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The Effects of Protected Feed Supplements on Estrus Response, Milk Production and Composition of Sapera Dairy Goats

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ABSTRACT

Soybean groats and earthworms (*Lumbricus rubellus*) are natural sources of protein, which are easy to obtain. Therefore, this research aims to evaluate the effects of protected soybean groats (PSG) and earthworm meal (PEM) in the diet on estrus response, milk production and composition of Saanen Peranakan Etawa (Sapera) dairy goats. These feed supplements were further protected by 1% formaldehyde. A completely randomized design with three treatments consisting of basal diet (P0), 75% basal diet + 25% PSG (P1), and 74.96% basal diet +24.99% PSG and 0.05% PEM (P2) was used in this research. Also, a total of 21 lactating Sapera goats were utilized and equally grouped into these treatments. Milk productions and composition were recorded and checked using a Lactoscan milk analyzer. Moreover, estrus synchronization was applied using the PGF2α and GnRH hormone using the intramuscular (IM) injection method and observing estrus. The data were analyzed using one-way analysis of variance while the differentiation of treatments was conducted with Duncan's multiple range test (DMRT). Electrical current resistance, vaginal temperature, vulva color, milk production, fat, total solids, and protein contents were observed to be significantly affected by the supplementation of PSG and PEM ($P<0.05$). In conclusion, supplementation of PSG and PEM in the diet could improve estrus response, milk production and composition of Sapera goats.

Keywords**:** Earthworm meal, Estrus response, Milk production, Sapera goat, Soybean groats

Introduction

In Indonesia, the production of milk is virtually derived from dairy cattle, which meets only 22% of the nation's demand. The bulk of this demand is provided by imports (BPS, 2021), hence, an increase in the population and productivity of dairy cattle is essential. Sapera, which is a Saneen and Etawa crossbreed dairy goat (Widiyono and Anggraeni, 2021), produces the highest milk average compared to other breeds and also generates a higher mineral, amino acid, and vitamin milk content in comparison to cattle (Hafidz *et al*., 2021). Nutrients play an important role in the maintenance and increase of milk production, specifically in lactating goats. Therefore, dairy goat farmers generally administer feed concentrate to increase the number of nutrients available in their diet. This method is considered less effective and economically less profitable due to its high production costs. Also, administering goats with excess feed concentrate may trigger a risk of metabolic diseases, such as acidosis, which increases the rate of fermentation in the rumen (Pramono *et al*., 2021).

Feed ingredients containing high protein, such as soybean groats and earthworm meal (L. rubellus) can be added to the diet to improve the milk quality. These ingredients should contain eco-friendly feedstuff resources to minimize agricultural waste. Due to their high amino acid contents, soybean groats and earthworm can also be used as an alternative feed additive for animal protein sources. This earthworm meal is produced from earthworms with a high protein content of 76%, 17% carbohydrates, 4.5% fat, and 1.5% ash. According to previous research, this food source also contains an essential amino acid index (EAAI) of 58.67%, which is higher than the value for fresh worms (Istiqomah *et al*., 2009). Protection of this soybean groats and earthworm meal is also necessary to optimize absorption and prevent protein degradation by rumen microbes, due to its high protein content. These protected protein sources further improve the peptides in the small intestine, which is required for milk synthesis, specifically to increase its fat and protein contents (Pramono *et al*., 2019).

Dairy goat reproductive management can be improved by applying estrus synchronization technology, so that difficulties in estrus detection and accuracy in breeding livestock can be solved.

Estrous synchronization is one method to regulate the reproductive cycle of livestock in order to achieve a high conception rate (Hafidz *et al*., 2021). Synchronization of estrus can be done by injection of the hormone PGF2α. The mechanism of action of the hormone PGF2α is to drastically inhibit blood flow in the corpus luteum, causing regression of the corpus luteum (Pramono, 2014). For optimum estrus synchronization, it is generally combined with injection of Gonadotrophin Releasing Hormone (GnRH). The GnRH hormone will stimulate the gonadotroph cells of the pituitary gland to secrete FSH and LH (Hafidz *et al*., 2021). Therefore, this research was aimed to evaluate the effects of diets containing feed supplements PSG and PEM on response estrus, milk production and chemical composition of Sapera goats.

Materials and Methods

Experimental design

A total of 21 lactating Sapera goats with an average weight of 32.96 + 3.14 kg were used. The soybean groats and earthworm meal were protected with 1% formaldehyde calculated from dry matter (DM). A completely randomized experimental design (CRD) was also employed with three treatment groups of 100% basal diet (P0), 75% basal diet + 25% PSG (P1), and 74.96% basal diet + 24.99% PSG and 0.05% PEM (P2). Subsequently, each treatment was repeated seven times. The dietary treatment given to Sapera goat was divided into concentrate and forage. In the daily basis, formulated concentrate was given in the morning at 6 a.m. (50%), while remaining 50% concentrate was given at 9.30 a.m. Similarly, the forage was given twice a day at 3 and 7 p.m. In addition, the amount of given feed is determined according to its average weight (4%). The access for water was given as *ad libitum*. The feed formulation is presented in Table 1.

Table 1. Feedstuff composition and nutrient content of feed supplement

Item	P0	P1	P2
Feedstuff composition, %			
Basal concentrate	100	75	74.96
Protected soybean groats	0	25	24.99
Protected earthworm Meal	0	0	0.05
Total amount	100	100	100
Nutrient content, %			
Dry matter (%)	66.30	72.41	72.41
Crude protein (%)	11.44	16.55	16.57
Crude fat (%)	5.18	7.33	7.33
Crude fibre (%)	4.81	4.58	4.58
Ash (%)	4.70	5.03	5.03
NFE (%)	73.80	64.26	64.26
Organic matter (%)	88.77	94.97	94.97
Total digestible nutrient	80.51	81.07	81.07

NFE: nitrogen free extract.

Synchronization treatment and estrus observation

After two weeks of feeding treatment, goats were received estrus synchronization using the PGF2α hormone through intramuscular (IM) injection and estrus was observed. PGF2α hormone injection was carried out using a double dosage method, PGF2α hormone was injected twice with a dose of 0.3 mL for each injection (according to the dose, containing dinoprost tromethamine 0.0789 mg/mL), and given an interval of 11 days, with the aim of knowing whether there is corpus luteum (Metodiev, 2015). After an interval of two days, GnRH hormone was also injected. Specifically, each goat was received 0.3 ml PGF2α at first day, 0.3 mL PGF2α at the 11th day, and 0.004 mL GnRH at the 13^{th} d (Figure 1). Detection of estrus after the first injection of PGF2α hormone until day 11 was observed every 24 h. The goat showing signs of estrus after the first injection of the PGF2α hormone will not be bred. Observations of estrus after the second injection on the first to third day were carried out every 8 h and on the fourth to fifth day every 3 h after the injection of GnRH hormone.

Milk production and measurement of milk chemical composition

A total of 21 Sapera dairy goats at the second lactation period were utilized in this study and their milk productions were recorded per day. The milking activities were conducted twice a day with the routinely recorded data at 6 a.m. and 3 p.m.

In addition, 30 mL of milk of Sapera dairy goats were individually collected. Milk samples were analyzed to evaluate milk composition of each goat using Lactoscan (SAP, Nova Zagora, Bulgaria).

Data analysis

The data collected was analyzed using the analysis of variance (ANOVA), through a custom script written in R programing. Duncan’s Multiple Range Test (DMRT) was also conducted to differentiate among treatments (Pramono, 2014).

Results and Discussion

The effects of PSG and PEM supplementations in the diet on estrus response

The PSG and PEM supplementation in the Sapera goat diet had a significant effect ($P<0.05$) on the value of the electric current resistance during estrus (Table 2). There was a difference in the time of occurrence and the value of the electric current resistance among the goat groups treatments, presumably due to differences in crude protein (CP) consumption in the Sapera goat group. Pramono (2014), stated that the high consumption of CP causes a series of interrelated processes that cause an increase in hormone precursors in the anterior pituitary. Therefore, FSH secretion increases that have functions to stimulate the growth of follicles in the ovaries, from primary follicles to De Graff follicles. The more mature follicles, the more estrogen secretion increases, resulting in an increase in estrogen concentration. High levels of estrogen cause vaginal mucosal secretion, causing a low value of electrical resistance in the heat estrus detector.

Statistical analysis also showed The PSG and PEM supplementation in the Sapera goat diet had a significant effect (P<0.05) on the value of vaginal temperature during estrus (Table 2). The occurrence of estrus in all treatments was affected by the injection of the hormone PGF2α which caused the lysis of the corpus luteum as a site for the production of the hormone progesterone and GnRH hormone after injection of PGF2α hormone causes better follicular development (Hafidz *et al*., 2021). The difference in the value of vaginal temperature in the goat group at the time of estrus was due to differences in the value of estrogen hormone levels which were influenced by the consumption of CP. The PSG and PEM supplementation in the Sapera goat diet can increase CP consumption (Arif *et al*., 2021), causing an increase in estrogen levels during estrus and can improve animal reproductive performance (Yanuartono *et al*., 2016). Sufficient nutrient needs and synchronization of estrus can result in an increase in FSH and LH hormones related to reproductive function. FSH and LH hormones are gonadotropin hormones produced by the anterior pituitary, optimally functioning these glands increase the secretion of FSH and LH hormones. The availability of arachidonic acid which is a precursor of the hormone PGF2α and protein as a biosynthetic precursor of the FSH and LH hormones contained in soybean groats and protected worm flour accelerates the process of follicle development. The developing follicle secrete the estrogen hormone, so that the level of the estrogen hormone in the blood increases. Pramono (2014), states that the protein content consumed causes a series of interrelated processes, namely an increase in the precursor of the hormone FSH-LH in the anterior pituitary and ovaries. This will affect the speed and size (size) of follicle development so that there is an increase in the concentration of the hormone estrogen.

The P2 and P1 groups of goats compared to P0 during estrus showed a higher vaginal temperature caused by an increase in the hormone estrogen which affects cell activity in the goat's vagina. The results of the study by Saputra (2018) showed that during estrus, Etawa Peranakan (PE) goats had a vulvar temperature between 38.08 – 39.44ºC. The increase in vulva temperature (warm) was caused by an increase in blood supply to the external organs of the vulva due to increased levels of estrogen during estrus. Increased vaginal blood flow results in higher heat production, resulting in an increase in vaginal temperature. This is in accordance with the opinion of Pramono (2014) which states that the high hormone estrogen during estrus causes blood circulation in the vaginal area to increase.

Based on statistical analysis showed that supplementary feeding in the form of soybean groats and protected earthworm meal had an effect (P<0.05) on the color score of the vulva during estrus (Table 2). A lower vulvar color score indicates a dark red color. This was due to the increased rate and concentration of blood around the vulva due to increased levels of the hormone estrogen in the P1 and P2 groups, which was affected by the increased consumption of CP in that group. The high consumption of CP increases the secretion of FSH hormone, so that follicle development also increases. Increased follicle development results in increased secretion of the hormone estrogen. This is in accordance with the statement that goats during estrus have a dark red color, which is affected by increasing levels of estrogen in their blood (Ridlo *et al*., 2018) and increasing levels of estrogen are affected by higher consumption of CP (Pramono, 2014). Besides, in order to supply energy and protein that is easily absorbed without experiencing a large drop once it enters the digestive tract, feed protections are made. It is a modest technical strategy to enhance animal performance, increase the amount of nutrients accessible in the rumen, and produce byproducts that are friendlier to the environment (Barido *et al*., 2021). Numerous investigations (Barido *et al.,* 2021; Lee *et al.,* 2023) have described pre-treatment employing coating materials to slow the pace at which vital nutrition degrades. The effects of PSG and PEM supplementations in the diet on milk production and composition.

Figure 2 presents the effect of feed supplements in milk production, while Table 3 displays the milk production and chemical composition. This result shows that supplementing

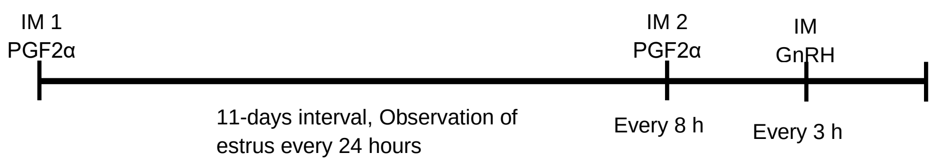

Figure 1. Estrus synchronization treatment.

Table 2. Estrus response of Sapera goat

Parameter	P0	P1	P2	*P*-value
Electrical current resistance	511,46^{a}	487,08^{b}	484,58^{b}	0,0281
Vaginal temperature	38,98^{b}	39,32^{b}	39,37^{a}	0,0147
Vulva color	19^{a}	18,33^{b}	18,35^{b}	0,0277

P0: basal diet, P1: 75% basal diet + 25% PSG, P2: 74.96% basal diet +24.99% PSG with 0.05% PEM, ^{a,b} Different superscript in the same row indicates significant difference (*P*<0.05).

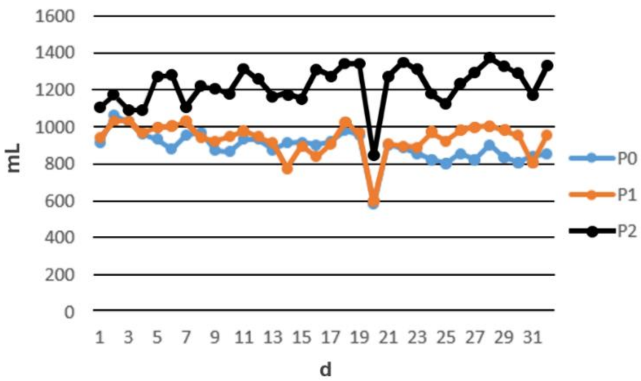

Figure 2. Sapera goat milk production for 32 d. (P0) is basal diet, (P1) is 75% basal diet + 25% PSG, (P2) is 74.96% basal diet +24.99% PSG with 0.05% PEM.

the diet with PSG and worm meal statistically improved milk production (P<0.05). Also, this increase in production and quality might be related to the improvement of protein density and energy through diet supplementation. These findings are in agreement with the previous research by (Pramono *et al*., 2017) which stated that the adequacy of energy and protein in animal feed are important factors in increasing milk production and quality. Previous reports also stated the need for protected soybean meal optimization, with protein that passes through the small intestine and is used by dairy cattle for milk synthesis (Pramono *et al*., 2019a).

The results showed that the fat content of milk differed significantly between treatments (P<0.01), where its composition increased with the addition of protected soybean groats and worm meal (Figure 2). This finding might be due to an improvement in feed protein consumption. Recorded feed intake data revealed that the average consumption of concentrate was at 1 kg/day, while forage was consumed at 3 kg/d by utilized Sapera goat. Furthermore, the number of enzymes in the digestive tract, such as the xanthine oxidase enzyme, improved with an increase in the provision of protected protein supplementation. The concentration of this enzyme, which assists in the synthesis of milk fat and is produced by the endoplasmic reticulum cell organelles, also determines the fat content (Utari *et al*., 2012). According to previous research, protein supplementation with protected fatty acids can be directly transferred to the milk fat synthesis system. This balance transfer of the long-chain fatty acid in the diet further affects milk fat through its synthesis in the mammary glands (Pramono *et al*., 2017).

Statistical analysis also showed that milk protein content differed significantly between treatments (Table 3). The results of this research indicated a decrease in milk protein content in P1 and P2 treatments compared to P0 due to its inverse relationship with fat composition. This phenomenon is in accordance with a previous report, which stated that the high-fat content of milk is a result of the inverse association between the protein and fat content value (Utari *et al*., 2012). Based on this research, an opportunity was created to evaluate the protein deposition of PSG and PEM on the amino acid profile in the milk protein synthesis of dairy goats. This incidence might be due to the rumen's incomplete digestion of the rationed protected protein, allowing the passage of certain molecules through the intestine and further conversion into amino acids. Consequently, the availability of amino acids in the intestines was increased, resulting in optimal absorption and transportation through the blood to the secretory cells of the udder, where these molecules are synthesized into milk protein (Pramono *et al*, 2019b).

The levels of milk total solid in this research also differed significantly between treatments (Table 3). This result might be due to high milk fat produced by goats receiving PSG and PEM treatments in their diet, implying a high total solid content. The total solid component of milk consists of solid non-fat and fat content, with the fat content having a direct effect on this factor. This result was in line with previous research, which stated that total solid components were obtained mostly from the contribution of high milk fat content (Saputra, 2018).

Table 3. Milk production and composition of Sapera goat

Parameter	P0	P1	P2	*P*-value
Milk production (mL)	864.65 [a]	1115.21[b]	1167.54[b]	0.001
Fat (%)	3.57[b]	3.73[b]	5.56[a]	0.001
SNF (%)	6.67	6.85	6.73	0.529
Protein (%)	3.47[a]	3.29[b]	3.23[b]	0.001
Lactose	3.21	3.12	3.26	0.544
Total solid (%)	10.23[b]	10.13[b]	12.29[a]	0.001

P1: basal diet, P2: 75% basal diet + 25% PSG, P3: 74.96% basal diet +24.99% PSG with 0.05% PEM, [a,b] Different superscript in the same row indicates significant difference (*P*<0.05).

In terms of milk lactose and SNF, the treatments did not differ significantly (P>0.05), due to the general relationship between these two factors, in which the effect of lactose on other components, such as SNF, increases with its amount in the milk. Furthermore, lactose, which is a glucose-forming milk derived from protein degradation during the gluconeogenesis process, may be used as the main energy source for fat and protein synthesis, as well as milk production. This compound is generally used as an energy source rather than the precursor for the synthesis of lactose in the mammary gland (Hames and Hooper, 2005).

Conclusion

Estrus response, milk production, fat and protein contents of milk of Sapera dairy goat improved with the supplementation with PSG and PEM as eco-friendly feedstuffs in diet.

Conflict of interest

There is no potential conflict of interest declared by the authors.

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Author's contribution

Conceptualization: Ahmad Pramono, Muhammad Cahyadi; Data curation: Muhammad, Fairuz Izzudin Riyadi; Formal analysis: Muhammad, Fairuz Izzudin Riyadi; Methodology: Ahmad Pramono, Muhammad, Fairuz Izzudin Riyadi; Software: Muhammad, Fairuz Izzudin Riyadi; Validation: Ahmad Pramono, Muhammad, Fairuz Izzudin Riyadi, Muhammad Cahyadi; Investigation: Muhammad, Fairuz Izzudin Riyadi; Writing - original draft: Ahmad Pramono; Muhammad, Fairuz Izzudin Riyadi; Writing - review and editing: Ahmad Pramono, Muhammad, Fairuz Izzudin Riyadi, Muhammad Cahyadi.

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