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Supplementation Effect of DFM, Protein Bypass, Ca-PUFA, And Organic Minerals on Milk Condition and Blood Profile of Friesan Holland Dairy Cows

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Abstract. The experiment was conducted on 20 lactating dairy cows for the 2nd-4th lactation period at 4-7 lactation months, which was carried out for three months at PT. CABS Malangbong Garut, West Java. The treatment was a complete feed with a combination of direct-fed microbials (DFM), protein bypass, Ca-PUFA, and organic mineral supplementation according to the experimental design. In vivo testing is carried out to obtain dairy cow productivity values, including milk production and milk quality, as well as hematological and biochemical testing of dairy cow blood to see an overview of livestock health based on blood conditions. Analysis of milk composition using a Lactoscan milk analyzer, while blood hematological analysis is done using an automated hematology analyzer (KT-6200 VET) and biochemical analysis of blood is done using a spectrophotometer. The research method used an experimental method with an experimental design that is a completely randomized design with five treatments with four replications. The research results were analyzed using MANOVA with further tests using DMRT $\alpha \leq 5\%$ in the SPSS 24 version. The results showed that the supplement combination affected milk fat and total solids content, which was apparent from the hematological and biochemical aspects profile before and after the treatment, which was relatively unaffected, thus proving there is no negative effect when given to dairy cows.

Key words: Ca-PUFA, DFM, organic minerals, protein by pass, supplementation

INTRODUCTION

Factors that affect the productivity of dairy cows include seeds, feed and maintenance management. The factor that has a big role in productivity is feed. The feed given should increase productivity and balanced between nutrient intake and the nutrient needs of livestock for basic living and production. The essential nutrients needed by dairy cows include amino acids, essential fatty acids, minerals and energy. Essential nutrients are obtained from feed sources of fiber (carbohydrates), protein, fat, and minerals.

Direct feeding has disadvantages including: (1) feeding protein sources without protection will not be effective because it will be degraded by microbes in the rumen; (2) feeding unprotected fat sources will cause disruption of the rumen ecosystem due to the defaunation process; (3) giving minerals directly to livestock will be difficult to absorb, so organic minerals are the choice because they are easily absorbed by the animal's body through the incorporation of minerals with microbes. In addition, the ability of livestock to digest feed ingredients must be considered. In order to optimize the digestive system in the rumen added beneficial, microorganisms added to digest fiber in fodders ingredients.

Based on these facts, the main environmental factors, especially feed, play an important role in physiological processes in the body of livestock (Salfer et al., 2019). In order to support the productivity and quality of dairy cows, it is necessary to add direct fed microbes, protein by pass, CaPUFA, and organic minerals. The addition is expected to be able to increase the quality and quantity of dairy cow's milk without disturbing the health of livestock. The addition of direct fed microbial (DFM) can improve and balance the rumen ecosystem (Tasripin et al., 2022).

The addition of DFM can be taken from the group of bacteria and fungi (yeast). The fungi commonly used are *Aspergillus oryzae* and *Saccharomyces cerevisiae* (Fuller, 1989; Horincar & Bahrim, 2017; Mutaqin et al., 2018, 2020; Mutaqin & Tanuwiria, 2020). The mechanism of DFM action includes balancing the rumen ecosystem, suppressing the production of lactic acid, and helping to break down cellulose. The balance of the rumen ecosystem that occurs will stimulate increased growth and productivity in livestock whose growth indicator is an increase in body weight. The use of DFM in ruminants is used between 0,2 – 0,6% dry matter (Mutaqin et al., 2018). As for other sources say the use of DFM of 4 x 10^7, 8 x 10^7 and 12 x 10^7 CFU/DM (Stanford et al., 2014). While the recommendation to use DFM in fodders for fattening is 1 x10^9 CFU/head/day (Vipham et al., 2015), 2 × 10^9 for yeast and 4 - 5 × 10^9 CFU for bacteria (AlZahal et al., 2014; Boyd et al., 2011; Luan et al., 2015; Nocek & Kautz, 2011; Shao et al., 2014).

On the other hand, the addition of oil to the diet has several benefits, such as increasing the energy of the diet, increasing the efficiency of energy use through inhibition of methanogenesis, as a defaunation agent, and a source of essential unsaturated fatty acids such as linoleic, linolenic and

arachidonic (Bayat et al., 2018). The addition of 3% Ca-PUFA to dairy cattle feed causes milk production to increase from 18,88 kg/day to 22.48 kg/day, improves reproduction and increases pregnancy (Maeng et al., 1993; Purushothaman et al., 2008; Reis et al., 2012).

The addition of supplements with a combination of direct fed microbial, protein by pass, Ca-PUFA, and organic minerals is something that has recently implemented in dairy cows with the aim of increasing milk productivity and quality without disturbing the health of livestock as seen from blood conditions (hematological and blood biochemical).

MATERIALS AND METHODS

Study area

The in vivo experiment was carried out for three months in PT. Citra Agro Buana Semesta (CABS) a dairy cow farm Malangbong Garut, West Java. Which has of about 800 m altitude and THI is 84 - 90. Feeding is in the form of complete feed based on cow body weight with the addition of supplements based on treatment and ad-libitum drinking water.

The research location is shown in Figure 1.

Figure 1. Location of PT. Citra Agro Buana Semesta (CABS) Dairy Farm. Research location in Malangbong Garut, West Java: point (7º01’25”S, 108º05’16”E) and the detected sites.

Experimental Design

The experiment was conducted on 20 lactating dairy cows for the 2-4th lactation period and 4-7 lactation months, with an average milk production of 16 liters/day. Research design was determined based on the coefficient of variation of milk production. The nutrient content of the complete feed (Table 1).

Table 1. The nutrient content of the complete feed

No	Nutrient	Complete Feed	Protein by-pass	Ca-PUFA	Organic Minerals
1	Water (%)	3,48	17,09	2,44	4,90
2	Ash (%)	9,28	32,29	6,48	4,83
3	Crude Protein (%)	15,08	28,56	1,66	20,74
4	Crude Fibre (%)	19,23	0,77	6,42	2,88
5	Crude Fat (%)	10,44	12,58	45,44	9,61
6	NNFE (%)	45,47	25,8	39,69	61,93
7	TDN (%)	69,61	78,77	71,87	78,68
8	Energy (Kkal/kg)	3.439	2.905	6.587	3.469

In vivo testing with using a completely randomized design with five treatments with four replications. Treatment as follows:

T0 = Complete Feed

T1 = Complete Feed + 0,4% DFM

T2 = Complete Feed + 0,4% DFM + 3% Protein *by-pass*

T3 = Complete Feed + 0,4% DFM + 3% Protein *by-pass* +2% Ca-PUFA

T4 = Complete Feed + 0,4% DFM + 3% Protein *by-pass* +2% Ca-PUFA + 2% Organic Minerals (Zn, Cu, Se, Cr-organic)

Procedures

In vivo testing was carried out to obtain dairy cow productivity values including milk production and milk quality. Hematological and biochemical testing of dairy cow blood. The analysis was carried out by taking 20 ml samples of milk from the morning and evening milking for each cow. Samples were taken immediately after milking and put into sample bottles. The data is then standardized into 4% FCM. The purpose of this standardization is to eliminate the effect of fat content on milk production which was previously analyzed statistically (Adriani & Mushawwir, 2009).

Measurement of milk quality is done by taking samples every two weeks and analyzing the quality of the milk. In the sampling process, stirring is performed first so that the condition of the milk is homogeneous. The composite milk samples were then analyzed by lactoscan. Milk composition analysis using a lactoscan milk analyzer. Measurement of milk composition using Lactoscan which previously had milk samples that had been composited between morning and evening milking once every two weeks. Milk samples were stored in closed and non-clear plastic vials. The collected samples were then processed lactoscan milk analyzer by recording all the milk content recorded. Blood samples were taken from the Coccigea Vein. This vein is located in the ventral area of the 2nd or 3rd tailbone, this is usually done for cows. Blood biochemical analysis method using a spectrophotometer with a wavelength of 200–800 nm (Adriani & Mushawwir, 2009).

Blood hematological measurement taken with automated hematology analyzer (KT-6200 VET)(Adriani et al., 2017). Automated hematology analyzer uses the principle of flow cytometry. Flow cytometry is used to analyze the physiological and chemical properties of cells providing information about the size, structure, and interior of cells. Blood biochemical analysis was performed using a spectrophotometer technique with a wavelength of 200–800 nm following a procedure based on the Biolabo Kit. The blood biochemical parameters that were calculated were blood glucose, cholesterol, triglycerides, creatinin, HDL, LDL, Total Protein and Albumin.

Data analysis

The research method used is an experimental method with Completely Randomized Design (CRD) experimental design performed through five treatments with four replications. The research results were analyzed using MANOVA. Further tests using the Duncan's Multiple Range Test (DMRT) $\alpha \leq 5\%$ in the SPSS IBM 24 version.

RESULTS AND DISCUSSION

Milk Production and Milk Quality Dairy Cows

Milk production is a manifestation of the interaction between genetic and physiological factors with environmental factors. Therefore, the milk production of dairy cows varies greatly depending on these two factors. Most of the variations caused by physiological factors can be controlled and some cannot be controlled.

Milk quality is the properties of milk that reflect the condition of the milk. By standard Codex Alimetarius Commision (CAC) about Code of hygienie practice for milk and milk products (CAC/RCP 57-2004), milk testing is very important and must be done. The test results must meet the requirements

as suitable for consumption milk according to SNI 01-3141.1-2011. Milk Production and milk quality dairy cows (Table 2).

Table 2. Milk Production and Milk Quality Dairy Cows

	1		3	4	
			Treatment		
Parameter					
Milk Production 4%FCM (kg)	14,85±5,030 [a]	14,37±3,194 [a]	15,58±2,603 [a]	12,85±3,739 [a]	17,82±5,280[a]
Milk Fat (%)	4,12±0,584 [b]	4,71±0,623 [ab]	4,60±0,534 [b]	4,39±0,458 [b]	5,34±0,401 [a]
Milk Protein (%)	3,01±0,151 [a]	2,99±0,062 [a]	3,15±0,256 [a]	3,06±0,077 [a]	2,97±0,160[a]
Milk Lactose (%)	4,21±0,209 [a]	4,10±0,417 [a]	4,33±0,246 [a]	4,27±0,110 [a]	4,15±0,281[a]
Total Solid (%)	12,18±0,900[b]	12,58±0,896[ab]	12,91±0,978 [ab]	12,59±0,428 [ab]	13,26±0,609[a]

Note: Different superscripts in the direction of the column indicate significantly different ($p<0,05$).

Milk production of 4% FCM based on Table 2 showed results that were not significantly different ($p>0.05$) in each treatment and group, however the average production of treatment (T4) showed a high yield of 17,82 kg. These results were obtained because the milk fat content in treatment (T4) was 5,34% higher than the other treatments. The condition of 4% FCM milk production with additional fat sources will not be significant to 4% FCM milk production, because the increase in 4% FCM milk production must also be seen in the BCS of cows. Skinny cows will produce more milk with less fat content and vice versa (Scott et al., 1995). The previous measurement of 4% FCM milk production must base on average milk fat content of each treatment. Milk fat content in each treatment will be an indicator of determining the average value of milk production standardized by 4% FCM (Tasripin et al., 2009).

The increase in the value of milk fat content is certainly influenced by the intake of given fodders. The type of fodders will affect the high and low value of the fat content of dairy cow's milk produced. Fatty acid content in fodders will affect milk fat content, because the increase in milk fat content is obtained from fatty acid synthesis. The higher the level of fatty acid synthesis, the higher the production of milk fat (Zurriyati et al., 2011). The percentage of milk lactose content in each treatment was in the range of 4,10 – 4,33%. This value has met the standards of Codex and SNI 2011. The value of protein content in each treatment ranged from 2,97 - 3,15%. The value of protein content is still passing the codex standard and SNI 2011. As with milk fat and lactose content, it turns out that complete feed supplementation affects the protein content of the low milk production.

The total solid content of milk ranges from 12,18 – 13,26%. This value has exceeded the codex and SNI standards which require a total solid milk content of at least 11% (SNI, 1998). High total solid milk is a determinant of whether or not dairy cow's milk is accepted, as well as an indicator of determining

milk prices (Yeamkong et al., 2010). Total solid milk is an element of milk consisting of solid non-fat and fat content, so the total solid content of milk depends on these elements (Marwah et al., 2012).

Blood Hematologist

Blood has a very complex role so that physiological processes run normally, so that livestock productivity is optimal (Adam et al., 2015). The function of blood is related to the transportation of components in the body such as nutrients, oxygen, carbon dioxide, metabolism, hormones and endocrine glands, heat, and immunity. Hemoglobin is a red pigmented protein that carries and exchanges oxygen and carbon dioxide in erythrocytes (Samuelson, 2007). Blood hemoglobin content is expressed in grams of hemoglobin per hundred milliliters of blood (g/100 ml) or grams/deciliter (Akbari et al., 2018). Blood Hematologist Before and After Treatment (Table 3 and Table 4).

Table 3. Blood Hematologist Before Treatment

	1		**3**	**4**	
Hemoglobin (gr/dl)	10,33±1,167[a]	10,25±1,103 [a]	10,40±0,663 [a]	10,08±0,670 [a]	9,25±0,759 [a]
Erytrocyites (10^6 cell/mm^3)	4,87±0,461[ab]	4,97±0,431[ab]	5,34±0,171 [a]	5,10±0,134[ab]	4,58±0,458[b]
Leukocyites (cell/mm^3)	6075±2054,87 [a]	6525±613,05 [a]	7500±2544,27 [a]	5475±1408,01 [a]	6800±489,90 [a]
Hematocrit (%)	30,50±3,109 [a]	31,25±3,500 [a]	32,00±0,816 [a]	30,00±2,000 [a]	28,50±2,517 [a]
Thrombocytes (10^4 pieces/mm^3)	44,03±8,394 [a]	44,05±16,239 [a]	60,83±14,759 [a]	78,30±70,097 [a]	38,78±12,189 [a]
Eosinofil (%)	8,50±1,000 [a]	7,50±1,000 [a]	6,50±3,109 [a]	6,25±2,217 [a]	7,50±1,291 [a]
Stab Neutrofil (%)	2,50±0,580 [a]	3,00±0,820 [a]	3,25±1,260 [a]	2,75±0,960 [a]	2,50±0,580 [a]
Segmen Neutrofil (%)	33,75±2,062[ab]	36,25±2,500[ab]	32,25±4,193[a]	31,00±3,559[ab]	29,00±6,481[b]
Lymphocytes (%)	47,75±1,708 [a]	47,75±5,500 [a]	50,25±7,365 [a]	53,50±6,245 [a]	52,00±5,292 [a]
Monocytes (%)	7,50±1,291 [a]	7,00±1,414 [a]	7,75±0,957 [a]	6,50±1,732 [a]	7,50±1,291 [a]
Parameter			**Treatment**		

Note: Different superscripts in the direction of the column indicate significantly different (p<0,05).

Table 4. Blood Hematologist After Treatment

	1		**3**	**4**	
Hemoglobin (gr/dl)	9,58±1,565[a]	9,63±0,842 [a]	10,13±1,014 [a]	9,33±0,754 [a]	9,68±0,911 [a]
Erytrocyites (10^6					

Parameter	Treatment				
cell/mm^3)	4,65±0,342 [a]	4,46±0,413 [a]	5,14±0,550 [a]	4,77±0,253 [a]	4,71±0,606 [a]
Leukocyites (cell/mm^3)	5750±903,7 [a]	6100±1695,09 [a]	6150±943,4 [a]	6125±1848,2 [a]	5975±838,15 [a]
Hematocrit (%)	30,75±3,096 [a]	30,75±2,630 [a]	32,75±4,031 [a]	30,5±3,416 [a]	31,75±3,948 [a]
Thrombocytes (10^4 pieces/mm^3)	48,48±17,109 [a]	40,78±5,797 [a]	58,35±7,301 [a]	73,18±72,558 [a]	36,38±5,972 [a]
Eosinofil (%)	8,50±0,577 [a]	7,00±1,414 [a]	7,00±1,826 [a]	6,50±1,291 [a]	7,00±0,816 [a]
Stab Neutrofil (%)	2,25±0,500 [a]	3,00±0,820 [a]	3,00±0,000 [a]	2,75±0,960 [a]	2,25±0,500 [a]
Segmen Neutrofil (%)	32,50±1,915 [a]	33,00±2,160 [a]	32,75±2,062 [a]	33,00±5,715 [a]	33,00±1,826 [a]
Lymphocytes (%)	49,75±1,500 [a]	49,50±0,577 [a]	48,75±5,439 [a]	51,25±8,382 [a]	48,75±0,957 [a]
Monocytes (%)	7,00±0,000 [a]	7,75±1,500 [a]	8,50±1,732 [a]	7,25±3,304 [a]	9,00±1,633 [a]

Note: The same superscript in the direction of the column indicates not significantly different (p>0,05).

The hemoglobin content before and after treatment based on Tables 3 and 4 showed that the complete feed supplementation was not significantly different (p>0,05) in each treatment. The content of hemoglobin is very important for survival, because it carries and regulates oxygen to body tissues. The hemoglobin content before treatment ranged from 9,25 – 10,40 g/dl and after treatment ranged from 9,33 – 10,13 g/dl. The hemoglobin content before and after treatment in lactating dairy cows under normal conditions ranged from 8-15 g/dl (Gavan et al., 2010).

Likewise, the hemoglobin content of lactating dairy cows in the subtropics is 8,60-11,90 g/dl (Divers & Peek, 2008). The level of hemoglobin in the blood is influenced by several factors. The most important factor is the adequacy of feed, especially protein in the ration and its digestibility in addition to age, sex, and type of livestock (Reece et al., 2015). Hemoglobin content in livestock will increase at low environmental temperatures and will decrease at high environmental temperatures (Nurrasyidah et al., 2012).

Measurement of erythrocytes number is an important part of research because erythrocytes are blood cells that have a function to bind and circulate oxygen to all body tissues (Ganong, 2003). The number of erythrocytes based on Tables 3 and 4 showed that the complete ration supplementation was significantly different (p<0,05) in the pre-treatment condition and not significantly different (p>0,05) in the post-treatment condition. The number of erythrocytes before treatment ranged from 4,58 – 5,34 x 10^6 cell/mm^3 and after treatment ranged from 4,45 – 5,14 x 10^6 cell/mm^3.

Leukocytes are the mobile unit of the body's defense system. The number of leukocytes can be used as a benchmark for the health condition of livestock. The benefit of white blood cells is that they provide a rapid defense against infection. The number of leukocyte cells before treatment ranged from 5.475 – 7.500 cells/mm^3 and after treatment ranged between 5.750 – 6.150 cells/mm^3. The number of leukocytes

before and after treatment was lower than the opinion of Sattar and Mirza (2009) which stated that the number of leukocytes ranged from 7.340-8.860 cells/µl and Mirzadeh et al. (2010) which stated that it was around 6.500-11.500 cells/µl. Leukocytes make up only 1% of the total blood in the body, but have a very important function in the immune system (Akers & Denbow., 2008).

Hematocrit or packed cell volume (PCV) is the percentage of blood volume consisting of red blood cells. Hematocrit is the percentage of red blood cells in 100 ml of blood. The percentage of hematocrit before treatment ranged from 28,50 – 32,00% and after treatment ranged from 30,50 - 32,75. This value is already inside the range of normal hematocrit percentage, which is between 24 - 46% (Jackson & Cockcroft, 2007; Reece, 2009). The results of other studies showed that the percentage of hematocrit ranged from 28,14-30,32% (Sattar & Mirza, 2009) and ranged from 25,89-36,01% (Mirzadeh et al., 2010). There is also those who stated that the perentage of normal hematocrit was even higher, namely 40% (Frandson, 1992). Abnormal hematocrit values can cause anemia due to the amount of fluid in the total blood. A decrease in the hematocrit value can occur due to a decrease in the degree of body activity (Guyton & Hall, 2014).

Thrombocytes are blood cells that function in hemostasis. Thrombocytes are cytoplasmic fragments of megakaryocytes that do not have a nucleus and are formed from the spinal cord (Kosasih, 2008). Thrombocytes counts before treatment ranged from 3,87 - 7,83 x 10^5 pieces/mm^3 and after treatment ranged from 3,63 to 7,31 x 10^5 pieces/mm3. Some research results state that the thrombocytes count is in the range of 2 - 5 x 10^5 pieces/microliter and is in the range of 2 - 3 x 10^5 pieces/microliter (Waterburry, 1998).

The percentage of eosinophils before treatment ranged from 6,25 - 8,5% and after treatment ranged from 6,50 – 8,50%. The percentage of eosinophils was in the normal range and some were categorized as high. Several studies stated that the percentage of eosinophils ranged from 4,16 - 6,64% (Sattar & Mirza, 2009) and some stated that it was between 5,50 -19,7% for dry dairy cows. The number of eosinophils under normal circumstances is in the range of 2% of the total leukocyte count (Guyton & Hall, 2003). Several research results stated that the condition of the eosinophil count of lactating dairy cows was lower than the results of each supplementation treatment on complete feed.

Granular leukocytes are divided into three types, namely neutrophils, eosinophils, and basophils. Neutrophil nuclei have various types of shapes and are segmented (Colville & Bassert, 2002). The percentage of stable neutrophils before treatment ranged from 2,5 - 3,25% and after treatment ranged from 2,25 – 3,00%. The neutrophil segment is the most mature neutrophilic granulocyte present in the circulating blood. Neutrophils are also known as polymorphonuclear (PMN), because their nuclei have various types of shapes and are segmented (Colville & Bassert, 2002; Kiswari, 2014). The percentage

of neutrophil segments before treatment ranged from 29,00 – 36,25% and after treatment ranged from 32,50 – 33,00%.

Lymphocytes are part of the leukocyte differential. Lymphocytes have a role in the immune system. The number of lymphocytes in the blood circulation can be influenced by the level of production (Jain, 1993). The percentage of lymphocytes before treatment ranged from 47,75 – 53,50% and after treatment ranged from 48,75 – 51,25%. The percentage before and after treatment was still in the same range, namely 45.9-52.3% (O'Driscoll et al., 2009) and 61,39-67,21% lower (Sattar & Mirza, 2009). A decrease in the number of lymphocytes (lymphopenia) can also occur due to a decrease in production which usually occurs in lactating cows that are close to dry drums. Increased lymphocytes in the blood circulation (lymphocytosis) can occur due to physiological, reactive, and proliferative (Jain, 1993).

Monocytes are part of the leukocyte differential. Monocytes act as macrophages that phagocytize foreign particles of microbes that attack the body and residual cells resulting from neutrophil activity(Lawhead & Baker, 2005). The percentage of monocytes before treatment ranged from 6,50 – 7,75% and after treatment ranged from 7,00 to 9,00%. The percentage before and after treatment was still in the same range, namely 5,42 - 15,10% (Sattar & Mirza, 2009). Meanwhile, the percentage of dry cow monocytes in dry drums was lower, ranging from 2.5-3.5% (O'Driscol et al., 2009). There are two types of leukocytes, namely polymorphonuclear leukocytes (granulocytes) and mononuclear leukocytes (agranulocytes). Granular leukocytes are divided into three types, namely neutrophils, eosinophils, and basophils. The three types have their own roles as body immunity (Lawhead & Baker, 2005; Theml et al., 2004).

Blood Biochemistry

Health aspects such as livestock immunity can be seen from the hematological and biochemical status of the blood, because this is influenced by the content and adequacy of the feed consumed (Lawhead & Baker, 2005; Reece et al., 2015; Theml et al., 2004). Blood Biochemistry Before and After Treatment (Table 5 and Table 6).

Table 5. Blood Biochemistry Before Treatment

	1		**3**	**4**	
			Treatment Parameter		
Blood Glucose (mg/dl)	34,00±5,416 [a]	27,00±2,582 [a]	26,50±1,291 [a]	35,00±2,828 [a]	31,00±8,165 [a]
Cholesterol					

(mg/dl)	178,50±47,205 [a]	183,25±41,524 [a]	199,75±31,181 [a]	244,50±75,412 [a]	168,25±58,163 [a]
Triglycerides					
(mg/dl)	29,75±2,986[b]	36,75±3,594 [a]	27,75±3,202[b]	27,75±0,500[b]	28,25±4,193[b]
HDL (mg/dl)	105,25±37,766 [a]	119,25±20,222 [a]	122,5±23,742 [a]	132,75±42,999 [a]	103,75±34,150 [a]
LDL (mg/dl)	67,25±16,235 [a]	56,75±22,984 [a]	71,75±35,584 [a]	98,5±42,304 [a]	78,25±15,370 [a]
Creatinin					
(mg/dl)	1,26±0,182 [a]	1,46±0,093 [a]	1,27±0,231 [a]	1,26±0,263 [a]	1,13±0,187 [a]
Total Protein					
(g/dl)	11,54±1,794 [a]	11,31±1,435 [a]	10,61±1,989 [a]	11,79±2,613 [a]	10,32±0,742 [a]
Albumin					
(g/dl)	4,97±0,724 [a]	5,11±0,673 [a]	4,9±0,809 [a]	5,09±0,972 [a]	4,45±0,428 [a]
Globulin					
(g/dl)	6,58±1,995 [a]	6,20±1,399 [a]	5,71±1,827 [a]	6,70±2,995 [a]	5,87±1,128 [a]

Note: Different superscripts in the direction of the column indicate significantly different (p<0,05).

Table 6. Blood Biochemistry After Treatment

	Treatment				
Parameter	**1**		**3**	**4**	
Blood Glucose					
(mg/dl)	55,00±7,789 [a]	50,75±13,720 [a]	49,75±10,626 [a]	55,25±9,179 [a]	56,50±6,856 [a]
Cholesterol					
(mg/dl)	192,25±59,191 [a]	140,25±38,922 [a]	208,75±28,253 [a]	173,75±33,589 [a]	181,50±44,576 [a]
Triglycerides					
(mg/dl)	31,00±2,160 [a]	33,75±7,042 [a]	30,50±2,646 [a]	28,25±2,986 [a]	33,50±5,916 [a]
HDL (mg/dl)	112,75±20,040 [a]	106,00±34,108 [a]	132,25±8,539 [a]	110,50±27,086 [a]	124,50±28,172 [a]
LDL (mg/dl)	70,75±45,617 [a]	27,75±14,751 [a]	70,50±20,632 [a]	57,50±6,856 [a]	52,25±16,297 [a]
Creatinin					
(mg/dl)	1,07±0,172 [a]	1,17±0,102 [a]	1,05±0,099 [a]	1,28±0,186 [a]	1,03±0,183 [a]
Total Protein					
(g/dl)	9,03±0,499[ab]	10,17±2,185[ab]	12,39±5,239 [a]	7,89±0,976[b]	10,56±2,632[ab]
Albumin					
(g/dl)	4,00±0,359 [a]	3,84±0,909 [a]	3,79±0,495 [a]	3,88±0,320 [a]	4,40±0,653 [a]
Globulin					
(g/dl)	5,16±0,530[ab]	6,32±1,533[ab]	8,33±5,214 [a]	4,02±0,744[b]	6,16±2,490[ab]

Note: Different superscripts in the direction of the column indicate significantly different (p<0,05).

The blood glucose content based on Tables 5 and 6 shows that the complete feed supplementation before and after treatment was not significantly different (p>0.05). Blood glucose content before treatment ranged from 26,50 – 35,00 mg/dl and after treatment ranged from 49,75 – 56,50%. Blood glucose content before treatment showed a very low content, while after treatment it showed an increase

according to normal blood glucose content, which ranged from 48,58 ± 6,67 mg/dl (Ramandani & Nururrozi, 2015) and blood glucose content which was classified as normal in ruminants was approx. 30-70 mg/dl (Anggorodi, 1995). Glucose is the most important metabolic substrate needed to support the body functions of dairy cows. Low glucose content can cause high concentrations of non-esterified fatty acids which have toxic effects on follicles, oocytes, embryos, and fetuses (Arthur, 2001).

Cholesterol is a lipid substance that is the result of metabolism that is found in the blood (Frandson, 1992) and is produced by the liver (Murray et al., 2012). Cholesterol is the main sterol in animal lipids and can produce a number of oxidation products under certain conditions. Cholesterol content before treatment ranged from 168.25 – 244.50 mg/dl and after treatment ranged from 140.25 – 208.75 mg/dl. The cholesterol content in each treatment before and after was included in the normal cholesterol content, which was around 130-252 mg/dl (Turk et al., 2004).

Triglycerides are a type of fat found in the blood. Triglycerides are produced by the liver, but most come from foods, such as meat, dairy, and oil. When the intake of triglycerides from food exceeds the amount needed by the body, there will be an increase in the content of triglycerides in the blood (Singh & Singh, 2016). The content of triglycerides before treatment ranged from 27,75 - 36,75 mg/dl and after treatment ranged from 28,25 – 33,75 mg/dl. The triglyceride content in each treatment before and after has a normal triglyceride content value of 7 - 30 mg/dl (Turk et al., 2004), although there are treatments that exceed 30 mg/dl because there are other opinions stating that the triglyceride content is normal in dairy cows if it is less than 150 mg/dl (Petkova et al., 2008)

HDL cholesterol is a component of the lipoprotein fraction formed from fat and protein components (Schlegel et al., 2012). HDL is a lipoprotein that maintains the balance of cholesterol so that it does not accumulate in cells, the balance is maintained by the removal of sterols from the membrane at a rate equal to the amount of cholesterol synthesized to the liver (Hasanuddin et al., 2013). HDL content before treatment ranged from 103,75 – 132,75 mg/dl and after treatment ranged from 106,00 – 132,25 mg/dl. The HDL content in several treatments before and after showed high HDL values, exceeds the results of research conducted (Petkova et al., 2008), which stated that the HDL content of dairy cows ranged from 93,44 – 111,58 mg/dl. HDL cholesterol is used to transport excess cholesterol from all body tissues to be carried to the liver.

LDL cholesterol is a component of the lipoprotein fraction formed from fat and protein components (Schlegel et al., 2012). LDL cholesterol is bad cholesterol because it has atherogenic properties, which is easily attached to the inner walls of blood vessels. LDL content before treatment ranged from 56,75 - 98,50 mg/dl and after treatment ranged from 27,75 – 70,75 mg/dl. LDL content before treatment had normal LDL content, which was in the range of 44 - 141 mg/dl (Turk et al., 2004), but after treatment

showed a decrease in LDL content in treatment (T1), treatment with DFM supplementation showed a fairly high decrease in LDL.

Supplementation on a complete ration on LDL content before and after treatment. The condition of decreasing LDL content in each treatment occurred after the complete feed supplementation treatment. High and low LDL content will affect the body function of dairy cows. Although LDL is considered as bad cholesterol, the content of LDL still has a role in body tissues. LDL plays an important role in the distribution of cholesterol to body tissues. The role of LDL is to provide cholesterol in body tissues because it is the main carrier for cholesterol from the liver into body tissues (Weatherby & Ferguson, 2002).

Creatinine is a metabolite that is excreted through the kidneys. Increased creatinine in the blood will interfere with kidney function. The effect of animal feed on kidney function can be checked based on the examination of creatinine levels in blood serum. Creatinine content before treatment ranged from 1,13 - 1,46 mg/dl and after treatment ranged from 1,03 - 1,28 mg/dl. Creatinine content before and after treatment showed normal values ranging from 0,2 - 2,6 mg/dl (Meyer & Harvey, 2004; Wahjuni & Bijanti, 2006).

Total protein content is very important because it relates to the body's health status or when experiencing a disease (Kaslow, 2010). The total protein content based on Tables 5 and 6 showed that the complete ration supplementation before treatment was not significantly different ($p>0.05$) and after treatment was significantly different ($p<0.05$). The total protein content before treatment ranged from 10,32 – 11,79 mg/dl and after treatment ranged from 7,89 – 12,39 mg/dl. The total protein content in each treatment before and after supplementation showed that the total protein content was relatively high compared to the normal content, namely 6,1 g/dl (Ogunsanmi & Taiwo, 2001), some also stated that the total protein content was normal at around 7,56 ± 0,500 g/dl (Mitruka and Rawnsley, 1981) and 6,81 ± 0,821g/dl (Ramandani & Nururrozi, 2015) and other studies say it is in the range of 7,2 – 8,0 g/dl (Kaslow, 2010).

Several studies show that the value of total protein content is lower than the results of the research carried out. This indicates that the total protein content before and after treatment has a high total protein content. However, the high total protein content will show its role and function in maintaining the body condition of dairy cows. The role of total blood protein content helps regulate blood osmotic pressure which is very essential for determining cell membrane permeability (Utari et al., 2012).

Albumin has a moderate salt content and easily coagulates when exposed to heat. Differences occurred in the milk production group after treatment. Albumin content before treatment ranged from 4,45 – 5,11 mg/dl and after treatment ranged from 3,79 - 4.40 mg/dl. Albumin content before and after treatment showed normal albumin content, namely 4.5 - 5 g/dl (Kaslow, 2010) and higher than several

other studies which stated lower albumin content ranging from 3,44 ± 0,250 – 3,78 ± 0,220 g/dl (Sudarman et al., 2019).

Globulins are components of immunoglobulins that function to maintain body immunity (Hicks et al., 1998). The globulin content before treatment ranged from 5,71 – 6,70 g/dl and after treatment ranged from 4,02 – 8,33 g/dl. The globulin content before and after treatment had a higher value than the content obtained from several studies, which ranged from 2.3 to 2.8 g/dl (Kaslow, 2010).

Hematological and biochemical conditions of abnormal blood will cause disturbances to livestock body. This condition is very important because it is related to the body's health status or is experiencing a disease (Kaslow, 2010). The expected condition of livestock is livestock that has optimum productivity with healthy conditions.

Overall, from observations, it is stated that the addition of DFM, protein by pass, Ca-PUFA, and Organic Minerals supplementation combination can improve the quality of dairy cow's milk in terms of milk fat content and total solids without disturbing the health of the livestock treated with the supplement, which can be seen from the blood condition (Blood Hematologist and Biochemistry).

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