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To cite this article: S Sani *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **757** 012051

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***Chlorella vulgaris* Supplementation as Mineral Source of Zinc and Selenium to Improve the Quality of Goat Milk as Health Drink in COVID-19 Pandemy**

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Abstract. Microalgae *Chlorella vulgaris* contains micronutrients such as mineral zinc and selenium having role to produce antibody and improve body immunity. However, the impact of *Chlorella vulgaris* supplementation to goat milks's chemical compositions has not being well documented. This research's objective is to identify *Chlorella vulgaris* suppmentation as zinc and selenium source to improve goat milk's quality as health drink in COVID-19 pandemy. Fifteen Etawah cross breed or Peranakan Etawa (PE) goats grouppped into 5 treatments dan 3 repetitions. The goats were fed with grass, tofu waste and tempe waste. *Chlorella* supplement is fed to treated goat feed every day with level 0g (P0 = control), 2,5g (P1), 5g (P2), 7,5g (P3), and 10g (P4). The research's result shows that zinc content in goat milk increase 2,12 ppm or 24,67% , while selenium increase 0,08 mcg or 5,5% after getting supplementation of *Chlorella* 10g/goat/day (P4). The provision of *Chlorella* also increase as solids total, protein and milk fat. Milk production increased by 28,36% with *Chlorella vulgaris* supplements (P4). Based on the results of this study it can be stated that *Chlorella vulgaris* supplementation in goat PE feed can be used as a source of natural zinc and selenium minerals to improve the quality of goat milk as a health drink to increase body immunity, especially during the Covid-19 pandemic.

Keywords: *Chorella vulgaris*, zinc, selenium, PE goat milk, immunity

1. Introduction

The end of Covid-19 pandemic is not yet known. It is important to keep the body healthy so that the immunity of human body or immune system will not decline. Nutrition plays an important role in boosting immunity. Goat milk is very good drink for maintaining body's health because it contains nutrients such as protein, vitamins, fatty acids and minerals that can increase body immunity.

The immune system is the body's ability to fight against infections and virulent factors that are antigenic and immunogenic. The body forms antigens, which are compounds that can stimulate the formation of specific antibodies that are protective in increasing cellular immunity [1]. If the immune system is weakened, the ability to protect the body is also reduced, so that pathogens, including viruses, can grow and develop in the body.

The role of nutrition in enhancing immunity because antigens are part of protein, fat, polysaccharides, lipopolysaccharides, lipoproteins or nucleic acids, while micro nutrients that support the immune system in fighting infection include the minerals zinc, Selenium, Ferum and vitamins A, B, C, D, and E.



Nucleic acids are essential compounds in cells, which play role in the function of cellular immunity. Zinc is a micro mineral that plays a role in the synthesis of these nucleic acids, so that the presence of zinc has an important role in the function of cellular immunity. This role is proven by the decreased activity of natural killer cells, CD4 + and CD8 + and decreased lymphocyte proliferation when the body suffer from lack of Zinc[2]. The role of zinc in immune function, namely in the function of T cells and in the formation of antibodies by B cells. Zinc is also needed in the activity of the enzyme SOD (superoxide dismutase) which has a role in the body's defense system.

Another micro-mineral that plays role in increasing immunity is Selenium. The mineral selenium works together with vitamin E act as an antioxidant in the body. Selenium plays role in breaking down peroxides into unreactive bonds to avoid damage to the unsaturated fatty acids that are mostly found in the membrane. Selenium also helps maintain membrane integrity and protects DNA from damage [3]. The research's results showed that selenium deficiency can cause a decrease in IgG and IgM titers, interfere with neutrophil chemotaxis and antibody production by lymphocytes, increase CD4 + and decrease CD8 +[4].

The role of minerals is also related to several vitamins, namely vitamins A, C, D and E. Vitamin A plays role in maintaining epithelial cells, namely the epithelial mucosa. Epithelial cells are one of the body tissues involved in the function of non-specific immunity and cellular immunity [5]. The role of vitamin C in the immune system is closely related to the role of vitamin C as an antioxidant. Because vitamin C easily donates electrons to free radicals so that immune cells are protected from damage caused by free radicals. Vitamin C also plays arole in enhancing immune function by stimulating the production of interferon (a protein that protects cells from virus attack) [6]. Vitamin E or α -tocopherol collaborates with the mineral selenium and is widely present in erythrocyte membranes and plasma lipoproteins. Vitamin E is known to be an antioxidant that is able to maintain integrity and to protect and maintain cell membrane permeability. The integrity of this cell membrane greatly affects the immune function, especially helper T cells in interacting with antigen presenting cells (APC)[7].

During the Covid-19 pandemic, so many medicines, supplements and health drinks were offered both organic and synthetic with the aim of increasing the body's immunity. However, milk is the best food, including goat milk which has been known to have medicinal properties because it contains nutrients that can increase body immunity. milk is also safe for consumption for anyone, especially for those who are intolerant of milk and do not cause bloating.

Payakumbuh and Limapuluh Kota Regency are goat breeding areas, both for meat and dairy from local and etawa crossbreed. Dairy goat farming continues to be developed socially, culturally and is an important part of the community's economy. Currently, milk production is only sufficient to meet local needs and has not been able to meet global needs. But, this sector has grown rapidly with the number of goats doubling [8]. However, the increase in population was not followed by an increase in milk production and quality. If the goats are reared properly the production can reach 2 liters per day. Currently, the production of PE goat milk belonging to the breeders is only around 0.4-0.8 liters per day and the quality has not met the SNI standard for fresh milk (SNI 01-3141-1998)[9].

The low production and quality of milk is generally caused by the low quality of the feed given. On a daily basis, breeders usually herd the goats on marginal land or are penned by providing adequate forage with or without additional feeding. To support production, sometimes synthetic feed additives are given.

Organic dairy goat production can be a viable alternative to produce quality milk. To improve the quality of milk can be done by modifying the composition of milk and milk fatty acids by various methods. The way to improve the composition of milk that is commonly done lately is by giving grains, but the provision of natural supplements from microalgae in food has not been widely used. *Chlorella vulgaris* is promising as a source of supplements, can be used as a source of minerals needed by the body to increase body immunity[10].

Chlorella vulgaris is a microalgae that has chlorophyll, which is a source of natural pigments that are safe to use as additives resulting from the photosynthesis process. Chlorophyll in the body acts as an antioxidant [11]. *Chlorella vulgaris* is a good source of long-chain PUFAs (CL-PUFAs) such as

eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), [12] which have beneficial effects on livestock and human health including to reduce the risk of coronary heart disease [13].

Chlorella vulgaris contains protein, carbohydrates, fats, vitamins and minerals [13]. The results of several studies indicate that *Chlorella* contains nucleic acids, amino acids, enzymes, vitamins, minerals, beta-carotene, and *Chlorella* growth factor (CGF). Fresh *Chlorella*, contains about 5% CGF. CGF plays a role in healing the wound, supports the immune response, as a growth promoter and to increase the probiotic population in the digestive tract [14]. *Chlorella* contains vitamins in every kilogram, namely; vitamin A 480 mg/kg, Thiamin 10 mg/kg, Riboflavin 36 mg/kg, Cobalamin 0.02 mg/kg, Vitamin C 20 mg/kg, Vitamin E 120 mg/kg, Biotin 0.15 mg/kg [15]. *Chlorella* is also a rich source of minerals, long-chain C24 fatty acid, and protein, which can provide a natural source of nutrition for livestock. Each 100g of *Chlorella* contains 972 mg of Calcium (Ca), 533 mg Potassium (K), 659 mg Sodium (Na), 6 mg Magnesium (Mg), 1 mg Zinc (Zinc), 136 mg Iron (Fe), 3.4 mg Manganese (Mn), 35.0 mg Copper (Cu), 0.22 mg Nickel (Ni), and 1 mg Cobalt (Co) [15].

Minerals are an important component in enzyme reactions and immune system function. It is stated that the minerals zinc and selenium play a role in producing antibodies and increasing body immunity. However, the impact of microalgae supplementation as a source of zinc and selenium minerals on the production and chemical composition of PE milk has not been well documented. The purpose of this study was to determine the supplementation of *Chlorella vulgaris* as a source of natural zinc and selenium to improve the quality of PE goat milk as a health drink during the Covid-19 pandemic.

2. Materials and Methods

The material used in this study was goat milk collected from 15 PE goats weighing about 45 kg who were lactating for 2 months. Field grass is given one kg/goat/day. Additional feed in the form of a mixture of tofu waste and tempe waste as much as 500 grams /goat/day (farmer formula). The supplementary feed in the form of *Chlorella vulgaris* powder was mixed with additional feed according to the treatment.

Equipments utilized in this study is spectrophotometer, atom absorber (SSA) GBC 932 AA, cathode lamp for metal analysis, erlenmeyer, spatula, milk analyser, funnel, digital scale and furnace.

Design applied in this study was completely randomized design consisted of 5 treatments of *Chlorella vulgaris* provision with repetition of 3 times consist of: P0 = 0 g *Chlorella* /goat/day (control); P1 = 2.5 g *Chlorella* /goat/day); P2 = 5.0 g *Chlorella* /goat/day); P3 = 7.5 g *Chlorella* /goat/day); P4 = 10 g *Chlorella* /goat/day.

2.1. Data analysis

Data were analyzed by one-way ANOVA using the SPSS program version 20. Differences between treatments were tested using the advanced Duncan's Multiple Range Test (DMRT). The variables observed were daily milk production in the morning, milk quality and mineral content of zinc and goat milk.

3. Results and Discussion

The effect of *Chlorella* sp supplementation on goat milk production in the morning is shown in Table 1 and figure 1.

Table 1. Daily goat milk production (ml)

Treatment	Repetition			Average
	1	2	3	
P0	420	420	410	416,67 ^a
P1	500	505	510	505,00 ^b
P2	530	510	510	516,67 ^b
P3	570	575	600	581,67 ^c
P4	580	590	590	586,67 ^c

3.1. Milk production

The production of PE goat milk in the morning after being fed with *Chlorella vulgaris* supplement had a significant effect ($P < 0.05$) on the production of PE goat milk. The average daily milk production value in the morning ranges from 500-580 ml / day. Increased milk production in line with increased supplementation. The supplementation of *Chlorella vulgaris* 2.5g /goat /day can increase milk production by 13% compared to without provision (P0). The provision of supplement 7.5g /goat/ day (P3) was able to increase the production of goat milk 28.36% compared to without provision (P0), but it was not significant ($P > 0.05$) to the supplementation of *Chlorella vulgaris* 10g/ day (P4). The increase in goat milk production with supplementation is probably due to an increase in rumen fermentation kinetics. *Chlorella vulgaris* contains substances that can increase the growth of rumen bacteria and affect digestibility [16]. *Chlorella vulgaris* supplementation in goat feed caused an increase in the concentration of several bacterial species in the rumen, such as *Butyrivibrio fibrisolvens* *Ruminococcus labus* and *Clostridium sticklandii* [10]

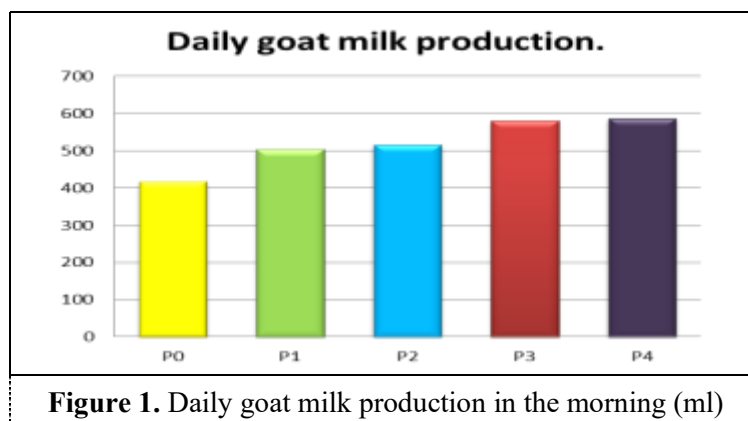


Figure 1. Daily goat milk production in the morning (ml)

3.2. Quality of PE goat milk

The quality of the goat milk produced is determined by the nutritional composition of the milk. The results of the analysis of the nutrient composition in goat milk supplemented with *Chlorella vulgaris* are shown in Table 2 and figure 2.

Table 2. Nutritional composition of goat milk in the morning supplemented with *Chlorella vulgaris*

Analysis	P0	P1	P2	P3	P4	Minimum standards (SNI:01-3141-1998)
Water content	86,70	85,58	85,76	85,55	85,55	
Ash matter	0,85	0,85	0,85	0,85	0,85	
Protein (%)	2,78	3,76	3,20	4,23	4,24	2,8
Total fat (%)	2,34	2,36	3,51	3,71	3,73	3.0
Dry matter without fat (%)	9,19	10,42	12,24	14,45	14,46	8,0
pH	6,35	6,31	6,36	6,47	6,50	6,3-6,8
Zinc (ppm)	6,47	6,80	6,13	8,59	8,59	Max 40 ppm
Se (mcg)	1,36	1,38	1,40	1,43	1,44	

3.3. Total Fat Content

The total fat content of milk is influenced by several factors such as the type of feed and the concentrate. In Table 2, it can be seen that the fat content of goat milk P0 (control) and P1 (*Chlorella* 2.5g) has not met the SNI standard for fresh cow milk (SNI standard for goat milk does not exist yet). The minimum quality standard of fresh milk fat is 3. (SNI: 01-3141-1998)[9]. *Chlorella vulgaris* supplementation can increase the total fat content of milk from 2.34 to 3.73. The results of this study are in line with the results of Poti at all [17], namely that the fat content of goat milk given microalgae supplementation was higher ($P < 0.05$) than control goats that were only given alfalfa forage (3.67 vs 3.04).

Giving *Chlorella vulgaris* supplement in goat feed can increase the concentration of several species of rumen bacteria so that will affect the amount of fatty acid synthesis which will then result in an increase in milk fat levels [10]. *Chlorella vulgaris* supplementation in ruminant feed would increase bacterial growth in the rumen so that the fermentative process in the form of VFA consisting of propionate, acetate, and butyrate would be converted into fatty acids which would then enter udder secretion cells and become fat milk. *Chlorella vulgaris* supplements cause ruminal biohydrogenation by stimulating the production of trans C18: 1, Trans -11 C18: 1 fatty acid, MUFA and PUFA, which are unsaturated fatty acids that are important for health that can reduce blood cholesterol levels, reduce the risk of heart disease and stroke [18].

3.4. Protein Content

Based on Table 2, it can be seen that the group of goats given *Chlorella vulgaris* supplementation has an effect on goat milk protein. Protein content increased from 2.78% (P0) to 4.24% (P4) or increased to 1,36 % compared to without supplementation. The protein content of milk is influenced by the type of feed given. *Chlorella* supplementation can increase milk protein because *Chlorella* contains high protein amounting 57,63 % [19]. *Chlorella* is also rich in essential amino acids. Amino acids in feed will increase milk protein synthesis [20]

3.5. Dry Matter Without Fat

The dry matter without fat (DMWF) of goat milk is influenced by the quality of the feed given. DMWF for treatment P1 (10.42) is above the SNI standard for milk (8.0). This indicates that feeding grass with tofu dregs and tempe waste supplemented with 2.5g *Chlorella* /goat/day is sufficient to meet the DMWF milk standard. DMWF is influenced by lactose and protein. If the levels of lactose and milk protein are high, the dry matter without milk fat will increase. DMWF determines the nutritional quality of milk [20].

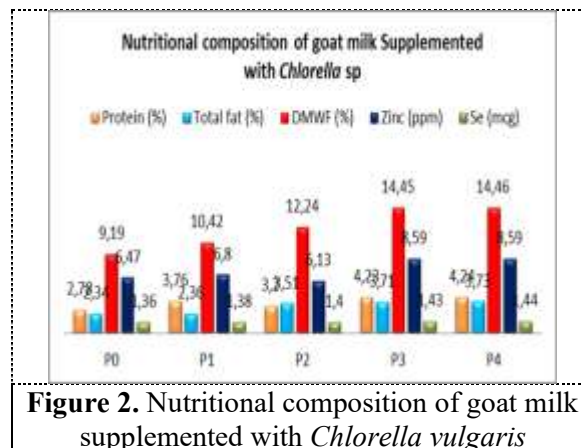


Figure 2. Nutritional composition of goat milk supplemented with *Chlorella vulgaris*

3.6. Zinc Minerals Content

Zinc is an essential mineral substance, which is needed by the body, but in excessive amounts it will cause poisoning. Zinc in small amounts is an essential element for metabolism, because zinc deficiency can cause slow growth.

In Table 2 and figure 3, it can be seen that the zinc concentration in goat milk is different for each treatment. *Chlorella vulgaris* supplementation can increase the zinc concentration in goat milk. The concentration of zinc minerals in the control treatment (P0) was 6.47 ppm, increased to 8.59 ppm, an increased to 2,12 ppm or 24,67% after getting supplementation of *Chlorella vulgaris* 10g/goat/day (P4). *Chlorella* is a rich source of minerals. Every 100g of *Chlorella* contains 1 mg of zinc (zinc), 136 g of iron (Fe), 972 mg of calcium (Ca), 533 mg of potassium (K)[21].

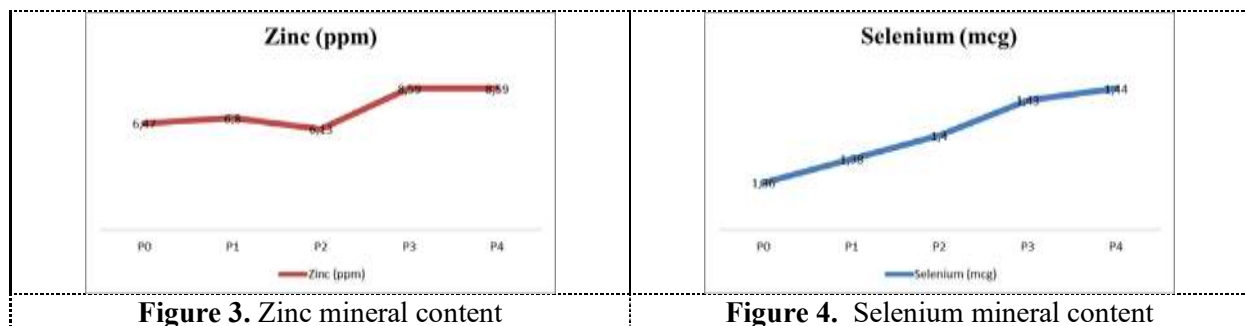
Mineral zinc is an important component in enzyme reactions and immune system function. Zinc and selenium act as antioxidants, helping to clear up some of the damage caused by oxidative stress. Zinc is needed for immune system function (immunity), cell membrane stability and expression of

certain genes in the body. Zinc is also needed for fetal development and milk production. From the research of the Animal Research Institute, organic minerals (Zn-methionine) can increase rumen fermentation as indicated by higher total gas and increase milk production [16].

3.7. Selenium minerals Content

In Table 2 and figure 4, it can be seen that *Chlorella vulgaris* supplementation can increase the Selenium content in goat milk. Selenium concentration was increased from 1,36 mcg (P0) to 1,44 mcg (P4), increased to 0,08 mcg or 5,5 % after getting supplementation of *Chlorella vulgaris* 10g/goat/day (P4). Selenium works together with vitamin E and acts as an antioxidant. Vitamin E protects cell membranes from free radicals by releasing hydrogen ions, while selenium plays a role in breaking down peroxides into unreactive bonds so as not to damage the unsaturated fatty acids that are abundant in the membrane. Selenium has role in protein synthesis and the activity of the enzyme glutathione peroxidase (GSH-PX) that act as catalyst in the breakdown of peroxides formed in the body into non-toxic bonds [22].

Adequacy of nutrients, especially vitamins and minerals, is necessary in maintaining an optimal immune system. *Chlorella vulgaris* supplementation in PE goat feed can increase the nutritional content of milk, namely protein, total fat, DMWF and zinc and selenium minerals which play a very important role in enzyme reactions and the function of the body's immune system. Zinc and selenium act as antioxidants, helping to clear up some of the damage caused by oxidative stress.



4. Conclusion

Based on the results of this study it can be stated that *Chlorella vulgaris* supplementation in goat PE feed can be used as a source of natural zinc and selenium minerals to improve the quality of goat milk as a health drink to increase body immunity, especially during the Covid-19 pandemic. Supplementation with *Chlorella vulgaris* 10g /goat/day increased milk production by 28.36%, zinc mineral levels increased by 2.12 ppm or 24.67%, and selenium increased by 0.08 mcg or 5.5%.

Acknowledgments

The authors would like to express a deep appreciation and thanks to the Director of Research and Community Service Ristek-BRIN and the head of LPPM Unand for its financial support of this work.

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