2011) explained the mechanism of excess protein and lack of energy that negatively affects fertility as follows. Urea and ammonia, which are by-products of protein metabolism causing lower uterine pH and mineral balance, decrease secretion of gonadotropin and progesterone hormones and damage the ovum and cause embryonic death. Feeding excess degradable protein to dairy goat can be deleterious to fertility, particularly if there is inadequate energy supplied to the rumen. Elrod and Buler (1993) reported that the negative effect on fertility may be mediated through an alteration in the uterine environment in which the embryo must grow.

The Saanen goat used in this study is the first lactating goat and the lactation period is 2 to 12 weeks. During this period, lactating goats, as is the case with lactating cows, will experience a negative energy balance (NEB) because peak production is reached before the peak consumption of dry matter and energy requirements for maintenance and milk production are very high compared to energy supply from feed (Macrae et al., 2019). The physiology of goat lactation is the same as that of lactating cows. When cows are in NEB, blood concentrations of IGF-1, glucose and insulin are low, while non-esterified fatty acids (NFAE), β-hydroxybutyrate (BHBA), and ammonia in plasma increase (More et al., 2020; Izquierdo, et al., 2021). Ammonia is believed to play a role in starting before ovulation, whereas urea mainly interferes negatively after fertilization (Albaaj et al., 2017). The first ovulation of a dairy cow is retarded by decreasing the luteinizing hormone (LH) pulsatility because of the low blood glucose (Jorritsma et al., 2003).

Conclusions

Incorporating of 20% *Indigofera zollingeriana* leaf meal (Izml) to substitute concentrate in feed could increase the production and fat content of Saanen goat milk, but the optimum reproduction achieved at level of *Indigofera zollingeriana* level was 10%.

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