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Effect of methionine supplementation on the production of Friesian Holstein dairy cows

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ABSTRACT

A lack of certain amino acids, particularly methionine can lead to a number of issues in dairy cow output. This research aimed to evaluate the impact of methionine supplementation on the productivity of Friesian Holstein dairy cows during their lactation phase. The observational study was carried from January 2022 to December 2023 at a dairy farm located in West Java. A total of 82 Friesian Holstein dairy cows that had recently calved or were in the early stages of lactation, with a BCS from 3.25 to 3.75. The cows were housed in one barn. They were fed a total mixed ration (TMR) consisting of corn silage, alfalfa hay, and concentrate, which had a crude protein content of 16% and a total digestible nutrient (TDN) level of 67%. The treatments applied were T0 = Ration without methionine supplementation (control) and T1 = Ration with 25g/head/day methionine supplementation. The treatment was given during lactation until the cows gave birth again in the next period. The parameters observed included average feed intake (AFI), milk yield, milk quality factors such as fat, protein, lactose and fat-to-protein ratio. The analysis used was descriptive analysis with the Independent Sample T-test using SPSS 25. The results showed that methionine supplementation (T1) had a significant effect ($P<0.05$) for increasing on average feed intake (AFI) and milk quality such as fat, protein, lactose and fat protein ratio and did not have an impact on milk yield.

Introduction

The development of the dairy industry in Indonesia has increased from year to year. This is in line with the increasing public awareness of the importance of consuming milk. The demand for high-quality milk continues to grow, but limited availability is a problem in the dairy industry. Dairy cows raised in Indonesia generally come from Friesian Holstein (FH) and Friesian Holstein Crossbreed (PFH). Generally, this breed has characterized by large, black and white or red and white, white tail and legs, a long head and does not face or extend forward, a white triangle-shaped forehead, milk production is high and has a calm and docile nature. Milk yield is the amount of milk yield, starting from the cow producing milk until the cow is stopped milking or goes through a dry period (Tribudi *et al.*, 2020). FH cows in Indonesia produce less milk than FH from another country especially from origin country. The average production of an FH cow in Indonesia is around 2,400–3,000 per lactation (Diwyanto *et al.*, , 2007). This low productivity is influenced by feeding and maintenance management that is not optimal and the environmental temperature that also affects it (Muwakhid *et al.*, 2024).

Feed for dairy cows has an important role, namely as a fulfillment of basic living needs (maintenance), milk yield and reproduction. Feeding that is not in accordance with the nutritional needs of livestock can cause nutrient deficiencies so that diseases are easy to infect (Erwanto, 1995). Small-scale farms or those that are usually maintained by the community in providing feed still do not pay attention to the nutritional needs of livestock where it is found that feeding, especially crude protein (CP) and total digestible nutrient (TDN) macronutrients, is still lacking compared to their needs. The provision of dry feed materials is also excessive add 5.7% of body weight from 3-4% recommended by the NRC (Despal *et al.*, 2013). This is possible due to the low quality of the feed provided.

One way to support these efforts is to improve the quality of feed nutrients. The nutrients in the feed are used to meet the staple life, production and reproduction. Normally, a dairy cow in one year can give birth to one calf with milk production according to its genetic potential. Ruminant feed engineering has been limited in the processing of effective and efficient feed ingredients so it still needs to be improved through the support of metabolic improvement in the body. The digestive system in ruminant livestock has a very complex process, which involves interactions between incoming feed, rumen microbial populations and the livestock itself. The digestive process is divided into mechanical digestion, fermentative digestion and hydrolytic digestion.

Fermentative digestion occurs in the rumen, in the fermentative digestion the feed protein will be degraded by the rumen microbial protease enzyme with the final result of ammonia (NH3) and α-keto acid. Protein cannot be replaced by other substances and in the body it always undergoes a "turn over" so it is not available in the form of reserves such as fat. The synthesis of amino acids itself requires 50% higher energy compared to the synthesis of triglycerides (Sigit, 1995). There are two types of feed proteins, rumen-degradable proteins (RDP) and non-degradable proteins (RUP). The degradation of proteins in the rumen results from microbial activity and is influenced by factors such as protein type, rumen dilution rate, rumen pH, fermented substrate, and the predominant species of rumen bacteria (Bach *et al.*, 2005). Methionine, an essential amino acid, is particularly significant as it serves as a limiting amino acid for protein synthesis. When amino acid requirements are not satisfied, optimal protein synthesis is hindered.

Methionine serves as one of the essential components required for protein biosynthesis due to its sulfur atom content. Methionine serves as the primary methyl donor in the body via S-adenosylmethionine through a transmethylation route that is crucial for metabolism. Methionine is involved in the production of cysteine and glutathione via transsulfurization pathways, which, along with methionine, contribute to protein synthesis and antioxidant mechanisms (Elango, 2020). Optimal protein biosynthesis will impact milk production because amino acids from digested feed proteins are absorbed in the small intestine, travel through the bloodstream, and then enter the uddeer's secretory cells to be transformed into milk protein.

Cows with high production that are pregnant, there are challenges because in addition to being susceptible to physiological stress, this period makes cows tend to experience higher nutrient stress (Bell *et al.*, 2000). Methionine helps optimize the energy use of feed thereby reducing nutrient stress because methionine can boost immunity. Increased immunity will increase the mRNA expression of DNA methyltransferases (DNMT) and increased cystathionine betasynthase (CBS) as well. This process provides signals to regulate the expression of many genes including in the process of steroidogenesis (Yang *et al.*, 2023).

Amino acid deficiency and imbalance affect protein synthesis which impacts milk production (Lee *et al.*, 2015). Based on this, it is necessary to supplement methionine in feed which aims to meet the needs of amino acids. Amino acid protection technology has developed rapidly to increase the availability of amino acids so that they do not degrade in the rumen. The high availability of amino acids, especially methionine, can support the process of protein biosynthesis and increase the production of good quality and productivity. Consequently, it is essential to carry out research related to the effect of protected methionine supplementation on the productivity of Friesian Holstein dairy cows.

Materials and methods

Animals and treatment

This research was conducted during the period from January 2022 – November 2023 through observation at one of the largest dairy companies in West Java, namely PT. Global Dairi Alami, Kalijati, Subang, West Java. A total 82 Friesian Holstein cows were used in this study. We used cows that had just given birth or the early lactation period with a BCS 3,25 – 3,75. Cows are divided into 2 groups where each group has 41 heads. Cows are kept in colonies and placed in one barn. The feed was provided as a total mixed ration (TMR) consisting of corn silage, alfalfa, hay and concentrate. The TDN wan 67% and the crude protein level was 16%. The treatment given was T0 = Ration without methionine supplementation (control) and T1 = Ration with protected methionine supplementation. The treatment was given during the lactation period until the cow gives birth again in the next period. Cows with the T0 treatment group were carried out in January – December 2022 while cows with the T1 treatment group were observed from January – December 2023.

Data collection

The tool used is the retsch particle size test to measure the quality of feed daily and lactoscan to measure milk quality. This research method is divided into three parts, including the implementation of the research, data collection procedures and data analysis. Data collection for average feed intake (AFI) is TMR with dry matter (DM) 55% taken from morning and evening feeding. Each feed is homogenized into feeding feed. The nutrient content is as shown in Table 1. Data samples of leftover feed were taken from leftover feed the next morning then each sample was homogenized into a sample of leftover. Observations were made on each sample for 7 days. Average Feed intake (AFI) can be calculated using the formula: Average Feed intake (kg) = Feed (kg) – Leftover Feed (kg).

Milk production data was taken at the same time as the time of taking milk quality data. Milk production is known from direct observation results on farms where milk production is in kilograms for 90 days. Data were taken at milking starting on the 60–150 days after the cow gave birth or at the time cows were milked for at least 60 days until 150 days. Lactoscan was used to analyze the samples and ascertain the milk's nutrient content, including its fat, protein, lactose, and fat-to-protein ratio.

Table 1. Feed nutrient analysis was carried out at the Dairy One Forage Laboratory.

Composition	Dry Ingredients
Crude Protein (%)	16.4
NDF (%)	30.3
NFC (%)	38.7
Crude Fat (%)	3.9
Ash (%)	10.77
TDN (%)	67
NEL (mcal/kg)	1.56
NEM (mcal/kg)	1.55
NEG (mcal/kg)	0.95
Crude Fiber (%)	12.4
IVTD 30 h, % of DM	84
NDFD 30 h, % of NDF	47

Neutral Detergent Fiber (NDF), Non-Fiber Carbohydrate (NFC), Total Digestible Nutrient (TDN), Net Energy for lactation (NEL), Net Energy for Maintenance (NEM), Net Enegry for Gain or Growth (NEG), Neutral Detergent Fiber Digestibility (NDFD) and In Vitro True Digestibility (IVTD).

Data analysis

Statistical analyses were carried out in SPSS (Version 25). Statistical analysis was performed using independent t-test to compare treated and control. The data were expressed as mean ± SD, and $P<0.05$ was considered significant or the treatment had an impact on the parameters.

Results

Average feed intake

Based on Table 2, the average feed intake for the T0 and T1 treatment groups differed statistically significantly. The average feed intake in the T1 group (34.35) was less than in the T0 group (38.22). The result of mean difference is 3.86745 This indicates that the average feed intake for the methionine group is higher compared to feed that is not supplemented by methionine.

Table 2. Average Feed Intake, Milk Yield and Milk Quality.

Parameters	Treatment		Sig.	P
	T0	T1		
AFI (Kg/Head/Day)	38.22±1,67	34.35±1.66	0.66	0
Milk Yield (l/Head/Day)	29.87±7.82	31.48±6.20	0.06	0.30
Fat (%)	3.73±0.31	3.60±0.27	0.34	0.05
Protein (%)	3.64±0.21	3.24±0.11	0.01	0
Lactose (%)	4.55±0.13	4.45±0.10	0.02	0
Fat/Protein (%)	1.03±0.09	1.12±0.10	0.20	0

Milk yield and milk quality

The result of the independent t-test showed on Table 2. Although there was a difference in the quantity of milk produced between the group of cows that were not supplemented with methionine and the feed supplemented with protected methionine, the difference was not statistically significant, indicating that protected methionine supplementation had no significant effect ($P>0.05$) on milk production.

Based on Table 2. descriptive analysis showed that feeds supplemented with methionine (T1) produced higher average production and fat/protein ratio compared to feeds not supplemented with methionine (T0). The protein, fat and lactose content of feed supplemented with methionine tended to be lower compared to the group not given methionine.

Discussion

The increase in average feed consumption in the T1 group proves that methionine supplementation has a positive effect on consumption. Methionine can improve various metabolic processes of the body, including in the process of glutathione production. This is in accordance with the opinion of Elango (2020) that methionine is the main methyl donor in the organism through S-adenosylmethionine through the trans-methylation pathway which is very important for metabolism. Through transsulfurization pathways, methionine also contributes to the synthesis of cysteine and glutathione, which together support antioxidant systems and protein synthesis. Thus, methionine plays a role in maintaining the health of the digestive system so that it affects appetite in livestock and causes feed consumption to increase.

Nevertheless, in certain earlier research, methionine supplementation in feed did not always indicate that it could impact consumption. When compared to the methionine group, the results of the Michelotti *et al.* (2021) research that protected methionine supplementation of 12 g/day did not significantly alter feed consumption. The findings of the meta-analysis by Wei *et al.* (2022) also support this, demonstrating that protected methionine supplementation had no discernible effect on feed intake and neither milk production nor lactose concentration.

On this research, milk yield did not shown the impact of giving protected methionine suplementation. This is parallel to the results of the research of Wei, *et al.* (2022) The use of protected methionine to feed does not significantly increase milk production, this can be influenced by several of factors, including the fact that the amino acid products used in the feed are not manufactured by the same company and the percentage of amino acids that pass through the cow's rumen varies, resulting in different metabolic amino acids that eventually reach the small intestine.

The results of the independent t-test showed that the administration of methionine in the ration had a real effect ($P<0.05$) on milk quality. This is caused by methionine, which is essential to produce casein, the primary protein found in milk. Furthermore, methionine has an impact on the synthesis of milk fat, albeit a smaller one than on milk protein. The impact of protected methionine supplementation on milk fat percentage has been the subject of numerous studies, with varying degrees of success. After adding protected methionine, the percentage of dairy fat in Frisian Holstein cows dropped in mid-lactation, as reported by Wang *et al.* (2003) study, whereas Wang *et al.* (2005) research showed the reverse outcome.

Milk fat is one of the most unstable ingredients in milk because it is readily impacted by the type of dairy cow, physiological condition, ration ratio, season, illness, and other variables.This is consistent with Wei *et al.* (2022) assertion that mastitis and rumen acidity will also result in a drop in milk fat, which is low in the summer and tends to rise in the winter. The decrease in milk fat caused by acidosis cannot be separated from the influence of lipid transport, which might prevent fat storage in the liver and hence affect the availability of lipids to mammalian tissues. This supports Liang *et al.* (2022) assertion that methionine's availability can promote choline synthesis and lipid transport as a very low-density lipoprotein, which can reduce hepatic fat accumulation and boost lipid availability in mammalian tissues. By providing methyl groups in trans-methylation reactions, it also acts as a precursor to protein synthesis, allowing methionine to aid in lipid biosynthesis and raise the fat content of milk.

Conclusion

Based on the study's findings, it can be concluded that adding 25 g/head/day of methionine can raise the average value in the majority of the parameters that were evaluated, but statistically only has a significant impact on average feed intake and milk quality not milk yield. Further research needs to be conducted to better understand the need for protected methionine for better production performance.

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Conflict of interest

The authors declare that they have no conflict of interest.

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