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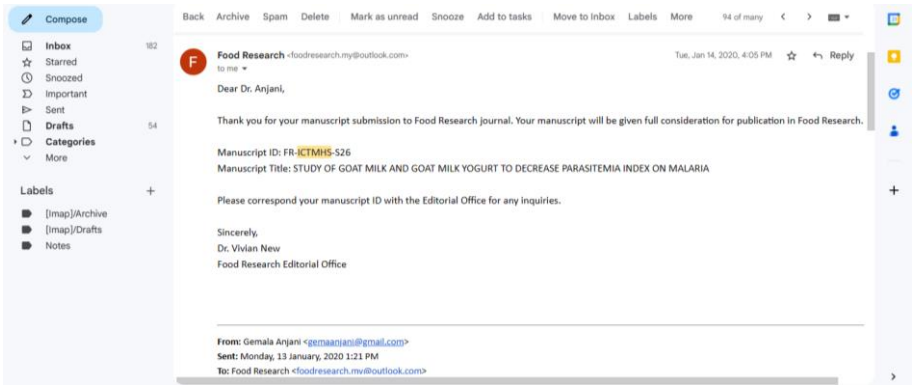

Artikel Awal

STUDY OF GOAT MILK AND GOAT MILK YOGURT TO DECREASE PARASITEMIA INDEX ON MALARIA

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Abstract

Malaria is an infectious disease caused by the Plasmodium Sp parasite. The last study, malaria has reached 219 million cases in 2017. Decreasing of parasitemia index indicates decreasing rate of infection in malaria. Goat milk and goat milk yogurt as immunomodulators have potential to decrease parasitemia index. This study aims to determine the effect of goat milk and goat milk yogurt on the index of parasitemia on malaria mice. This research is true experimental study with a post-test only group design. Thirty five female Balb/c mice were divided into: K- (control negative group), K+ (infected malaria), K+DHP (malaria, DHP drug), X1 (malaria, goat milk), X2 (malaria, goat milk, DHP), X3 (malaria, goat milk yogurt), X4 (malaria, goat milk yogurt, DHP). Inoculation of Plasmodium was given as much as 10^7/0.2 ml. The intervention was given 24 days. Parasitemia index data collection was performed on the seventh day post inoculation. Dose of goat milk and goat milk yogurt is 0.5 ml/20gBw. Data was analyzed using Kruskal-Wallis with Post Hoc Mann-Whitney. The result showed a significant decreased on parasitemia index ($p < 0.05$). The mean parasitemia index in each group: 0% (K-); 13,9% (K+); 0.60% (K+DHP); 4.68% (X1); 3.74% (X2); 3.66% (X3); 0.82% (X4). The group that effectively reduces parasitemia index is group K+DHP and X4. Goat milk yogurt (X3) is more effective in decreasing parasitemia index than goat milk (X1). Giving goat milk yogurt can be considered an additional therapy in the treatment of malaria.

Keywords: Malaria; Parasitemia index; Goat milk; Goat milk yogurt

1. Introduction

Malaria is a disease caused by protozoan parasite from the genus Plasmodium (Okpe et.al, 2016; Isah, 2014; Khalid, 2013; Percário S, 2012) Malaria is transmitted by the bite of a female

Anopheles mosquito that contains Plasmodium (Sucipto, 2015; Pusat Data dan Informasi Kementerian Kesehatan Republik Indonesia, 2016). The type of Plasmodium that most caused malaria in tropical and subtropical regions is Plasmodium falciparum.[7] Malaria causes oxidative changes effect of plasmodium infection. Plasmodium infection will cause an imbalance of antioxidant mechanisms.(Khalid, 2013; Gomes QBA, 2015; Fabbri, 2013) In malaria infections will increase parasitemia index. An increase in the parasitemia index can indicate increased infection rates in malaria (Tyagi AG,2017). Under these conditions, the use of antioxidants is expected to help reduce oxidative damage and prevent further development of malaria (Gomes QBA, 2015).

Goat milk has been shown have positive effect on biological functions in the human body. This relates to the nutritional content contained in goat milk. Goat milk also has a low allergen effect and easily digested by the body (Yangilar F,2013; Aristya AL, 2013; Banjare K, 2017). Protein in goat milk is the main source of active biopeptides that can act as antioxidants (Park, 2010, Alyaqoubi S, 2014; Alyaqoubi et.al, 2014). Goat milk contains natural antioxidant-forming agents so can prevent the lysis of erythrocyte cells(Alyaqoubi S, 2015). Protein in goat milk also has anti-inflammatory properties that are known as one of the body's responses to infection. Protein in goat milk is an important source of angiotensin converting enzyme (ACE) which functions as an antihypertensive peptide and can also help control infections from pathogenic microbes (Afiarahma AI,2015).

One of the processed fermented goat milk products is yogurt. Yogurt contains bioactive peptides and has antioxidant activity(Anggraeni RH, 2013; Gahruie, 2015; Nguyen L, 2016). The antioxidant activity of goat milk yogurt is higher than cow's milk yogurt (Holvik S Ltd,2013; Muniandy P, 2016). Increased antioxidant activity in goat milk yogurt comes from the activity of lactic acid bacteria (LAB) contained therein. Several studies have shown the effects of LAB in responding to oxidative stress (Fardet A, 2017; Padaga MC, 2018). The potential of LAB for human health is as a stimulant of the immune system, able to balance the intestinal flora, reduce cholesterol, have anti-aging activity and antioxidant activity (Padaga MC, 2018; Nakagawa H, 2016). LAB produces exopolysaccharides (EPS) which specifically has immunostimulant activity and is able to increase the digestive tract colonization (Polak, 2013). LAB will hydrolyze casein to a bioactive peptide that has various biological functions. Based on previous research, casein in goat milk yogurt can prevent an increase in Malodialdehide (MDA) (Padaga MC, 2018). This study aims to determine the effect of giving goat milk and goat milk yogurt on the parasitemia index in mice infected with malaria. This research is expected to be useful in the development of food and health science related to the role of antioxidants in the treatment of malaria.

2. Materials and Methods

The research design used in this study is true experimental with post test only randomized control group design. This study used female Balb/c mice which were inoculated by P. Berghei ANKA (PbA). The study was conducted by dividing 7 groups: 3 control groups and 4 treatment groups. K group (negative control group that is given standard feed); K + (positive control group fed standard, inoculated PbA and not treated; K (+) DHP (positive control group fed standard, inoculated PbA and receiving anti-malaria therapy (DHP); X1 (treatment group 1, mice that were inoculated PbA, and given goat milk X2 (treatment group 2, mice that were inoculated PbA, received anti-malaria therapy (DHP) and were given goat milk; X3 (treatment group 3, mice that were inoculated PbA and given goat milk yogurt); X4 (X4 (mice) treatment group 4, mice that were inoculated with PbA, received anti-malaria therapy (DHP) and were given goat milk yogurt.

Parasitemia index data collection was performed on the seventh day post inoculation. Dose of goat milk and goat milk yogurt is 0.5 ml / 20gBw.

Research location for making yogurt, analysis of protein content and antioxidant activity of goat milk and goat milk yogurt were conducted at the Integrated Laboratory of Diponegoro University, Semarang. The location of mice maintenance, malaria inoculation, and measurement of the parasitemia index were carried out at the Integrated Biomedical Laboratory (IBL) Faculty of Medicine, Sultan Agung Islamic University Semarang (FK-UNISSULA). The goat milk used was obtained from the Kuncen Farm Farmers Group located in the Bubakan Village, Mijen District, Semarang City. This study was approved by the Health Research Ethics Commission of the Sultan Agung University School of Medicine with No. 196 / III / 2019 / Bio-commission.

3. Results

The mice used in this study were obtained from the Biology Laboratory of Semarang State University in the number of 28, 25 for treatment and 3 for donor mice. Data normality test of parasitemia index uses the Saphiro-Wilk test. The results of normality test are $p < 0.05$ ($p = 0.00$) so the data have not normal distribution. Data have abnormal distribution so using the Kruskal-Wallis test. The test results can be seen in Table 2.

Table 2. Statistical Analysis of the Parasitemia Index

Treatment Group	Parasitemia index (%)	p^a	p^b						
			K-	K+	K+DHP	X1	X2	X3	X4
K-	0,00±0,00	0,00*	-	0,005*	0,005*	0,005*	0,005*	0,005*	0,005*
K+	13,9±0,58		-	-	0,009*	0,009*	0,009*	0,009*	0,009*
K+ DHP	0,60±0,27		-	-	-	0,009*	0,009*	0,009*	0,289
X1	4,68±0,53		-	-	-	-	0,059	0,602	0,008*
X2	3,74±0,74		-	-	-	-	-	0,834	0,005*
X3	3,60±1,80		-	-	-	-	-	-	0,008*
X4	0,70(0,70-1,20)		-	-	-	-	-	-	-

Information :
a = Kruskall Wallis
b = Post Hoc Mann-Whitney
* = p value <0.005 (significantly)
K^- = Control Group Negative, Healthy
K^+ = Control Group Positive, Infected Malaria
K^+ DHP = Control Group, Infected Malaria and DHP Drug Administration
X1 = Treatment Group, Goat milk- Infected Malaria- Goat milk
X2 = Treatment Group, Goat milk - Infected Malaria- Goat milk and DHP
X3 = Treatment Group, Goat milk Yogurt- Infected Malaria- Goat milk Yogurt
X4 = Treatment Group, Goat milk Yogurt- Infected Malaria- Goat milk Yogurt and DHP

The results normality test of parasitemia index using the Saphiro-Wilk test obtained p value <0.005 (p = 0.00) shows that the parasitemia index data have not normal distribution. Then the Kruskal-Wallis test is performed. Based on Table 2, there is a statistically significant difference in the parasitemia (p <0.05). Treatment with Giving goat milk and goat milk yogurt or a combination of both with DHP drugs has an effect on the parasitemia index. Next, Post Hoc tests were carried out with Mann-Whitney.

Based on Table 8, the groups that not have a significantly different on parasitemia index were the K^{+}DHP and X4 groups with a p value = 0.289. In groups X1 and X2 there was also no significant difference in the parasitemia index with a value of p = 0.059. In group X1 and group X3 there was also no significant difference with a value of p = 0.602. Group which have not significantly different on parasitemia index showed that the two groups had not much different effectiveness in suppressing the parasitemia index. To be able to see the ability of each group to suppress the parasitemia index can be seen based on the mean parasitemia index in Figure 1.

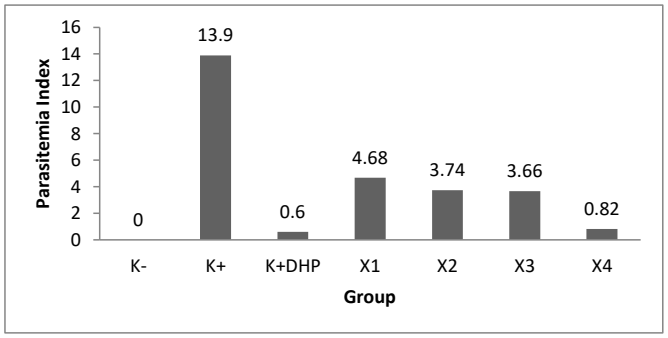

Figure 1. Graph of Average Parasitemia Index

Information :
K^{-} = Control Group Negative, Healthy
K^{+} = Control Group Positive, Infected Malaria
K^{+} DHP = Control Group, Infected Malaria and DHP Drug Administration
X1 = Treatment Group, Goat milk- Infected Malaria- Goat milk
X2 = Treatment Group, Goat milk - Infected Malaria- Goat milk and DHP
X3 = Treatment Group, Goat milk Yogurt- Infected Malaria- Goat milk Yogurt
X4 = Treatment Group, Goat milk Yogurt- Infected Malaria- Goat milk Yogurt and DHP

4. Discussion

Malaria is one of endemic disease in Indonesia and still being health problem which need concern. Malaria usually treated with antimalarial drugs. Standard treatment for malaria use Artemisinin-based combination therapies (ACTs). These therapies can combine artemisinin or one of its derivatives with another antimalarial. One of the combination is Dihidroartemisinin Piperaquin (DHP). Regions in Indonesia which are still endemic areas, one of which is in Papua and West Papua. When malaria cases occur, not all sufferers have close access to health facilities and get DHP medication as early as possible. Utilization of functional food is considered to be an

additional therapy for malaria sufferers. One of the functional foods that can be developed is goat milk and goat milk yogurt (Sucipto CD, 2015; Subdit Malaria Direktorat KR, 2018; Yangilar F, 2013).

This research aimed to know the effect of goat milk and goat milk yogurt to decrease parasitemia index. Result this research can see from figure 1. Figure 1 shows that the group with the lowest parasitemia index is the group K- (0%). the group K- have 0% parasitemia index because the K- group is a group of healthy mice that not inoculated with malaria, so they have a parasitemia index of 0%. Group K+ had the h ighest parasitemia index among all groups (13.9%). K+ group had the highest parasitemia index because this group was inoculated with malaria without treatment so the plasmodium continued to increase. Increasing of plasmodium will caused increasing of parasitemia index. K+ DHP group (0.6%) having the lowest mean of parasitemia index ompared to the K +, X1, X2, X3 and X4 groups. This shows the effectiveness of the use of DHP drugs. DHP drugs include an artemisinin group that is known to be effective in killing plasmodium quickly in all stages including gametocytes (Douglas NM, 2010).

The mean parasitemia index results in groups X1 and X3 were lower compared with K +. Goat milk yogurt can reduce parasitemia index. Goat milk and goat milk yogurt contain casein and whey. Several studies have shown that casein and whey contain antioxidants (Sabeena FKH, 2010). Casein has antioxidants such as radical scavengers and cation chatorators that can inhibit lipid oxidation, using proteins containing lactoferrin, beta-lactoglobulin, alpha-lactalbumin, glycomacropeptides, and immunoglobulins. This protein component can enhance the immune system. the main protein boosts the immune system by converting the cysteine intracellular amino acids into glutathione, glutathione acts as an intracellular antioxidant. Lactoferrin provided in goat milk and goat milk yogurt can stimulate the immune system through the activation of T and B lymphocyte cells (Zapata CR, 2017).

Group X3 was more effective in reducing the parasitemia index than Group X1. Yogurt generally contains lactic acid bacteria that have the enzyme Bile Salt Hydrolase (BSH) which is able to de-conjugate bile salts to produce free bile salts that are less absorbed by the small intestine. Bile salt that returns to the liver becomes reduced so the body will use cholesterol as a precursor to balance the amount of bile salt so that the amount of cholesterol decreases. The decrease in cholesterol levels will affect on decreasing in the amount of lipids that are exposed to free radicals. Decreasing the amount of lipids exposed to free radicals causing the lipid peroxidation process to decrease. This causes a decrease in the number of MDA levels and a decrease in the parasitemia index (El-Dein NA, 2017).

The mean parasitemia index of groups X2 and X4 was higher than the K + group. This proves that the contribution of food sources of protein (goat milk or goat milk yogurt) is effective as an adjuvant treatment in the treatment of malaria. Giving goat milk or goat milk yogurt with DHP drugs is more effective in reducing the parasitemia index. Group X4 was more effective in reducing the parasitemia index than group X2. The group with DHP and goat milk yogurt was more effective in reducing the parasitemia index than the group with DHP and goat milk. The administration of DHP drugs is recommended to be consumed together with milk or fatty foods, (WHO,2015) but previous studies have shown that the administration of DHP drugs together with 200ml of milk (containing 6.4 grams of fat) does not improve pharmacokinetic parameters (including absorption of DHP drugs) when compared with the control group (Annerberg A,2011). Giving goat milk with DHP drugs might inhibit the bioavailability of the drug. This is because goat milk contains higher calcium and magnesium than cow's milk (Park, 2010). Calcium and magnesium have divalent ionic bonds which will cause inhibition of drug absorption (Bushra R,

2011). On the other hand, administration of DHP with goat milk yogurt can reduce the parasitemia index. This shows that goat milk yogurt is more effective in reducing the parasitemia index than goat milk, so that goat milk yogurt is more effective if it becomes a malaria adjuvant therapy than goat milk. The decrease in parasitemia index is due to the content of lactic acid bacteria in goat milk yogurt. In accordance with previous studies (Villarinoa et.al, 2016) that intestinal microbiota can influence the pathogenesis of malaria. The difference in the intestinal bacterial community affects the parasitic load and death after Plasmodium infection. Provision of yogurt can modulate intestinal microbiota so that it can reduce the burden of parasites(Villarinoa et.al, 2016).

Yogurt undergoes a fermentation process, the process will release bioactive peptides from the main protein of milk. The fermentation process will produce antioxidant peptides consisting of 5-11 hydrophobic amino acids. Hydrophobic amino acids include proline, histidine, tyrosine or tryptophan. These amino acids will prevent the formation of free radicals and inhibit the process of lipid peroxidation. Lactic acid bacteria have antioxidant activity and are able to reduce the accumulation of ROS during the process of digestion of food and have the ability to degrade superoxide anions and hydrogen peroxide (Gjorgievski N, 2015).

Giving goat milk yogurt can reduce the parasitemia index. One potential mechanism of intestinal microbiota in influencing the severity of malaria is by giving a direct effect on the parasite itself, where the products of intestinal microbiota inhibit the growth of parasites. Another more potential mechanism is that intestinal microbiota impacts malaria severity by modulating the host immune response to Plasmodium. Previous research showed that intestinal microbiota will give signals to monocytes / macrophages to prepare these cells to respond and help control infections (Denny, 2018).

An important mechanism of yogurt during Plasmodium infection through the ability of intestinal microbiota to express the glycemic molecule in the form of α-gal (Gal1 α1-3Galβ1-4GlnAc-R). The α-gal antibody binds to the surface of the sporozoite, induces lysis order and prevents the spread of Plasmodium to liver cells. The provision of yogurt can trigger an α-gal response to inhibit the transmission of Plasmodium sporozoite (Burgess SL,2017). Decreased parasitemia index after malaria inoculation indicate that diet (intake) plays an important role. Diet has a major role in shaping the composition and activity of intestinal microbiota. In accordance with previous studies that the species Lactobacillus and Bifidobacterium have a protective role by modulating the burden of parasites and reducing the severity of malaria so that the administration of yogurt containing Lactobacillus bacteria can cause a decrease in parasitemia. Differences in intestinal microbiota can prevent malaria severity and accelerate the healing process of malaria. These results also support the possibility that conditioning the intestinal microbiota by giving goat milk yogurt cannot prevent malaria, but has the potential to control the severity of malaria in humans (Shasteen MA, 2015).

5. Conclusion

The addition of goat milk yogurt is more effective in lowering the parasitemia index than goat milk. Giving goat milk yogurt with a dose of 0.5 ml / 20gBw can be considered an additional therapy in the treatment of malaria.

Conflict of Interest - None.

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References

Afiarahma AI, Witjahyo RBB. (2015). Pengaruh Pemberian Susu KAmbing Terhadap Gambaran Mikroskopis Paru dan Kadar Hemoglobin (Hb) Tikus. *Media Med Muda*.4(4):282-292.

Alyaqoubi S, Abdullah A, Addai ZR. (2014). Antioxidant activity of goat milk from three different locations in Malaysia. *AIP Conf Proc* ; 198-201. doi:10.1063/1.4895195

Alyaqoubi S, Abdullah A, Samudi M, Abdullah N, Radhi Z, Al-ghazali M.(2014). Effect of Different Factors on Goat Milk Antioxidant Activity Ministry of Regional Municipalities and Water Resource. *Int J Chem Tech Res*. 6(5) : 974-4290. http://sphinxsai.com/2014/vol6pt5/9/(3191-3196)S-2014.pdf.

Alyaqoubi S, Abdullah A, Samudi M, Abdullah N, Addai ZR, Al-ghazali M. (2015). Physicochemical properties and antioxidant activity of milk samples collected from five goat breeds in malaysia. *Adv J Food Sci Technol*.7(4):235-241. doi:10.19026/ajfst.7.1301

Annerberg A, Lwin KM, Lindegardh N, Khrutsawadchai S, Ashley E, Day NP, et al. (2011). Small Amount of Fat Does Not Affect Piperaquine Exposure in Patients with Malaria. Antimicrobial Agents And Chemotherapy. *American Society for Microbiology*.(55).9.3971–3976 doi:10.1128/AAC.00279-11

Anggraeni RH, Legowo AM, Al-Baarri AN. (2013). Intensitas Warna Dan Aroma Pada Susu Skim Kambing Akibat Proses Glikasi Dengan Penambahan Gula D-Fruktosa, L-Fruktosa, Dan D-Tagatosa. *J Aplikasi Teknologi Pangan*. 2(3):156-158.

Aristya AL, Legowo AM, Al-baarri AN. (2013). Karakteristik fisik, kimia, dan mikrobiologis kefir susu kambing dengan penambahan jenis dan konsentrasi gula yang berbeda. *J Aplikasi Teknologi Pangan*. 2(3):139-143.

Banjare K, Kumar M, Kumar R, Kartikyen S. (2017). Perspective role of goat milk and products : A review. *Inter J of Chem Studies*. 5(4):1328-1338.

Burgess SL, Gilchrist CA, Lynn TC, Petri WA. (2017). Parasitic Protozoa and Interactions with the Host Intestinal Microbiota. *Infect Immun* 85. https://doi.org/10 .1128 / IAI.00101-17.

Bushra R, Aslam N, Khan YA. (2011).Food-Drug Interactions. *Oman Medical Journal*. 26(2): 77-83

Denny, Joshua E. (2018). "Characterizing the gut microbiota during plasmodium infection and antimalarial treatment". *Electronic Theses and Dissertations*. https://doi.org/10.18297/etd/3109

Douglas NM, Ansley NM, Angus BJ, Nosten F, Price RN. (2010). Artemisinin combination therapy for

vivax malaria?. *Lancet Infect Dis.* 10(6): 405–416. doi:10.1016/S1473-3099(10)70079-7

El-Dein NA, El-Deen NMA, Tolba MS, El-Shatoury HE, Awad AG, Ibrahim KM, et al. (2017). Probiotic Properties and Bile Salt Hydrolase Activity of Some Isolated Lactic Acid Bacteria. Egypt. *J. Micro*. (52)

Fabbri C, De-Cássia MNR, Lalwani P. (2013). Lipid peroxidation and antioxidant enzymes activity in Plasmodium vivax malaria patients evolving with cholestatic jaundice. *Malar J*. 12(1). doi:10.1186/1475-2875-12-315

Fardet A, Rock E.(2017). In vitro and in vivo antioxidant potential of milks, yoghurts, fermented milks and cheeses: a narrative review of evidence. *Nutr Res Rev*..1-19. doi:10.1017/S0954422417000191

Gahruie, Hashemi H, Eskandari, Hadi M, Mesbahi G, Hanifpour, Amin M. (2015). Scientific and Technical Aspects of Yogurt Fortification: A Review. *Food Sci Hum Wellness*.(4):1-8. doi:http://dx.doi.org/10.1016/j.fshw.2015.03.002

Gjorgievski N, Ansley NM, Angus BJ, Nosten F, Price RN.(2015). Determination Of The Antioxidant Activity In Yogurt. *Journal of Hygienic Engineering and Design*.

Gomes QBA, Da-Silva LFD, Gomes QAR.(2015). N-acetyl cysteine and mushroom Agaricus sylvaticus supplementation decreased parasitaemia and pulmonary oxidative stress in a mice model of malaria. *Malar J*.14(1):1-12. doi:10.1186/s12936-015-0717-0

Holvik S Ltd. (2013).Introduction Macronutrient Nutritional Composition of Goat Milk. *Happy Days Dairies*:1-12.

Isah MB, Ibrahim MA. (2014). The role of antioxidants treatment on the pathogenesis of malarial infections: A review. *Parasitol Res*. 113(3):801-809. doi:10.1007/s00436-014-3804-1

Khalid M, Alam R, Khan S, Prakash V. (2013). Oxidative Stress Marker and Antioxidant Status In Falciparum Malaria In Relation To The Intensity Of Parasitaemia. *Int J. Biol Med Res*.3(1):3469-3471.

Muniandy P, Shori AB, Baba AS. (2016). Influence of green, white and black tea addition on the antioxidant activity of probiotic yogurt during refrigerated storage. *Food Packag Shelf Life*.8:1-8. doi:10.1016/j.fpsl.2016.02.002

Nakagawa H, Miyazaki T. (2017). Beneficial effects of antioxidative lactic acid bacteria. *AIMS Microbiol*. 3(1):1-7. doi:10.3934/microbiol.2017.1.1

Nguyen L, Hwang ES. (2016). Quality characteristics and antioxidant activity of yogurt supplemented with aronia (Aronia melanocarpa) juice. *Prev Nutr Food Sci*. 21(4):330-337. doi:10.3746/pnf.2016.21.4.330

Okpe O, Habila N, Ikwebe J, Upev VA, Okoduwa SIR, Isaac OT.(2016). Antimalarial Potential of Carica papaya and Vernonia amygdalina in Mice Infected with Plasmodium berghei. *J Trop Med*:6.

doi:10.1155/2016/8738972

Padaga MC, Erika A, Haskito P, Irawan M.(2018). Efek Antioksidatif Kasein Yogurt Susu Kambing Terhadap Pencegahan Reprotoksik Pada Hewan Model Antioxidative Effect of Casey Goat Milk Yogurt against Reprotoxic Prevention in Animal Model Rattus norvegicus Display 2, 3, 7, 8-Tetrachlorinedibenzo-P- Dio. J*urnal Ilmu dan Teknologi Hasil Ternak*.13(2):72-80.

Park, Young. (2010). Goat Milk : Composition, Characteristics. Encyclopedia of Animal Science.

Park, Y.W. (2010). Goat Milk: Composition, Characteristics. Encyclopedia of Animal Science. W.G. Pond and N. Bell. 2nd ed. Taylor and Francis. CRC Press. Boca Raton, FL. In Press

Percário S, Moreira DR, Gomes BAQ, Ferreira MES.(2012) Oxidative Stress in Malaria Review. *Int J Mol Sci*. 13:16346-16372. doi:10.3390/ijms131216346

Polak, M., A. Berecka, D. Wasko, Szwajgier & A. Choma.(2013). Bifidogenic and antioxidant activity of exopolysaccharides produced by Lactobacillus rhamnosus E/N cultivated on different carbon sources. *Journal of Microbiology*. 62(2): 181–189

Pusat Data dan Informasi Kementerian Kesehatan Republik Indonesia. (2016). InfoDatin-Malaria-2016.pdf.

Sabeena FKH, Baron CP, Nielsen NS and Jacobsen C. (2010). Antioxidant activity of yoghurt peptides: Part 1-in vitro assays and evaluation in ω-3 enriched milk. *Food Chemistry.123(4),1081–1089*.doi:10.1016/j.foodchem.2010.05.067

Shasteen MA, Lebeis WS, Schmidt N. (2015). "Lessening Malaria Parasite Burden with Yogurt". MicrobiologyPublications and Other Works.http://trace.tennessee.edu/utk_micrpubs/74

Subdit Malaria Direktorat KR.(2018) *Buku Saku Tatalaksana Malaria 2018*:Kemenkes RI

Sucipto CD. *Manual Lengkap Malaria*. Yogyakarta: Gosyen Publishing; 2015

Tyagi AG, Tyagi RA, Choudhary PR, Shekhawat JS. (2017). Study of antioxidant status in malaria patients. *Int J Res Med Sci*. 5(4):1649-1654. doi:10.18203/2320-6012.ijrms20171281

Villarinoa FN, Lecleir RG, Denny EJ, Dearth PS, Harding LC, Sloan SS, et.al. (2016). Composition of the gut microbiota modulates the severity of malaria. *PNAS*. 113(8): 2235–2240 www. pnas. org/cgi/doi/10. 1073/pnas. 1504887113

WHO.(2015). Guidelines for the treatment of malaria, 3rd edition.

Yangilar F.(2013). As a Potentially Functional Food: Goats' Milk and Products. *J Food Nutr Res*. 1(4):68-81. doi:10.12691/jfnr-1-4-6

Zapata CR, Singh A, Pezeshki A, Nibber T, Chelikani KP. (2017).Whey Protein Components - Lactalbumin and Lactoferrin - Improve Energy Balance and Metabolism. *Scientific Reports*. (7):9917 doi: 10.1038/s41598-017-09781-2

Tables and Figures –

Table 2. Statistical Analysis of the Parasitemia Index

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K+	13,9±0,58		-	-	0,009*	0,009*	0,009*	0,009*	0,009*
K+ DHP	0,60±0,27		-	-	-	0,009*	0,009*	0,009*	0,289
X1	4,68±0,53		-	-	-	-	0,059	0,602	0,008*
X2	3,74±0,74		-	-	-	-	-	0,834	0,005*
X3	3,60±1,80		-	-	-	-	-	-	0,008*
X4	0,70(0,70-1,20)		-	-	-	-	-	-	-

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X1 = Treatment Group, Goat milk- Infected Malaria- Goat milk
X2 = Treatment Group, Goat milk - Infected Malaria- Goat milk and DHP
X3 = Treatment Group, Goat milk Yogurt- Infected Malaria- Goat milk Yogurt
X4 = Treatment Group, Goat milk Yogurt- Infected Malaria- Goat milk Yogurt and DHP

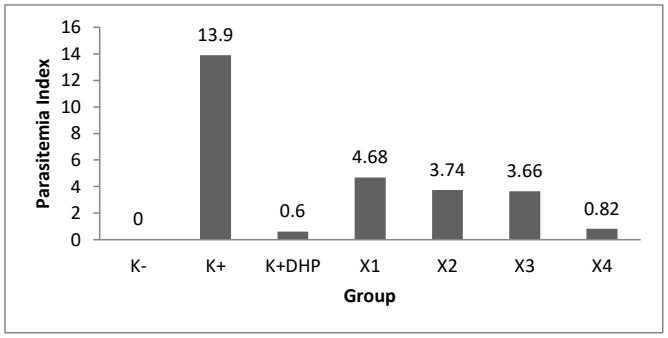

Figure 1. Graph of Average Parasitemia Index

Information :

K^- = Control Group Negative, Healthy

K^+ = Control Group Positive, Infected Malaria

K^+ DHP = Control Group, Infected Malaria and DHP Drug Administration

X1 = Treatment Group, Goat milk- Infected Malaria- Goat milk

X2 = Treatment Group, Goat milk - Infected Malaria- Goat milk and DHP

X3 = Treatment Group, Goat milk Yogurt- Infected Malaria- Goat milk Yogurt

X4 = Treatment Group, Goat milk Yogurt- Infected Malaria- Goat milk Yogurt and DHP

Table 1. Statistical analysis of the parasitemia index

Treatment Group	Parasitemia index (%)	P^1	P^2						
			a	**b**	**c**	**d**	**e**	**f**	**g**
a	0,00±0,00	0.00*	-	0.005*	0.005*	0.005*	0.005*	0.005*	0.005*
b	13,9±0,58		-	-	0.009*	0.009*	0.009*	0.009*	0.009*
c	0,60±0,27		-	-	-	0.009*	0.009*	0.009*	0.289
d	4,68±0,53		-	-	-	-	0.059	0.602	0.008*
e	3,74±0,74		-	-	-	-	-	0.834	0.005*
f	3,60±1,80		-	-	-	-	-	-	0.008*
g	0,70(0,70-1,20)		-	-	-	-	-	-	-

[1] = Kruskall Wallis, [2] = Post Hoc Mann-Whitney,* = p value <0.005 (significantly)

a = Control Group Negative, Healthy, b = Control Group Positive, Infected Malaria, c = Control Group, Infected Malaria and DHP Drug Administration, d = Treatment Group, Goat's milk- Infected Malaria- Goat's milk, e = Treatment Group, Goat's milk - Infected Malaria- Goat's milk and DHP, f = Treatment Group, Goat's milk Yogurt- Infected Malaria- Goat's milk Yogurt, g = Treatment Group, Goat's milk Yogurt- Infected Malaria- Goat's milk Yogurt and DHP

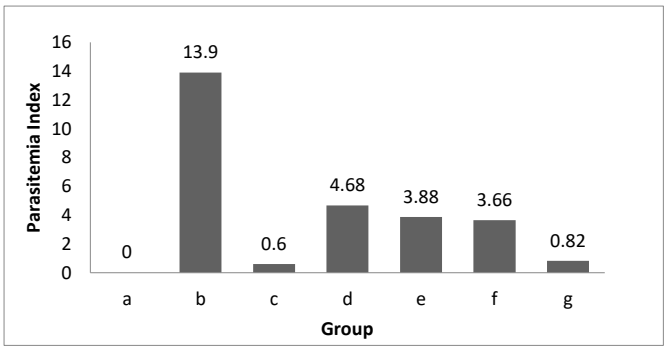

Figure 1. Graph of average parasitemia index. a = Control Group Negative, Healthy, b = Control Group Positive, Infected Malaria, c = Control Group, Infected Malaria and DHP Drug Administration, d = Treatment Group, Goat's milk- Infected Malaria- Goat's milk, e = Treatment Group, Goat's milk - Infected Malaria- Goat's milk and DHP, f = Treatment Group, Goat's milk Yogurt- Infected Malaria- Goat's milk Yogurt, g = Treatment Group, Goat's milk Yogurt- Infected Malaria- Goat's milk Yogurt and DHP

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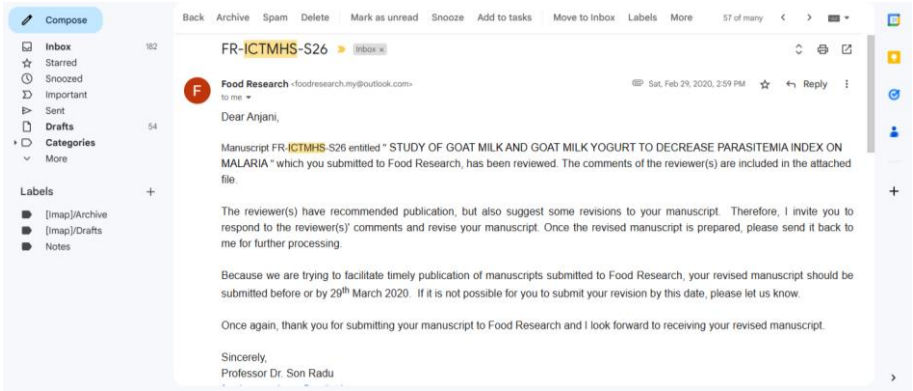

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FR-ICTMHS-S26 Inbox ×

Food Research <foodresearch.my@outlook.com>
to me

Sat, Feb 29, 2020, 2:59 PM Reply

Dear Anjani,

Manuscript FR-ICTMHS-S26 entitled " STUDY OF GOAT MILK AND GOAT MILK YOGURT TO DECREASE PARASITEMIA INDEX ON MALARIA " which you submitted to Food Research, has been reviewed. The comments of the reviewer(s) are included in the attached file.

The reviewer(s) have recommended publication, but also suggest some revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript. Once the revised manuscript is prepared, please send it back to me for further processing.

Because we are trying to facilitate timely publication of manuscripts submitted to Food Research, your revised manuscript should be submitted before or by 20th March 2020. If it is not possible for you to submit your revision by this date, please let us know.

Once again, thank you for submitting your manuscript to Food Research and I look forward to receiving your revised manuscript.

Sincerely,
Professor Dr. Son Radu

STUDY OF GOAT MILK AND GOAT MILK YOGURT TO DECREASE PARASITEMIA INDEX ON MALARIA

Abstract

Malaria is an infectious disease caused by the Plasmodium Sp parasite. The last study, malaria has reached 219 million cases in 2017. Decreasing of parasitemia index indicates decreasing rate of infection in malaria. Goat milk and goat milk yogurt as immunomodulators have potential to decrease parasitemia index. This study aims to determine the effect of goat milk and goat milk yogurt on the index of parasitemia on malaria mice. This research is true experimental study with a post-test only group design. Thirty five female Balb/c mice were divided into: K- (control negative group), K+ (infected malaria), K+DHP (malaria, DHP drug), X1 (malaria, goat milk), X2 (malaria, goat milk, DHP), X3 (malaria, goat milk yogurt), X4 (malaria, goat milk yogurt, DHP). Inoculation of Plasmodium was given as much as 10^7/0.2 ml. The intervention was given 24 days. Parasitemia index data collection was performed on the seventh day post inoculation. Dose of goat milk and goat milk yogurt is 0.5 ml/20gBw. Data was analyzed using Kruskal-Wallis with Post Hoc Mann-Whitney. The result showed a significant decreased on parasitemia index ($p < 0.05$). The mean parasitemia index in each group: 0% (K-); 13,9% (K+); 0.60% (K+DHP); 4.68% (X1); 3.74% (X2); 3.66% (X3); 0.82% (X4). The group that effectively reduces parasitemia index is group K+DHP and X4. Goat milk yogurt (X3) is more effective in decreasing parasitemia index than goat milk (X1). Giving goat milk yogurt can be considered an additional therapy in the treatment of malaria.

Keywords: Malaria; Parasitemia index; Goat milk; Goat milk yogurt

6. Introduction

Malaria is a disease caused by protozoan parasite from the genus Plasmodium (Okpe et.al, 2016; Isah, 2014; Khalid, 2013; Percário S, 2012) Malaria is transmitted by the bite of a female Anopheles mosquito that contains Plasmodium (Sucipto, 2015; Pusat Data dan Informasi Kementerian Kesehatan Republik Indonesia, 2016). The type of Plasmodium that most caused malaria in tropical and subtropical regions is Plasmodium falciparum.[7] Malaria causes oxidative changes effect of plasmodium infection. Plasmodium infection will cause an imbalance of antioxidant mechanisms.(Khalid, 2013; Gomes QBA, 2015; Fabbri, 2013) In malaria infections will increase parasitemia index. An increase in the parasitemia index can indicate increased infection rates in malaria (Tyagi AG,2017). Under these conditions, the use of antioxidants is expected to help reduce oxidative damage and prevent further development of malaria (Gomes QBA, 2015).

Goat milk has been shown have positive effect on biological functions in the human body. This relates to the nutritional content contained in goat milk. Goat milk also has a low allergen effect and easily digested by the body (Yangilar F,2013; Aristya AL, 2013; Banjare K, 2017). Protein in goat milk is the main source of active biopeptides that can act as antioxidants (Park, 2010, Alyaqoubi S, 2014; Alyaqoubi et.al, 2014). Goat milk contains natural antioxidant-forming agents so can prevent the lysis of erythrocyte cells(Alyaqoubi S, 2015). Protein in goat milk also has anti-inflammatory properties that are known as one of the body's responses to infection.

Protein in goat milk is an important source of angiotensin converting enzyme (ACE) which functions as an antihypertensive peptide and can also help control infections from pathogenic microbes (Afiarahma AI,2015).

One of the processed fermented goat milk products is yogurt. Yogurt contains bioactive peptides and has antioxidant activity(Anggraeni RH, 2013; Gahruie, 2015; Nguyen L, 2016). The antioxidant activity of goat milk yogurt is higher than cow's milk yogurt (Holvik S Ltd,2013; Muniandy P, 2016). Increased antioxidant activity in goat milk yogurt comes from the activity of lactic acid bacteria (LAB) contained therein. Several studies have shown the effects of LAB in responding to oxidative stress (Fardet A, 2017; Padaga MC, 2018). The potential of LAB for human health is as a stimulant of the immune system, able to balance the intestinal flora, reduce cholesterol, have anti-aging activity and antioxidant activity (Padaga MC, 2018; Nakagawa H, 2016). LAB produces exopolysaccharides (EPS) which specifically has immunostimulant activity and is able to increase the digestive tract colonization (Polak, 2013). LAB will hydrolyze casein to a bioactive peptide that has various biological functions. Based on previous research, casein in goat milk yogurt can prevent an increase in Malodialdehide (MDA) (Padaga MC, 2018). This study aims to determine the effect of giving goat milk and goat milk yogurt on the parasitemia index in mice infected with malaria. This research is expected to be useful in the development of food and health science related to the role of antioxidants in the treatment of malaria.

7. Materials and Methods

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The research design used in this study is true experimental with post test only randomized control group design. This study used female Balb/c mice which were inoculated by P. Berghei ANKA (PbA). The study was conducted by dividing 7 groups: 3 control groups and 4 treatment groups. K group (negative control group that is given standard feed); K + (positive control group fed standard, inoculated PbA and not treated; K (+) DHP (positive control group fed standard, inoculated PbA and receiving anti-malaria therapy (DHP); X1 (treatment group 1, mice that were inoculated PbA, and given goat milk X2 (treatment group 2, mice that were inoculated PbA, received anti-malaria therapy (DHP) and were given goat milk; X3 (treatment group 3, mice that were inoculated PbA and given goat milk yogurt); X4 (X4 (mice) treatment group 4, mice that were inoculated with PbA, received anti-malaria therapy (DHP) and were given goat milk yogurt. Parasitemia index data collection was performed on the seventh day post inoculation. Dose of goat milk and goat milk yogurt is 0.5 ml / 20gBw.

Research location for making yogurt, analysis of protein content and antioxidant activity of goat milk and goat milk yogurt were conducted at the Integrated Laboratory of Diponegoro University, Semarang. The location of mice maintenance, malaria inoculation, and measurement of the parasitemia index were carried out at the Integrated Biomedical Laboratory (IBL) Faculty of Medicine, Sultan Agung Islamic University Semarang (FK-UNISSULA). The goat milk used was obtained from the Kuncen Farm Farmers Group located in the Bubakan Village, Mijen District, Semarang City. This study was approved by the Health Research Ethics Commission of the Sultan Agung University School of Medicine with No. 196 / III / 2019 / Bio-commission.

8. Results

The mice used in this study were obtained from the Biology Laboratory of Semarang State University in the number of 28, 25 for treatment and 3 for donor mice. Data normality test of

parasitemia index uses the Saphiro-Wilk test. The results of normality test are p <0.05 (p = 0.00) so the data have not normal distribution. Data have abnormal distribution so using the Kruskal-Wallis test. The test results can be seen in Table 2.

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Table 2. Statistical Analysis of the Parasitemia Index

Treatment Group	Parasitemia index (%)	p^a	p^b						
			K-	K+	K+DHP	X1	X2	X3	X4
K-	0,00±0,00	0,00*	-	0,005*	0,005*	0,005*	0,005*	0,005*	0,005*
K+	13,9±0,58		-	-	0,009*	0,009*	0,009*	0,009*	0,009*
K+ DHP	0,60±0,27		-	-	-	0,009*	0,009*	0,009*	0,289
X1	4,68±0,53		-	-	-	-	0,059	0,602	0,008*
X2	3,74±0,74		-	-	-	-	-	0,834	0,005*
X3	3,60±1,80		-	-	-	-	-	-	0,008*
X4	0,70(0,70-1,20)		-	-	-	-	-	-	-

Information :
a = Kruskall Wallis
b = Post Hoc Mann-Whitney
* = p value <0.005 (significantly)
K^- = Control Group Negative, Healthy
K^+ = Control Group Positive, Infected Malaria
K^+ DHP = Control Group, Infected Malaria and DHP Drug Administration
X1 = Treatment Group, Goat milk- Infected Malaria- Goat milk
X2 = Treatment Group, Goat milk - Infected Malaria- Goat milk and DHP
X3 = Treatment Group, Goat milk Yogurt- Infected Malaria- Goat milk Yogurt
X4 = Treatment Group, Goat milk Yogurt- Infected Malaria- Goat milk Yogurt and DHP

The results normality test of parasitemia index using the Saphiro-Wilk test obtained p value <0.005 (p = 0.00) shows that the parasitemia index data have not normal distribution. Then the Kruskal-Wallis test is performed. Based on Table 2, there is a statistically significant difference in the parasitemia (p <0.05). Treatment with Giving goat milk and goat milk yogurt or a combination of both with DHP drugs has an effect on the parasitemia index. Next, Post Hoc tests were carried out with Mann-Whitney.

Based on Table 8, the groups that not have a significantly different on parasitemia index were the K^+DHP and X4 groups with a p value = 0.289. In groups X1 and X2 there was also no significant difference in the parasitemia index with a value of p = 0.059. In group X1 and group X3 there was also no significant difference with a value of p = 0.602. Group which have not significantly different on parasitemia index showed that the two groups had not much different effectiveness in suppressing the parasitemia index. To be able to see the ability of each group to suppress the parasitemia index can be seen based on the mean parasitemia index in Figure 1.

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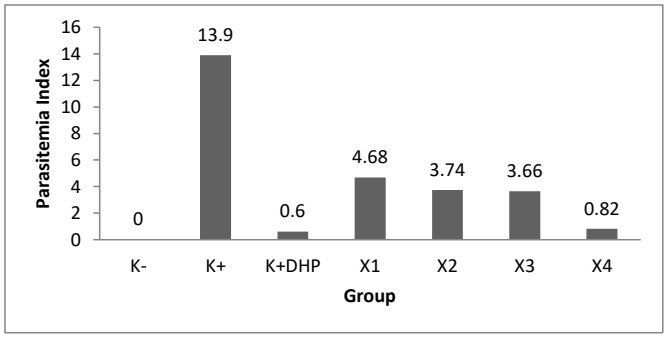

Figure 1. Graph of Average Parasitemia Index

Information :
K^- = Control Group Negative, Healthy
K^+ = Control Group Positive, Infected Malaria
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X1 = Treatment Group, Goat milk- Infected Malaria- Goat milk
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X3 = Treatment Group, Goat milk Yogurt- Infected Malaria- Goat milk Yogurt
X4 = Treatment Group, Goat milk Yogurt- Infected Malaria- Goat milk Yogurt and DHP

9. Discussion

Malaria is one of endemic disease in Indonesia and still being health problem which need concern. Malaria usually treated with antimalarial drugs. Standard treatment for malaria use Artemisinin-based combination therapies (ACTs). These therapies can combine artemisinin or one of its derivatives with another antimalarial. One of the combination is Dihidroartemisinin Piperaquin (DHP). Regions in Indonesia which are still endemic areas, one of which is in Papua and West Papua. When malaria cases occur, not all sufferers have close access to health facilities and get DHP medication as early as possible. Utilization of functional food is considered to be an additional therapy for malaria sufferers. One of the functional foods that can be developed is goat milk and goat milk yogurt (Sucipto CD, 2015; Subdit Malaria Direktorat KR, 2018; Yangilar F, 2013).

This research aimed to know the effect of goat milk and goat milk yogurt to decrease parasitemia index. Result this research can see from figure 1. Figure 1 shows that the group with the lowest parasitemia index is the group K- (0%). the group K- have 0% parasitemia index because the K- group is a group of healthy mice that not inoculated with malaria, so they have a parasitemia index of 0%. Group K+ had the h ighest parasitemia index among all groups (13.9%). K+ group had the highest parasitemia index because this group was inoculated with malaria without treatment so the plasmodium continued to increase. Increasing of plasmodium will caused increasing of parasitemia index. K+ DHP group (0.6%) having the lowest mean of parasitemia index ompared to the K +, X1, X2, X3 and X4 groups. This shows the effectiveness of the use of DHP drugs. DHP drugs include an artemisinin group that is known to be effective in killing plasmodium quickly in all stages including gametocytes (Douglas NM, 2010).

The mean parasitemia index results in groups X1 and X3 were lower compared with K +. Goat milk yogurt can reduce parasitemia index. Goat milk and goat milk yogurt contain casein and whey. Several studies have shown that casein and whey contain antioxidants (Sabeena FKH, 2010). Casein has antioxidants such as radical scavengers and cation chatorators that can inhibit lipid oxidation, using proteins containing lactoferrin, beta-lactoglobulin, alpha-lactalbumin, glycomacropeptides, and immunoglobulins. This protein component can enhance the immune system. the main protein boosts the immune system by converting the cysteine intracellular amino acids into glutathione, glutathione acts as an intracellular antioxidant. Lactoferrin provided in goat milk and goat milk yogurt can stimulate the immune system through the activation of T and B lymphocyte cells (Zapata CR, 2017).

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Group X3 was more effective in reducing the parasitemia index than Group X1. Yogurt generally contains lactic acid bacteria that have the enzyme Bile Salt Hydrolase (BSH) which is able to de-conjugate bile salts to produce free bile salts that are less absorbed by the small intestine. Bile salt that returns to the liver becomes reduced so the body will use cholesterol as a precursor to balance the amount of bile salt so that the amount of cholesterol decreases. The decrease in cholesterol levels will affect on decreasing in the amount of lipids that are exposed to free radicals. Decreasing the amount of lipids exposed to free radicals causing the lipid peroxidation process to decrease. This causes a decrease in the number of MDA levels and a decrease in the parasitemia index (El-Dein NA, 2017).

The mean parasitemia index of groups X2 and X4 was higher than the K + group. This proves that the contribution of food sources of protein (goat milk or goat milk yogurt) is effective as an adjuvant treatment in the treatment of malaria. Giving goat milk or goat milk yogurt with DHP drugs is more effective in reducing the parasitemia index. Group X4 was more effective in reducing the parasitemia index than group X2. The group with DHP and goat milk yogurt was more effective in reducing the parasitemia index than the group with DHP and goat milk. The administration of DHP drugs is recommended to be consumed together with milk or fatty foods, (WHO,2015) but previous studies have shown that the administration of DHP drugs together with 200ml of milk (containing 6.4 grams of fat) does not improve pharmacokinetic parameters (including absorption of DHP drugs) when compared with the control group (Annerberg A,2011). Giving goat milk with DHP drugs might inhibit the bioavailability of the drug. This is because goat milk contains higher calcium and magnesium than cow's milk (Park, 2010). Calcium and magnesium have divalent ionic bonds which will cause inhibition of drug absorption (Bushra R, 2011). On the other hand, administration of DHP with goat milk yogurt can reduce the parasitemia index. This shows that goat milk yogurt is more effective in reducing the parasitemia index than goat milk, so that goat milk yogurt is more effective if it becomes a malaria adjuvant therapy than goat milk. The decrease in parasitemia index is due to the content of lactic acid bacteria in goat milk yogurt. In accordance with previous studies (Villarinoa et.al, 2016) that intestinal microbiota can influence the pathogenesis of malaria. The difference in the intestinal bacterial community affects the parasitic load and death after Plasmodium infection. Provision of yogurt can modulate intestinal microbiota so that it can reduce the burden of parasites(Villarinoa et.al, 2016).

Yogurt undergoes a fermentation process, the process will release bioactive peptides from the main protein of milk. The fermentation process will produce antioxidant peptides consisting of 5-11 hydrophobic amino acids. Hydrophobic amino acids include proline, histidine, tyrosine or tryptophan. These amino acids will prevent the formation of free radicals and inhibit the process of lipid peroxidation. Lactic acid bacteria have antioxidant activity and are able to

reduce the accumulation of ROS during the process of digestion of food and have the ability to degrade superoxide anions and hydrogen peroxide (Gjorgievski N, 2015).

Giving goat milk yogurt can reduce the parasitemia index. One potential mechanism of intestinal microbiota in influencing the severity of malaria is by giving a direct effect on the parasite itself, where the products of intestinal microbiota inhibit the growth of parasites. Another more potential mechanism is that intestinal microbiota impacts malaria severity by modulating the host immune response to Plasmodium. Previous research showed that intestinal microbiota will give signals to monocytes / macrophages to prepare these cells to respond and help control infections (Denny, 2018).

An important mechanism of yogurt during Plasmodium infection through the ability of intestinal microbiota to express the glycemic molecule in the form of α-gal (Gal1 α1-3Galβ1-4GlnAc-R). The α-gal antibody binds to the surface of the sporozoite, induces lysis order and prevents the spread of Plasmodium to liver cells. The provision of yogurt can trigger an α-gal response to inhibit the transmission of Plasmodium sporozoite (Burgess SL,2017). Decreased parasitemia index after malaria inoculation indicate that diet (intake) plays an important role. Diet has a major role in shaping the composition and activity of intestinal microbiota. In accordance with previous studies that the species Lactobacillus and Bifidobacterium have a protective role by modulating the burden of parasites and reducing the severity of malaria so that the administration of yogurt containing Lactobacillus bacteria can cause a decrease in parasitemia. Differences in intestinal microbiota can prevent malaria severity and accelerate the healing process of malaria. These results also support the possibility that conditioning the intestinal microbiota by giving goat milk yogurt cannot prevent malaria, but has the potential to control the severity of malaria in humans (Shasteen MA, 2015).

10. Conclusion

The addition of goat milk yogurt is more effective in lowering the parasitemia index than goat milk. Giving goat milk yogurt with a dose of 0.5 ml / 20gBw can be considered an additional therapy in the treatment of malaria.

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Conflict of Interest - None.

Acknowledgments

This research funding by agency of health human resources development and empowerment as a part of Ministry of Health Republic Indonesia. Public health office Jayapura City contributed DHP drug.

References

Afiarahma AI, Witjahyo RBB. (2015). Pengaruh Pemberian Susu KAmbing Terhadap Gambaran Mikroskopis Paru dan Kadar Hemoglobin (Hb) Tikus. *Media Med Muda*.4(4):282-292.

Alyaqoubi S, Abdullah A, Addai ZR. (2014). Antioxidant activity of goat milk from three different locations in Malaysia. *AIP Conf Proc* ; 198-201. doi:10.1063/1.4895195

Alyaqoubi S, Abdullah A, Samudi M, Abdullah N, Radhi Z, Al-ghazali M.(2014). Effect of Different Factors on Goat Milk Antioxidant Activity Ministry of Regional Municipalities and Water Resource. *Int J Chem Tech Res*. 6(5) : 974-4290. http://sphinxsai.com/2014/vol6pt5/9/(3191-3196)S-2014.pdf.

Alyaqoubi S, Abdullah A, Samudi M, Abdullah N, Addai ZR, Al-ghazali M. (2015). Physicochemical properties and antioxidant activity of milk samples collected from five goat breeds in malaysia. *Adv J Food Sci Technol*.7(4):235-241. doi:10.19026/ajfst.7.1301

Annerberg A, Lwin KM, Lindegardh N, Khrutsawadchai S, Ashley E, Day NP, et al. (2011). Small Amount of Fat Does Not Affect Piperaquine Exposure in Patients with Malaria. Antimicrobial Agents And Chemotherapy. *American Society for Microbiology*.(55).9.3971–3976 doi:10.1128/AAC.00279-11

Anggraeni RH, Legowo AM, Al-Baarri AN. (2013). Intensitas Warna Dan Aroma Pada Susu Skim Kambing Akibat Proses Glikasi Dengan Penambahan Gula D-Fruktosa, L-Fruktosa, Dan D-Tagatosa. *J Aplikasi Teknologi Pangan*. 2(3):156-158.

Aristya AL, Legowo AM, Al-baarri AN. (2013). Karakteristik fisik, kimia, dan mikrobiologis kefir susu kambing dengan penambahan jenis dan konsentrasi gula yang berbeda. *J Aplikasi Teknologi Pangan*. 2(3):139-143.

Banjare K, Kumar M, Kumar R, Kartikyen S. (2017). Perspective role of goat milk and products : A review. *Inter J of Chem Studies*. 5(4):1328-1338.

Burgess SL, Gilchrist CA, Lynn TC, Petri WA. (2017). Parasitic Protozoa and Interactions with the Host Intestinal Microbiota. *Infect Immun* 85. https://doi.org/10 .1128 / IAI.00101-17.

Bushra R, Aslam N, Khan YA. (2011).Food-Drug Interactions. *Oman Medical Journal*. 26(2): 77-83

Denny, Joshua E. (2018). "Characterizing the gut microbiota during plasmodium infection and antimalarial treatment". *Electronic Theses and Dissertations*. https://doi.org/10.18297/etd/3109

Douglas NM, Ansley NM, Angus BJ, Nosten F, Price RN. (2010). Artemisinin combination therapy for vivax malaria?. *Lancet Infect Dis.* 10(6): 405–416. doi:10.1016/S1473-3099(10)70079-7

El-Dein NA, El-Deen NMA, Tolba MS, El-Shatoury HE, Awad AG, Ibrahim KM, et al. (2017). Probiotic Properties and Bile Salt Hydrolase Activity of Some Isolated Lactic Acid Bacteria. Egypt. *J. Micro*. (52)

Fabbri C, De-Cássia MNR, Lalwani P. (2013). Lipid peroxidation and antioxidant enzymes activity in Plasmodium vivax malaria patients evolving with cholestatic jaundice. *Malar J.* 12(1). doi:10.1186/1475-2875-12-315

Fardet A, Rock E.(2017). In vitro and in vivo antioxidant potential of milks, yoghurts, fermented milks and cheeses: a narrative review of evidence. *Nutr Res Rev*..1-19. doi:10.1017/S0954422417000191

Gahruie, Hashemi H, Eskandari, Hadi M, Mesbahi G, Hanifpour, Amin M. (2015). Scientific and

Technical Aspects of Yogurt Fortification: A Review. *Food Sci Hum Wellness*.(4):1-8. doi:http://dx.doi.org/10.1016/j.fshw.2015.03.002

Gjorgievski N, Ansley NM, Angus BJ, Nosten F, Price RN.(2015). Determination Of The Antioxidant Activity In Yogurt. *Journal of Hygienic Engineering and Design*.

Gomes QBA, Da-Silva LFD, Gomes QAR.(2015). N-acetyl cysteine and mushroom Agaricus sylvaticus supplementation decreased parasitaemia and pulmonary oxidative stress in a mice model of malaria. *Malar J*.14(1):1-12. doi:10.1186/s12936-015-0717-0

Holvik S Ltd. (2013).Introduction Macronutrient Nutritional Composition of Goat Milk. *Happy Days Dairies*:1-12.

Isah MB, Ibrahim MA. (2014). The role of antioxidants treatment on the pathogenesis of malarial infections: A review. *Parasitol Res*. 113(3):801-809. doi:10.1007/s00436-014-3804-1

Khalid M, Alam R, Khan S, Prakash V. (2013). Oxidative Stress Marker and Antioxidant Status In Falciparum Malaria In Relation To The Intensity Of Parasitaemia. *Int J. Biol Med Res*.3(1):3469-3471.

Muniandy P, Shori AB, Baba AS. (2016). Influence of green, white and black tea addition on the antioxidant activity of probiotic yogurt during refrigerated storage. *Food Packag Shelf Life*.8:1-8. doi:10.1016/j.fpsl.2016.02.002

Nakagawa H, Miyazaki T. (2017). Beneficial effects of antioxidative lactic acid bacteria. *AIMS Microbiol*. 3(1):1-7. doi:10.3934/microbiol.2017.1.1

Nguyen L, Hwang ES. (2016). Quality characteristics and antioxidant activity of yogurt supplemented with aronia (Aronia melanocarpa) juice. *Prev Nutr Food Sci*. 21(4):330-337. doi:10.3746/pnf.2016.21.4.330

Okpe O, Habila N, Ikwebe J, Upev VA, Okoduwa SIR, Isaac OT.(2016). Antimalarial Potential of Carica papaya and Vernonia amygdalina in Mice Infected with Plasmodium berghei. *J Trop Med*:6. doi:10.1155/2016/8738972

Padaga MC, Erika A, Haskito P, Irawan M.(2018). Efek Antioksidatif Kasein Yogurt Susu Kambing Terhadap Pencegahan Reprotoksik Pada Hewan Model Antioxidative Effect of Casey Goat Milk Yogurt against Reprotoxic Prevention in Animal Model Rattus norvegicus Display 2, 3, 7, 8-Tetrachlorinedibenzo-P- Dio. J*urnal Ilmu dan Teknologi Hasil Ternak*.13(2):72-80.

Park, Young. (2010). Goat Milk : Composition, Characteristics. Encyclopedia of Animal Science.

Park, Y.W. (2010). Goat Milk: Composition, Characteristics. Encyclopedia of Animal Science. W.G. Pond and N. Bell. 2nd ed. Taylor and Francis. CRC Press. Boca Raton, FL. In Press

Percário S, Moreira DR, Gomes BAQ, Ferreira MES.(2012) Oxidative Stress in Malaria Review. *Int J Mol Sci*. 13:16346-16372. doi:10.3390/ijms131216346

Polak, M., A. Berecka, D. Wasko, Szwajgier & A. Choma.(2013). Bifidogenic and antioxidant activity of exopolysaccharides produced by Lactobacillus rhamnosus E/N cultivated on different carbon sources. *Journal of Microbiology*. 62(2): 181–189

Pusat Data dan Informasi Kementerian Kesehatan Republik Indonesia. (2016). InfoDatin-Malaria-2016.pdf.

Sabeena FKH, Baron CP, Nielsen NS and Jacobsen C. (2010). Antioxidant activity of yoghurt peptides: Part 1-in vitro assays and evaluation in ω-3 enriched milk. *Food Chemistry.123(4),1081–1089*.doi:10.1016/j.foodchem.2010.05.067

Shasteen MA, Lebeis WS, Schmidt N. (2015). "Lessening Malaria Parasite Burden with Yogurt". MicrobiologyPublications and Other Works.http://trace.tennessee.edu/utk_micrpubs/74

Subdit Malaria Direktorat KR.(2018) *Buku Saku Tatalaksana Malaria 2018*:Kemenkes RI

Sucipto CD. *Manual Lengkap Malaria*. Yogyakarta: Gosyen Publishing; 2015

Tyagi AG, Tyagi RA, Choudhary PR, Shekhawat JS. (2017). Study of antioxidant status in malaria patients. *Int J Res Med Sci*. 5(4):1649-1654. doi:10.18203/2320-6012.ijrms20171281

Villarinoa FN, Lecleir RG, Denny EJ, Dearth PS, Harding LC, Sloan SS, et.al. (2016). Composition of the gut microbiota modulates the severity of malaria. *PNAS*. 113(8): 2235–2240 www. pnas. org/cgi/doi/10. 1073/pnas. 1504887113

WHO.(2015). Guidelines for the treatment of malaria, 3rd edition.

Yangilar F.(2013). As a Potentially Functional Food: Goats' Milk and Products. *J Food Nutr Res*. 1(4):68-81. doi:10.12691/jfnr-1-4-6

Zapata CR, Singh A, Pezeshki A, Nibber T, Chelikani KP. (2017).Whey Protein Components - Lactalbumin and Lactoferrin - Improve Energy Balance and Metabolism. *Scientific Reports*. (7):9917 doi: 10.1038/s41598-017-09781-2

Tables and Figures –

Table 2. Statistical Analysis of the Parasitemia Index

Treatment Group	Parasitemia index (%)	p^a	p^b						
			K-	K+	K+DHP	X1	X2	X3	X4
K-	0,00±0,00	0,00*	-	0,005*	0,005*	0,005*	0,005*	0,005*	0,005*
K+	13,9±0,58		-	-	0,009*	0,009*	0,009*	0,009*	0,009*
K+ DHP	0,60±0,27		-	-	-	0,009*	0,009*	0,009*	0,289
X1	4,68±0,53		-	-	-	-	0,059	0,602	0,008*
X2	3,74±0,74		-	-	-	-	-	0,834	0,005*
X3	3,60±1,80		-	-	-	-	-	-	0,008*
X4	0,70(0,70-1,20)		-	-	-	-	-	-	-

Information :
a = Kruskall Wallis
b = Post Hoc Mann-Whitney
* = p value <0.005 (significantly)
K^- = Control Group Negative, Healthy
K^+ = Control Group Positive, Infected Malaria
K^+ DHP = Control Group, Infected Malaria and DHP Drug Administration
X1 = Treatment Group, Goat milk- Infected Malaria- Goat milk
X2 = Treatment Group, Goat milk - Infected Malaria- Goat milk and DHP
X3 = Treatment Group, Goat milk Yogurt- Infected Malaria- Goat milk Yogurt
X4 = Treatment Group, Goat milk Yogurt- Infected Malaria- Goat milk Yogurt and DHP

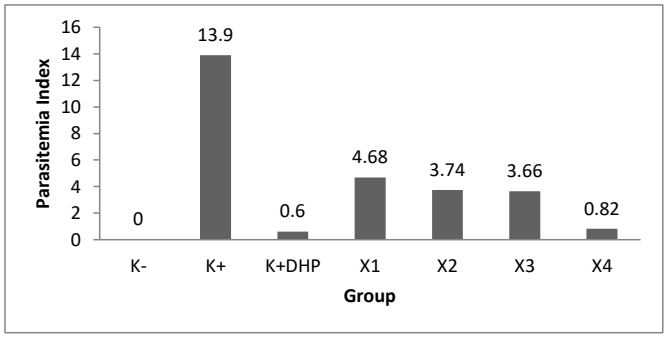


Figure 1. Graph of Average Parasitemia Index

Information :

K^- = Control Group Negative, Healthy

K^+ = Control Group Positive, Infected Malaria

K^+ DHP = Control Group, Infected Malaria and DHP Drug Administration

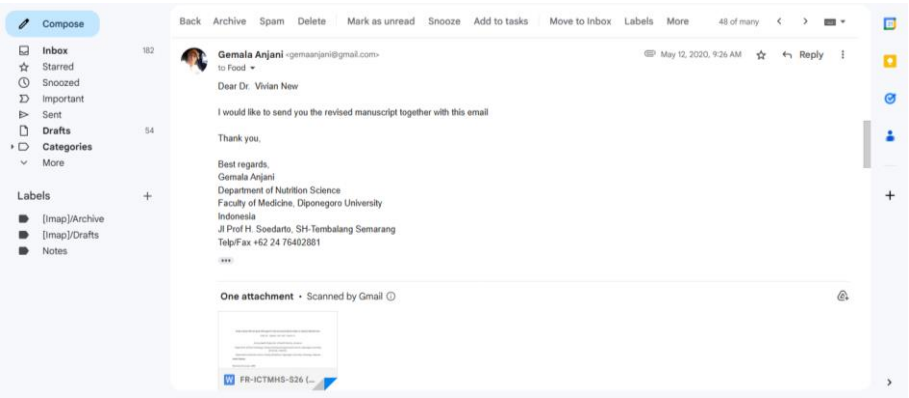
X1 = Treatment Group, Goat milk- Infected Malaria- Goat milk

X2 = Treatment Group, Goat milk - Infected Malaria- Goat milk and DHP

X3 = Treatment Group, Goat milk Yogurt- Infected Malaria- Goat milk Yogurt

X4 = Treatment Group, Goat milk Yogurt- Infected Malaria- Goat milk Yogurt and DHP

Hasil Perbaikan Artikel



Study of goat milk and goat milk yogurt to decrease parasitemia index on malaria-infected mice

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Abstract

Malaria is an infectious disease caused by the *Plasmodium* sp. parasite. In 2017, malaria has reached 219 million cases. The decline of parasitemia index indicates a lower degree of infection in malaria patients. Goat milk and goat milk yogurt as immunomodulators have the potential to reduce parasitemia index. This study was aimed to determine the effect of goat milk and goat milk yogurt on the index of parasitemia in malaria-infected mice. This research was a true experimental study with a post-test only group design. A total of thirty-five female Balb/c mice was divided into the following groups: a (negative control group), b (malaria), c (malaria, Dihydroartemisinin/piperaquine (DHP) drug), d (malaria, goat milk), e (malaria, goat milk, DHP), f (malaria, goat milk yogurt), and g (malaria, goat milk yogurt, DHP). Inoculation dose of *Plasmodium* was 10^7/0.2 mL. The intervention was studied for 24 days. Parasitemia index data was collected on the seventh day post-inoculation. The dose of goat milk and goat milk yogurt given was 0.5 mL/20 g BW. Data were analyzed using Kruskal-Wallis with Mann-Whitney post-hoc test. The results showed a significant decrease in the parasitemia index ($p<0.05$). The mean parasitemia index in each group were: 0% (a); 13.9% (b); 0.60% (c); 4.68% (d); 3.74% (e); 3.66% (f); 0.82% (g). The group that exhibited effective reduction of parasitemia index were group c and g. Goat milk yogurt (f) was more effective in decreasing the parasitemia index than goat milk (d). Giving goat milk yogurt can be considered an additional therapy for the treatment of malaria.

Keywords: Malaria, Parasitemia index, Goat milk, Goat milk yogurt

11. Introduction

Malaria is a disease caused by a protozoan parasite from the genus *Plasmodium* (Percário *et al*., 2012; Khalid *et al*., 2013; Isah and Ibrahim 2014; Okpe *et al*., 2016). Malaria is transmitted by the bite of a female Anopheles mosquito that carries the *Plasmodium* (Mota et al., 2019; Mala

et al., 2016). The type of *Plasmodium* that is responsible for most cases of malaria in tropical and subtropical regions is *Plasmodium falciparum* (Emmanuel *et al*., 2016). *Plasmodium* infection from malaria will cause oxidative changes and an imbalance of antioxidant mechanisms. (Khalid *et al*., 2013; Fabbri *et al*., 2013; Gomes *et al*., 2015). Malaria infection increases parasitemia index, an indicator of the severity of infection in malaria patients (Tyagi *et al*., 2017). The use of antioxidants is expected to help reduce oxidative damage and prevent further development of malaria (Gomes *et al*., 2015).

Goat milk is known for its positive effects on the biological functions of the human body due to its nutritional content. Goat milk also has low allergen levels and is easily digested by the body (Yangilar, 2013; Aristya *et al*., 2013; Banjare *et al*., 2017). Furthermore, it contains natural antioxidant-forming agents which can prevent the lysis of erythrocyte cells (Alyaqoubi *et al*., 2015). Protein in goat milk is the main source of active biopeptides that can act as antioxidants (Park, 2010: Alyaqoubi *et al*., 2014; Alyaqoubi *et al*., 2014). In addition, it demonstrates anti-inflammatory properties and serves as an important source of angiotensin-converting enzyme (ACE) which functions as an antihypertensive peptide and helps alleviate infections caused by pathogenic microbes (Lad *et al*., 2017).

Goat milk can be processed and fermented into yogurt. Yogurt contains bioactive peptides and has antioxidant activity (Gahruie *et al*., 2015; Nguyen and Hwang, 2016). The antioxidant activity of goat milk yogurt is known to be higher than cow milk yogurt (Holvik, 2013; Muniandy *et al*., 2016). Increased antioxidant properties in goat milk yogurt results from the activity of lactic acid bacteria (LAB). Several studies have reported the effects of LAB in response to oxidative stress (Fardet and Rock, 2017; Shu *et al*., 2018; Moreno-Fernandez *et al*., 2019). The potential health benefits of LAB includes stimulating the immune system, maintaining the balance of the intestinal flora, reducing cholesterol levels, and promoting anti-aging and antioxidant activity (Nakagawa and Miyazaki, 2016). LAB produces exopolysaccharides (EPS) which specifically has immunostimulant activity and increases the digestive tract colonization (Polak *et al*., 2013). LAB hydrolyzes casein into a bioactive peptide with various biological functions. Based on previous researches, casein in goat milk yogurt is capable of preventing the increase of Malondialdehyde (MDA) levels (Mahdi *et al*., 2018). This study is aimed to determine the effects of consuming goat milk and goat milk yogurt on the parasitemia index of mice infected with malaria. This research is expected to be useful in the development of food and health science in relation to the role of antioxidants in the treatment of malaria.

12. Materials and methods

2.1 Inoculation Plasmodium berghei ANKA (PbA)

This research used female Balb/c mice that have been inoculated with *Plasmodium berghei* ANKA (PbA). Three donor mice were obtained from the parasitology laboratory of the Faculty of Medicine, Nursing and Public Health, Gadjah Mada University (FKKMK UGM). Each mouse was inoculated with 10^7 mL of PbA parasite that was given at 0.2 mL intraperitoneally (Tafor *et al*., 2013)

2.2 Animal and treatments

The research design used in this research is true experimental with random post-test controlled group. Infected mice was divided into 7 groups: 3 control groups and 4 treatment groups. Group A (negative control group fed with normal food); group B (positive control group fed with normal food, inoculated with PbA and not receiving treatment); group C

(positive control group with normal food, inoculated with PbA and given the anti-malaria therapy (DHP)); group D (treatment group 1, inoculated with PbA and given goat milk); group E (treatment group 2, inoculated with PbA, given the anti-malaria therapy (DHP) and goat milk); group F (treatment group 3, inoculated with PbA and given goat milk yogurt); group G (treatment group 4, inoculated with PbA, given the anti-malaria therapy (DHP) and goat milk yogurt).

Parasitemia index data was collected at the 7th day post-inoculation. The goat milk and goat milk yogurt dose are 0,5 ml/20 g BW. The DHP dose used the combination of 3 mg/kg BW/days of Dihydroartemisinin and 24 mg/kg BW/day of Piperaquinine (Ariani *et al*., 2018).

Yogurt production as well as analysis of protein content and antioxidant activity of goat milk and goat milk product was done at the Integrated Laboratory of Diponegoro University, Semarang. Animal experiments including malaria inoculation and parasitemia index measurement were performed in Integrated Biomedic Laboratory (IBL) Medical Faculty of Sultan Agung Moeslem University Semarang (FK-UNISSULA). Goat milk was obtained from Kuncen Farmer Group at Bubakan Village, Mijen Semarang.

2.3 Goat Milk Yogurt Preparation

Before the yogurt making process, subculture starter was made to activate the Lactic Acid Bacteria (LAB) in commercial starter (manufactured yogurt). The manufactured yogurt contained *Streptococcus thermophiles* culture, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus,* and *Bifidobacterium*.

16 grams of skim milk was dissolved in 100 ml goat milk and pasteurized at 75°C for 15 seconds. Next, skim milk was mixed with commercial starter (manufactured yogurt) at the ratio of 1:1 and incubated at 40°C for 24 hours.

Goat milk yogurt production used 500 ml fresh goat milk. Goat milk was pasteurized at 75°C for 15 seconds and later cooled down until 43°C. Afterwards, the mixture was incubated with bacteria starter made from 5% fresh goat milk and incubated at 42°C for 6 hours before it was cooled in room temperature. Prepared yogurt was packed and kept in the refrigerator.

2.4 Goat milk and goat milk yogurt intervention

Goat milk and goat milk yogurt was given orally using feeding tube (sonde). The dose was determined based on the maximum capacity of the mice stomach which was 1 mL/20 g BW (Ngatidjan, 2006). Ideal amount is 0.25-0.5 ml and the given amount in this research was 0.5 ml/20 g BW/day. The goat milk and goat milk yogurt were given for 24 days, consisting of 21 days pre-inoculation and 3 days post-inoculation.

2.5 Parasitemia index examination

Object glass was thoroughly cleaned and coded in the top right corner. Blood sample was dropped on the object glass to make a smear that was repetitively washed and dried before applied with Giemsa stain. Samples were examined under the microscope with a 10x10 visual field enlargement, then 10x100 enlargement with immerse oil. Parasite erythrocytes were calculated and stated in percentage and compared with the studied blood (Torres *et al*., 2018).

2.6 Statistical analysis

The results were described as median±SD (for normally distributed data) or median (min-max) if otherwise. The statistical difference was analyzed using Kruskall Wallis and Mann Whitney Post-hoc test.

2.7 Ethical clearance

This research was approved by the Health Research Ethics Commission of the Sultan Agung University School of Medicine with No. 196/III/2019/Bio-commission.

13. Results

Twenty-eight mice were obtained from the Biology Laboratory of Semarang State University with 25 for treatment and 3 as donor mice. The results of normality test of parasitemia index using the Saphiro-Wilk test obtained p-value <0.005 (p = 0.00), indicating that the parasitemia index data was not normally distributed thus the Kruskal-Wallis test was performed. Based on Table 1, there is a statistically significant difference in the parasitemia index (p <0.05). Next, Post Hoc tests were carried out with Mann-Whitney and the results are as seen in Table 1. Treatment by giving goat milk and goat milk yogurt or a combination of both with DHP drugs has been proven to positively affect the parasitemia index.

Based on Table 1, the groups with no significant difference on the parasitemia index were the c and g groups with a value of p = 0.289. In the d and e groups, there was also no significant difference in the parasitemia index with a value of p = 0.059. Similarly, group d and group f also showed no significant difference with a value of p = 0.602. These results indicate that the two groups did not have different effectiveness in suppressing the parasitemia index. The ability of each group to suppress the parasitemia index can be seen based on the average parasitemia index in Figure 1.

14. Discussion

Malaria is one of the endemic diseases in Indonesia which is still a major health concern. Malaria is usually treated with antimalarial drugs. Standard treatment for malaria uses Artemisinin-based combination therapies (ACTs). These therapies can combine artemisinin or one of its derivatives with another antimalarial drug. One of the combinations is Dihidroartemisinin Piperaquin (DHP). Papua and West Papua are regions in Indonesia that are still endemic for malaria (Subdit Malaria Direktorat KR, 2018). When malaria cases occur, not all patients have immediate access to health facilities and DHP medication as early as possible. Utilization of functional food is considered to be an additional therapy for malaria patients. One of the functional foods that can be developed is goat milk and goat milk yogurt (Yangilar, 2013).

This research aimed to determine the effect of goat milk and goat milk yogurt in decreasing parasitemia index. The results of this research can be seen in Figure 1. Figure 1 shows that the group with the lowest parasitemia index is group a (negative control group, healthy) with 0% because they were not inoculated with malaria. Group b (positive control group, malaria-infected) had the highest parasitemia index among all groups (13.9%) as they were inoculated with malaria and not given any treatment thus the *Plasmodium* continued to multiply and the parasitemia index increased. Group c (malaria-infected, DHP drug administration) (0.6%) was found to have the lowest mean of parasitemia index compared with other groups, further proving the effectiveness of the use of DHP drugs. DHP drugs include an artemisinin group that is known to be effective in killing *Plasmodium* quickly in all life stages including gametocytes (Douglas *et al*., 2010).

The mean parasitemia index values in groups d (malaria-infected, given goat milk) and f (malaria-infected, given goat milk yogurt) were lower compared with group b, suggesting that

goat milk yogurt may play a role in lowering parasitemia index. Goat milk and goat milk yogurt both contain casein and whey which has been shown to possess antioxidant activity (Sabeena *et al*., 2010). Casein can work as radical scavengers and cation chelators which can inhibit lipid oxidation. In goat milk yogurt, the components of whey protein consisting of lactoferrin, beta-lactoglobulin, alpha-lactalbumin, glyomropropeptides, and immunoglobulins can boost the immune system. Lactoferrin binds the iron in the intestinal mucosa and acts as a bacteriostatic agent by suppressing populations of harmful bacteria and modulating the body's immunity. Lactoferrin also repairs cell damage by inhibiting the production of ROS and works with vitamin E in limiting membrane lipid oxidation by ROS. β-Lactoglobulin plays a role in the transfer of pro-vitamin A, while α-Lactalbumin is involved in lactose synthesis. Immunoglobulin is important for an antigen-antibody reaction. This protein component can enhance the immune system. The major protein boosts the immune system by converting the cysteine intracellular amino acids into glutathione that acts as an intracellular antioxidant. Lactoferrin provided in goat milk and goat milk yogurt can stimulate the immune system through the activation of T and B lymphocyte cells (Queiroz *et al*., 2013; Zapata *et al*., 2017; Mahdi *et al*., 2018).

Group f (malaria-infected, given goat milk yogurt) was more effective in reducing the parasitemia index than group d (malaria-infected, given goat milk). Yogurt generally contains lactic acid bacteria that have Bile Salt Hydrolase (BSH) enzyme. BSH is known to be able to de-conjugate bile salts into free bile salts that are less absorbable by the small intestine, leading to reduced amount of bile salts returning to the liver. Consequently, cholesterol would be used as a precursor to balance the amount of bile salt so overall cholesterol levels would decrease. Lower cholesterol levels meant less amount of lipid exposed to free radicals, resulting in decreased lipid peroxidation and eventually a decline in MDA levels and parasitemia index (El-Dein *et al*., 2017).

The mean parasitemia index of group e (malaria-infected, given the anti-malaria therapy and goat milk) and g (malaria-infected, given the anti-malaria therapy and goat milk yogurt) were higher than group b. This finding supports the contribution of food sources of protein (goat milk or goat milk yogurt) as an effective adjuvant treatment in the treatment of malaria. Giving goat milk or goat milk yogurt alongside DHP drugs was found to be more effective in reducing the parasitemia index. Interestingly, the mice receiving DHP and goat milk yogurt experienced a more significant reduction of parasitemia index than the group receiving DHP and goat milk. The administration of DHP drugs is recommended to be consumed together with milk or fatty foods (WHO, 2015) but previous studies have shown that the administration of DHP drugs together with 200mL of milk (containing 6.4 grams of fat) did not improve pharmacokinetic parameters (including absorption of DHP drugs) when compared with the control group (Annerberg *et al*., 2011). Giving goat milk with DHP drugs might inhibit the bioavailability of the drug because goat milk contains higher calcium and magnesium than cow milk (Park, 2010). Calcium and magnesium have divalent ionic bonds which may inhibit drug absorption (Bushra *et al*., 2011). On the other hand, administration of DHP with goat milk yogurt can decrease the parasitemia index better than goat milk, making it a more favorable option for malaria adjuvant therapy. The decrease in parasitemia index is due to the content of lactic acid bacteria in goat milk yogurt which may affect intestinal microbiota profile. In accordance with previous studies, intestinal microbiota influenced the pathogenesis of malaria and altering the intestinal bacterial community affected the parasitic load and death risk after *Plasmodium* infection. Providing yogurt helped modulate the intestinal microbiota so that it can reduce the burden of parasites (Villarino *et al*., 2016).

Yogurt is made by fermentation that causes the release of bioactive peptides from the major protein of milk. The fermentation process produced antioxidant peptides consisting of 5-11 hydrophobic amino acids which include proline, histidine, tyrosine or tryptophan. These amino acids prevent the formation of free radicals and inhibit the process of lipid peroxidation.

Lactic acid bacteria exhibit antioxidant activity through suppressing the accumulation of ROS during the food digestion process and destroying superoxide anions and hydrogen peroxide (Gjorgievski, 2015).

Giving goat milk yogurt was proven to help reduce the parasitemia index in our study. One potential mechanism to explain this finding is that intestinal microbiota influenced the severity of malaria by giving a direct effect on the parasite itself, where the products of intestinal microbiota inhibit the growth of parasites. Another potential mechanism is that intestinal microbiota may have modulated the host immune response to *Plasmodium*. Previous researches showed that intestinal microbiota may send signals for monocytes/macrophages to respond to and control the infection (Denny, 2018).

An important mechanism of yogurt during *Plasmodium* infection is through the ability of intestinal microbiota to express the glycemic molecule in the form of α-gal (Gal1 α1-3Galβ1-4GlnAc-R). The α-gal antibody binds to the surface of the sporozoite, induces lysis order and prevents the spread of *Plasmodium* to liver cells. The administration of yogurt can trigger an α-gal response to inhibit the transmission of *Plasmodium* sporozoite (Burgess *et al*., 2017). The decreased parasitemia index after malaria inoculation implied that diet (intake) played an important role in shaping the composition and activity of intestinal microbiota. Based on previous studies, *Lactobacillus* and *Bifidobacterium* have a protective role by modulating the burden of parasites and reducing the severity of malaria. Therefore, the administration of yogurt containing *Lactobacillus* bacteria can cause a decrease in parasitemia. Changes in intestinal microbiota can prevent malaria severity and accelerate the healing process of malaria. These results also support the possibility that altering the intestinal microbiota profile by giving goat milk yogurt cannot prevent malaria but has the potential to control the severity of malaria in humans (Shasteen *et al*., 2015).

15. Conclusion

The addition of yogurt and DHP resulted in higher parasitemia index in malaria-infected mice in comparison with DHP-only treatment, but the mechanism is unknown. The addition of goat milk yogurt is more effective in lowering the parasitemia index than goat milk only. The administration of goat milk yogurt at a dose of 0.5 mL / 20gBW can be considered an additional therapy in the treatment of malaria.

Conflict of Interest

The authors declared that they have no competing interests.

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References

Alyaqoubi, S., Abdullah, A. and Addai, Z.R. (2014). Antioxidant activity of goat milk from three different

locations in Malaysia. *AIP Conference Proceedings,* 198-201. doi:10.1063/1.4895195

Alyaqoubi, S., Abdullah, A., Samudi, M., Abdullah, N., Radhi, Z. and Al-ghazali, M. (2014). Effect of Different Factors on Goat milk Antioxidant Activity Ministry of Regional Municipalities and Water Resource. *International Journal of Chem Tech Research,* 6(5), 974-4290. http://sphinxsai.com/2014/vol6pt5/9/(3191-3196)S-2014.pdf.

Alyaqoubi, S., Abdullah, A., Samudi, M., Abdullah, N., Addai, Z. R. and Al-ghazali, M. (2015). Physicochemical properties and antioxidant activity of milk samples collected from five goat breeds in Malaysia. *Advance Journal of Food Science and Technology,* 7(4), 235-241. doi:10.19026/ajfst.7.1301.

Annerberg, A., Lwin, K. M., Lindegardh, N., Khrutsawadchai, S., Ashley, E., Day, N. P., Singhasivanon, P., Tarning, J., White, N.J. and Nosten, F. (2011). Small Amount of Fat Does Not Affect Piperaquine Exposure in Patients with Malaria. Antimicrobial Agents And Chemotherapy. *American Society for Microbiology,* (55), 3971–3976. doi:10.1128/AAC.00279-11.

Ariani, Anjani, G., Achasan, M. A. S. and Djamiatun, K. (2018). Tepung ulat sagu (*Rhyinchophorus ferrugineus*) imunomodulator Nitric Oxide (NO) sirkulasi mencit terapi antimalaria standar. *Jurnal Gizi Indonesia*, 6(2), 131-138.

Aristya, A. L., Legowo, A. M. and Al-baarri, A. N. (2013). Karakteristik fisik, kimia, dan mikrobiologis kefir susu kambing dengan penambahan jenis dan konsentrasi gula yang berbeda. *Jurnal Aplikasi Teknologi Pangan,* 2(3), 139-143.

Banjare, K., Kumar, M., Kumar, R. and Kartikyen, S. (2017). Perspective role of goat milk and products : A review. *International Journal of Chemical Studies,* 5(4), 1328-1338.

Burgess, S. L., Gilchrist, C. A., Lynn, T. C. and Petri, W. A. (2017). Parasitic Protozoa and Interactions with the Host Intestinal Microbiota. *Infection and Immunity,* 85, 1-12. https://doi.org/10.1128/IAI.00101-17.

Bushra, R., Aslam, N. and Khan, Y. A. (2011). Food-Drug Interactions. *Oman Medical Journal,* 26(2), 77-83.

Denny, J.E. (2018). Characterizing the gut microbiota during plasmodium infection and antimalarial treatment. *Electronic Theses and Dissertations*. 3109. https://doi.org/10.18297/etd/3109

Douglas, N. M., Ansley, N. M., Angus, B. J., Nosten, F. and Price, R. N. (2010). Artemisinin combination therapy for vivax malaria?. *Lancet Infectious Diseases,* 10(6), 405–416. doi:10.1016/S1473-3099(10)70079-7

El-Dein, N. A., El-Deen, N. M. A., Tolba, M. S., El-Shatoury, H. E., Awad, A. G., Ibrahim, K. M. and Mohamed, A. F. (2017). Probiotic Properties and Bile Salt Hydrolase Activity of Some Isolated Lactic Acid Bacteria. *Egyptian Journal of Microbiology,* (52), 87-100.

Emmanuel, A. N., Oliver, N. O. and Angela, U.N. (2016). *Using Plant Materials for Treatment of Malaria in Imo State, Nigeria*. *American Journal of Life Science Researches,* 4(2), 67-71. doi:10.20286/ajlsr-040287

Fabbri, C., De-Cássia, M. N. R. and Lalwani, P. (2013). Lipid peroxidation and antioxidant enzymes activity in Plasmodium vivax malaria patients evolving with cholestatic jaundice. *Malaria Journal,* 12(1), 315. doi:10.1186/1475-2875-12-315

Fardet, A. and Rock, E. (2017). In vitro and in vivo antioxidant potential of milks, yoghurts, fermented milks and cheeses: a narrative review of evidence. *Nutrition Research Reviews,* 31(1), 1-19. doi:10.1017/S0954422417000191

Gahruie, Hashem, H., Eskandari, Hadi, M., Mesbahi, G. and Hanifpour, A. M. (2015). Scientific and Technical Aspects of Yogurt Fortification: A Review. *Food Science and Human Wellness,* (4), 1-8. doi:http://dx.doi.org/10.1016/j.fshw.2015.03.002

Gjorgievski, N., Ansley, N. M., Angus, B. J., Nosten, F. and Price, R. N. (2015). Determination of The Antioxidant Activity In Yogurt. *Journal of Hygienic Engineering and Design,* 8, 88-92.

Gomes, Q. B. A., Da-Silva, L. F. D. and Gomes, Q. A. R. (2015). N-acetyl cysteine and mushroom *Agaricus sylvaticus* supplementation decreased parasitaemia and pulmonary oxidative stress in a mice model of malaria. *Malar Journal,* 14(1), 1-12. doi:10.1186/s12936-015-0717-0

Holvik, S. L. (2013). Introduction Macronutrient Nutritional Composition of Goat milk. *Happy Days Dairies,* 1-12.

Isah, M. B. and Ibrahim, M. A. (2014). The role of antioxidants treatment on the pathogenesis of malarial infections: A review. *Parasitology Research,* 113(3), 801-809. doi:10.1007/s00436-014-3804-1

Khalid, M., Alam, R., Khan, S. and Prakash, V. (2013). Oxidative Stress Marker and Antioxidant Status in Falciparum Malaria in Relation to the Intensity of Parasitaemia. *International Journal of Biological and Medical Research,* 3(1), 3469-3471.

Lad, S. S., Aparnathi, D. K., Mehta, B. and Velpula, S. (2017). Goat milk in Human Nutrition and Health – A Review. *International Journal of Current Microbiology and Applied Sciences,* 6(5), 1781-1792. https://doi.org/10.20546/ijcmas.2017.605.194

Mahdi, C., Untari, H., Padaga, M. C. and Raharjo, S. J. (2018). The characterization of bioactive peptide of goat milk fermented to activities as anti-hypercholesterolemia. *International Food Research Journal*, 25(1), 17-23

Mala, M., Imam, M. and Hassan, K. (2016). Interaction between parasite and vector for Malaria disease transmission-a review on Malaria. *Progressive Agriculture*, 27(2), 168-174. https://doi.org/10.3329/pa.v27i2.29327

Moreno-Fernandez, J., Alférez, M.J.M., López-Aliaga, I. and Javier, D. C. (2019). Protective effects of fermented goat milk on genomic stability, oxidative stress and inflammatory signalling in testis during anaemia recovery. *Science Report,* 9, 2232. https://doi.org/10.1038/s41598-018-37649-6

Mota, M. M. and Mello-Vierra, J. (2019). Parasitism: Anopheles Mosquitoes and Plasmodium Parasites Share Resources. *Current Biology*, 29(3), 632-634. doi:10.1016/j.cub.2019.05.030

Muniandy, P., Shori, A.B. and Baba, A. S. (2016). Influence of green, white and black tea addition on the antioxidant activity of probiotic yogurt during refrigerated storage. *Food Packag Shelf Life,* 8, 1-8. doi:10.1016/j.fpsl.2016.02.002

Nakagawa, H. and Miyazaki, T. (2017). Beneficial effects of antioxidative lactic acid bacteria. *AIMS Microbiology,* 3(1), 1-7. doi:10.3934/microbiol.2017.1.1

Ngatidjan. (2006). *Metode Laboratorium Dalam Toksikologi*. I. (Hakim L, ed.). Yogyakarta: Bagian Farmakologi & Toksikologi Fakultas Kedokteran Universitas Gadjah Mada.

Nguyen, L. and Hwang, E. S. (2016). Quality characteristics and antioxidant activity of yogurt supplemented with aronia (*Aronia melanocarpa*) juice. *Preventive Nutrition and Food Science,* 21(4), 330-337. doi:10.3746/pnf.2016.21.4.330

Okpe, O., Habila, N., Ikwebe, J., Upev, V. A., Okoduwa, S. I. R. and Isaac, O. T. (2016). Antimalarial Potential of *Carica papaya* and *Vernonia amygdalina* in Mice Infected with *Plasmodium berghei*. *Journal of Tropical Medicine, Volume 2016,* Article ID 8738972, 1-6. https://doi.org/10.1155/2016/8738972

Park, Y.W. (2010). Goat milk: Composition, Characteristics. Encyclopedia of Animal Science. W.G. Pond and N. Bell. 2nd ed. Taylor and Francis. CRC Press. Boca Raton, FL. In Press

Percário, S., Moreira, D. R., Gomes, B. A. Q. and Ferreira, M. E S. (2012). Oxidative Stress in Malaria Review. *International Journal of Molecular Sciences,* 13, 16346-16372. doi:10.3390/ijms131216346

Polak, M., A. Berecka, D., Wasko, S. and Choma, A. (2013). Bifidogenic and antioxidant activity of exopolysaccharides produced by *Lactobacillus rhamnosus* E/N cultivated on different carbon sources. *Journal of Microbiology,* 62(2), 181–189.

Queiroz, V. V. O., Assis, A. N. O. and Junior, H. C. R. (2013). Protective effect human lactoferrin in the gastrointestinal tract. *Revista Paulista de Pediatria*, 31(1), 90-95.

Sabeena, F. K. H., Baron, C. P., Nielsen, N. S. and Jacobsen, C. (2010). Antioxidant activity of yoghurt peptides: Part 1-in vitro assays and evaluation in ω-3 enriched milk. *Food Chemistry*, 123(4), 1081–1089. doi:10.1016/j.foodchem.2010.05.067

Shasteen, M. A., Lebeis, W. S. and Schmidt, N. (2015). Lessening Malaria Parasite Burden with Yogurt. Microbiology Publications and Other Works. http://trace.tennessee.edu/utk_micrpubs/74

Shu, G., Shi, X., Chen, L., Kou, J., Meng, J. and Chen, H. (2018). Antioxidant Peptides from Goat milk Fermented by *Lactobacillus casei* L61: Preparation, Optimization, and Stability Evaluation in Simulated Gastrointestinal Fluid. *Nutrients, 10*(6), 797**.** https://doi.org/10.3390/nu10060797

Subdit Malaria Direktorat KR. (2018). *Buku Saku Tatalaksana Malaria 2018* : Kemenkes RI.

Tafor, D., Djunaidi, A., Wasityastuti, W. and Sholikhah, N. E. (2013). Tumor Necrosis Factor-Alpha (TNF-Alpha) and Intercellular Adhesion Molecule-1 (ICAM-1) Expression of *Plasmodium berghei* Infected Swiss Mice Treated with Red Fruit (*Pandanus conoideus* Lam) Ethanol Extract. *Tropical Medicine Journal*, 3(2), 155-165.

Torres, K., Bachman, C.M., Delahunt, C.B., Jhonatan, A.B., Freddy, A., Dionicia, G. V., Stephane, P., Courosh, M., Shawn, K. McG., Clay, M. T., Travis, O., Liming, H., Mayoore, S.J., Victoria, M. H. and David, B. (2018). Automated microscopy for routine malaria diagnosis: a field comparison on Giemsa-stained blood films in Peru. *Malaria Journal,* 17**,** 339. https://doi.org/10.1186/s12936-018-2493-0

Tyagi, A. G., Tyagi, R. A., Choudhary, P. R. and Shekhawat, J. S. (2017). Study of antioxidant status in malaria patients. *International Journal of Research in Medical Sciences,* 5(4), 1649-1654. doi:10.18203/2320-6012.ijrms20171281

Villarino, F. N., Lecleir, R. G., Denny, E. J., Dearth, P. S., Harding, L. C., Sloan, S. S., Jennifer, L. G., Shawn, R. C., Steve,n W. W. and Nathan, W. S. (2016). Composition of the gut microbiota modulates the severity of malaria. *Proceedings of the National Academy of Sciences,* 113(8), 2235–2240 www.pnas. org/cgi/doi/10. 1073/pnas. 1504887113

WHO. (2015). Guidelines for the treatment of malaria, 3rd edition.

Yangilar, F. (2013). As a Potentially Functional Food: Goats' Milk and Products. *Journal of Food and Nutrition Research,* 1(4), 68-81. doi:10.12691/jfnr-1-4-6

Zapata, C. R., Singh, A., Pezeshki, A., Nibber, T. and Chelikani, K. P. (2017). Whey Protein Components - Lactalbumin and Lactoferrin - Improve Energy Balance and Metabolism. *Scientific Reports,* (7), 9917 doi: 10.1038/s41598-017-09781-2

Table 1. Statistical analysis of the parasitemia index

Treatment Group	Parasitemia index (%)	P^1	P^2						
			a	b	c	d	e	f	g
a	0,00±0,00	0.00*	-	0.005*	0.005*	0.005*	0.005*	0.005*	0.005*
b	13,9±0,58		-	-	0.009*	0.009*	0.009*	0.009*	0.009*
c	0,60±0,27		-	-	-	0.009*	0.009*	0.009*	0.289
d	4,68±0,53		-	-	-	-	0.059	0.602	0.008*
e	3,74±0,74		-	-	-	-	-	0.834	0.005*
f	3,60±1,80		-	-	-	-	-	-	0.008*
g	0,70(0,70-1,20)		-	-	-	-	-	-	-

[1] = Kruskall Wallis, [2] = Post Hoc Mann-Whitney,* = p value <0.005 (significantly)

a = Negative Control Group, Healthy; b = Positive Control Group, Malaria-infected; c = Control Group, Malaria-infected and DHP Drug Administration; d = Treatment Group, Goat milk - Malaria-infected - Goat milk; e = Treatment Group, Goat milk - Malaria-infected - Goat milk and DHP; f = Treatment Group, Goat milk Yogurt - Malaria-infected - Goat milk Yogurt; g = Treatment Group, Goat milk Yogurt - Malaria-infected - Goat milk Yogurt and DHP.

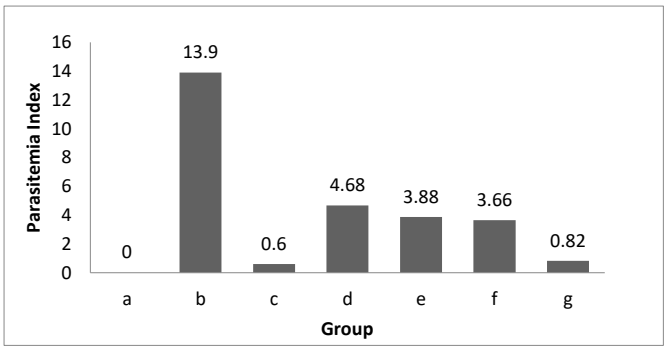

Figure 1. Average parasitemia index results. a = Negative Control Group, Healthy; b = Positive Control Group, Malaria-infected; c = Control Group, Malaria-infected and DHP Drug Administration; d = Treatment Group, Goat milk - Malaria-infected - Goat milk; e = Treatment Group, Goat milk - Malaria-infected - Goat milk and DHP; f = Treatment Group, Goat milk Yogurt - Malaria-infected - Goat milk Yogurt; g = Treatment Group, Goat milk Yogurt - Malaria-infected - Goat milk Yogurt and DHP.

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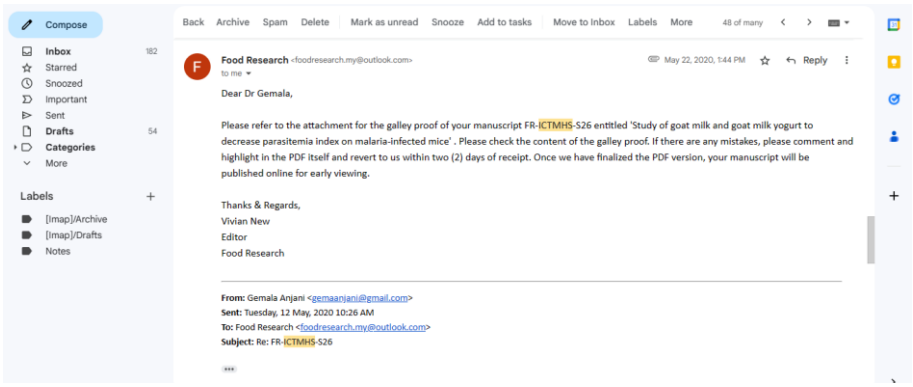

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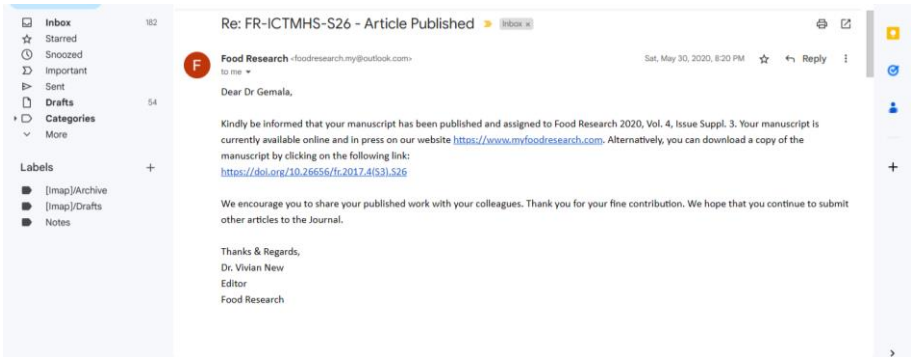

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FOOD RESEARCH

FULL PAPER

Study of goat milk and goat milk yogurt to decrease parasitemia index on malaria-infected mice

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Abstract

Malaria is an infectious disease caused by the *Plasmodium* sp. parasite. In 2017, malaria has reached 219 million cases. The decline of parasitemia index indicates a lower degree of infection in malaria patients. Goat milk and goat milk yogurt as immunomodulators have the potential to reduce parasitemia index. This study was aimed to determine the effect of goat milk and goat milk yogurt on the index of parasitemia in malaria-infected mice. This research was a true experimental study with a post-test only group design. A total of thirty-five female Balb/c mice was divided into the following groups: a (negative control group), b (malaria), c (malaria, Dihydroartemisinin/piperaquine (DHP) drug), d (malaria, goat milk), e (malaria, goat milk, DHP), f (malaria, goat milk yogurt), and g (malaria, goat milk yogurt, DHP). Inoculation dose of *Plasmodium* was 10^7/0.2 mL. The intervention was studied for 24 days. Parasitemia index data was collected on the seventh day post-inoculation. The dose of goat milk and goat milk yogurt given was 0.5 mL/20 g BW. Data were analyzed using Kruskal-Wallis with Mann-Whitney post-hoc test. The results showed a significant decrease in the parasitemia index ($p<0.05$). The mean parasitemia index in each group were: 0% (a); 13.9% (b); 0.60% (c); 4.68% (d); 3.74% (e); 3.66% (f); 0.82% (g). The group that exhibited effective reduction of parasitemia index were group c and g. Goat milk yogurt (f) was more effective in decreasing the parasitemia index than goat milk (d). Giving goat milk yogurt can be considered an additional therapy for the treatment of malaria.

1. Introduction

Malaria is a disease caused by a protozoan parasite from the genus *Plasmodium* (Percário *et al*., 2012; Khalid *et al*., 2013; Isah and Ibrahim 2014; Okpe *et al*., 2016). Malaria is transmitted by the bite of a female Anopheles mosquito that carries the *Plasmodium* (Mota et al., 2019; Mala et al., 2016). The type of *Plasmodium* that is responsible for most cases of malaria in tropical and subtropical regions is *Plasmodium falciparum* (Emmanuel *et al*., 2016). *Plasmodium* infection from malaria will cause oxidative changes and an imbalance of antioxidant mechanisms. (Khalid *et al*., 2013; Fabbri *et al*., 2013; Gomes *et al*., 2015). Malaria infection increases parasitemia index, an indicator of the severity of infection in malaria patients (Tyagi *et al*., 2017). The use of antioxidants is expected to help reduce oxidative damage and prevent further development of malaria (Gomes *et al*., 2015).

Goat milk is known for its positive effects on the biological functions of the human body due to its nutritional content. Goat milk also has low allergen levels and is easily digested by the body (Yangilar, 2013; Aristya *et al*., 2013; Banjare *et al*., 2017). Furthermore, it contains natural antioxidant-forming agents which can prevent the lysis of erythrocyte cells (Alyaqoubi *et al*., 2015). Protein in goat milk is the main source of active biopeptides that can act as antioxidants (Park, 2010: Alyaqoubi *et al*., 2014; Alyaqoubi *et al*., 2014). In addition, it demonstrates anti-inflammatory properties and serves as an important source of angiotensin-converting enzyme (ACE) which functions as an antihypertensive peptide and helps alleviate infections caused by pathogenic microbes (Lad *et al*., 2017).

Goat milk can be processed and fermented into yogurt. Yogurt contains bioactive peptides and has antioxidant activity (Gahruie *et al*., 2015; Nguyen and

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Hwang, 2016). The antioxidant activity of goat milk yogurt is known to be higher than cow milk yogurt (Holvik, 2013; Muniandy *et al*., 2016). Increased antioxidant properties in goat milk yogurt results from the activity of lactic acid bacteria (LAB). Several studies have reported the effects of LAB in response to oxidative stress (Fardet and Rock, 2017; Shu *et al*., 2018; Moreno-Fernandez *et al*., 2019). The potential health benefits of LAB includes stimulating the immune system, maintaining the balance of the intestinal flora, reducing cholesterol levels, and promoting anti-aging and antioxidant activity (Nakagawa and Miyazaki, 2016). LAB produces exopolysaccharides (EPS) which specifically has immunostimulant activity and increases the digestive tract colonization (Polak-Berecka *et al*., 2013). LAB hydrolyzes casein into a bioactive peptide with various biological functions. Based on previous researches, casein in goat milk yogurt is capable of preventing the increase of Malondialdehyde (MDA) levels (Mahdi *et al*., 2018). This study is aimed to determine the effects of consuming goat milk and goat milk yogurt on the parasitemia index of mice infected with malaria. This research is expected to be useful in the development of food and health science in relation to the role of antioxidants in the treatment of malaria.

2. Materials and methods

2.1 Inoculation Plasmodium berghei ANKA (PbA)

This research used female Balb/c mice that have been inoculated with *Plasmodium berghei* ANKA (PbA). Three donor mice were obtained from the parasitology laboratory of the Faculty of Medicine, Nursing and Public Health, Gadjah Mada University (FKKMK UGM). Each mouse was inoculated with 10^7 mL of PbA parasite that was given at 0.2 mL intraperitoneally (Tafor *et al*., 2013)

2.2 Animal and treatments

The research design used in this research is true experimental with random post-test controlled group. Infected mice were divided into 7 groups: 3 control groups and 4 treatment groups. Group A (negative control group fed with normal food); group B (positive control group fed with normal food, inoculated with PbA and not receiving treatment); group C (positive control group with normal food, inoculated with PbA and given the anti-malaria therapy (DHP)); group D (treatment group 1, inoculated with PbA and given goat milk); group E (treatment group 2, inoculated with PbA, given the anti-malaria therapy (DHP) and goat milk); group F (treatment group 3, inoculated with PbA and given goat milk yogurt); group G (treatment group 4, inoculated with PbA, given the anti-malaria therapy (DHP) and goat milk yogurt).

Parasitemia index data was collected at the 7th day post-inoculation. The goat milk and goat milk yogurt dose are 0.5 mL/20 g BW. The DHP dose used the combination of 3 mg/kg BW/days of Dihydroartemisinin and 24 mg/kg BW/day of Piperaquinine (Ariani *et al*., 2018).

Yogurt production as well as analysis of protein content and antioxidant activity of goat milk and goat milk product was done at the Integrated Laboratory of Diponegoro University, Semarang. Animal experiments including malaria inoculation and parasitemia index measurement were performed in Integrated Biomedic Laboratory (IBL) Medical Faculty of Sultan Agung Moeslem University Semarang (FK-UNISSULA). Goat milk was obtained from Kuncen Farmer Group at Bubakan Village, Mijen Semarang.

2.3 Goat Milk Yogurt Preparation

Before the yogurt making process, subculture starter was made to activate the Lactic Acid Bacteria (LAB) in commercial starter (manufactured yogurt). The manufactured yogurt contained *Streptococcus thermophiles* culture, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus,* and *Bifidobacterium*.

About 16 g of skim milk was dissolved in 100 mL goat milk and pasteurized at 75°C for 15 s. Next, the milk was mixed with commercial starter (manufactured yogurt) at the ratio of 1:1 and incubated at 40°C for 24 hours.

Goat milk yogurt production used 500 mL fresh goat milk. Goat milk was pasteurized at 75°C for 15 s and later cooled down until 43°C. Afterwards, the mixture was incubated with bacteria starter made from 5% fresh goat milk and incubated at 42°C for 6 hrs before it was cooled in room temperature. Prepared yogurt was packed and kept in the refrigerator.

2.4 Goat milk and goat milk yogurt intervention

Goat milk and goat milk yogurt was given orally using feeding tube (sonde). The dose was determined based on the maximum capacity of the mice stomach which was 1 mL/20 g BW (Ngatidjan, 2006). Ideal amount is 0.25-0.5 mL and the given amount in this research was 0.5 mL/20 g BW/day. The goat milk and goat milk yogurt were given for 24 days, consisting of 21 days pre-inoculation and 3 days post-inoculation.

2.5 Parasitemia index examination

Object glass was thoroughly cleaned and coded in the top right corner. Blood sample was dropped on the

object glass to make a smear that was repetitively washed and dried before applied with Giemsa stain. Samples were examined under the microscope with a 10x10 visual field enlargement, then 10x100 enlargement with immerse oil. Parasite erythrocytes were calculated and stated in percentage and compared with the studied blood (Torres *et al*., 2018).

2.6 Statistical analysis

The results were described as median±SD (for normally distributed data) or median (min-max) if otherwise. The statistical difference was analyzed using Kruskall Wallis and Mann Whitney Post-hoc test.

2.7 Ethical clearance

This research was approved by the Health Research Ethics Commission of the Sultan Agung University School of Medicine with No. 196/III/2019/Bio-commission.

3. Results

A total of 28 mice were obtained from the Biology Laboratory of Semarang State University with 25 for treatment and 3 as donor mice. The results of normality test of parasitemia index using the Saphiro-Wilk test obtained p-value < 0.005 (p = 0.00), indicating that the parasitemia index data was not normally distributed thus the Kruskal-Wallis test was performed. Based on Table 1, there is a statistically significant difference in the parasitemia index (p < 0.05). Next, Post Hoc tests were carried out with Mann-Whitney and the results are as seen in Table 1. Treatment by giving goat milk and goat milk yogurt or a combination of both with DHP drugs has been proven to positively affect the parasitemia index.

Based on Table 1, the groups with no significant difference on the parasitemia index were the c and g groups with a value of p = 0.289. In the d and e groups, there was also no significant difference in the parasitemia index with a value of p = 0.059. Similarly, group d and group f also showed no significant difference with a value of p = 0.602. These results indicate that the two groups did not have different effectiveness in suppressing the parasitemia index. The ability of each group to suppress the parasitemia index can be seen based on the average parasitemia index in Figure 1.

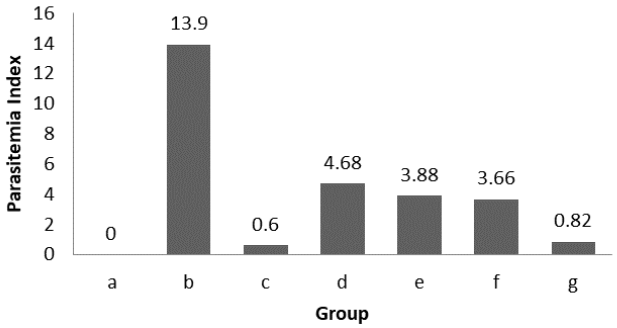

Figure 1. Average parasitemia index results. a = Negative Control Group, Healthy; b = Positive Control Group, Malaria-infected; c = Control Group, Malaria-infected and DHP Drug Administration; d = Treatment Group, Goat milk - Malaria-infected - Goat milk; e = Treatment Group, Goat milk - Malaria-infected - Goat milk and DHP; f = Treatment Group, Goat milk Yogurt - Malaria-infected - Goat milk Yogurt; g = Treatment Group, Goat milk Yogurt - Malaria-infected - Goat milk Yogurt and DHP.

4. Discussion

Malaria is one of the endemic diseases in Indonesia which is still a major health concern. Malaria is usually treated with antimalarial drugs. Standard treatment for malaria uses Artemisinin-based combination therapies (ACTs). These therapies can combine artemisinin or one of its derivatives with another antimalarial drug. One of

Table 1. Statistical analysis of the parasitemia index

Treatment Group	Parasitemia index (%)	P^1	P^2						
			a	b	c	d	e	f	g
a	0.00±0.00		-	0.005*	0.005*	0.005*	0.005*	0.005*	0.005*
b	13.9±0.58		-	-	0.009*	0.009*	0.009*	0.009*	0.009*
c	0.60±0.27		-	-	-	0.009*	0.009*	0.009*	0.289
d	4.68±0.53	0.00*	-	-	-	-	0.059	0.602	0.008*
e	3.74±0.74		-	-	-	-	-	0.834	0.005*
f	3.60±1.80		-	-	-	-	-	-	0.008*
g	070(0.70-1.20)		-	-	-	-	-	-	-

[1] = Kruskall Wallis, [2] = Post Hoc Mann-Whitney,* = p value <0.005 (significantly)

a = Negative Control Group, Healthy; b = Positive Control Group, Malaria-infected; c = Control Group, Malaria-infected and DHP Drug Administration; d = Treatment Group, Goat milk - Malaria-infected - Goat milk; e = Treatment Group, Goat milk - Malaria-infected - Goat milk and DHP; f = Treatment Group, Goat milk Yogurt - Malaria-infected - Goat milk Yogurt; g = Treatment Group, Goat milk Yogurt - Malaria-infected - Goat milk Yogurt and DHP.

the combinations is Dihidroartemisinin Piperaquin (DHP). Papua and West Papua are regions in Indonesia that are still endemic for malaria (Subdit Malaria Direktorat KR, 2018). When malaria cases occur, not all patients have immediate access to health facilities and DHP medication as early as possible. Utilization of functional food is considered to be an additional therapy for malaria patients. One of the functional foods that can be developed is goat milk and goat milk yogurt (Yangilar, 2013).

This research aimed to determine the effect of goat milk and goat milk yogurt in decreasing parasitemia index. The results of this research can be seen in Figure 1. Figure 1 shows that the group with the lowest parasitemia index is group a (negative control group, healthy) with 0% because they were not inoculated with malaria. Group b (positive control group, malaria-infected) had the highest parasitemia index among all groups (13.9%) as they were inoculated with malaria and not given any treatment thus the *Plasmodium* continued to multiply and the parasitemia index increased. Group c (malaria-infected, DHP drug administration) (0.6%) was found to have the lowest mean of parasitemia index compared with other groups, further proving the effectiveness of the use of DHP drugs. DHP drugs include an artemisinin group that is known to be effective in killing *Plasmodium* quickly in all life stages including gametocytes (Douglas *et al*., 2010).

The mean parasitemia index values in groups d (malaria-infected, given goat milk) and f (malaria-infected, given goat milk yogurt) were lower compared with group b, suggesting that goat milk yogurt may play a role in lowering parasitemia index. Goat milk and goat milk yogurt both contain casein and whey which has been shown to possess antioxidant activity (Sabeena *et al*., 2010). Casein can work as radical scavengers and cation chelators which can inhibit lipid oxidation. In goat milk yogurt, the components of whey protein consisting of lactoferrin, beta-lactoglobulin, alpha-lactalbumin, glyomropropeptides, and immunoglobulins can boost the immune system. Lactoferrin binds the iron in the intestinal mucosa and acts as a bacteriostatic agent by suppressing populations of harmful bacteria and modulating the body's immunity. Lactoferrin also repairs cell damage by inhibiting the production of ROS and works with vitamin E in limiting membrane lipid oxidation by ROS. β-Lactoglobulin plays a role in the transfer of pro-vitamin A, while α-Lactalbumin is involved in lactose synthesis. Immunoglobulin is important for an antigen-antibody reaction. This protein component can enhance the immune system. The major protein boosts the immune system by converting the cysteine intracellular amino acids into glutathione that acts as an intracellular antioxidant. Lactoferrin provided in goat milk and goat milk yogurt can stimulate the immune system through the activation of T and B lymphocyte cells (Queiroz *et al*., 2013; Zapata *et al*., 2017; Mahdi *et al*., 2018).

Group f (malaria-infected, given goat milk yogurt) was more effective in reducing the parasitemia index than group d (malaria-infected, given goat milk). Yogurt generally contains lactic acid bacteria that have Bile Salt Hydrolase (BSH) enzyme. BSH is known to be able to de-conjugate bile salts into free bile salts that are less absorbable by the small intestine, leading to reduced amount of bile salts returning to the liver. Consequently, cholesterol would be used as a precursor to balance the amount of bile salt so overall cholesterol levels would decrease. Lower cholesterol levels meant less amount of lipid exposed to free radicals, resulting in decreased lipid peroxidation and eventually a decline in MDA levels and parasitemia index (El-Dein *et al*., 2017).

The mean parasitemia index of group e (malaria-infected, given the anti-malaria therapy and goat milk) and g (malaria-infected, given the anti-malaria therapy and goat milk yogurt) were higher than group b. This finding supports the contribution of food sources of protein (goat milk or goat milk yogurt) as an effective adjuvant treatment in the treatment of malaria. Giving goat milk or goat milk yogurt alongside DHP drugs was found to be more effective in reducing the parasitemia index. Interestingly, the mice receiving DHP and goat milk yogurt experienced a more significant reduction of parasitemia index than the group receiving DHP and goat milk. The administration of DHP drugs is recommended to be consumed together with milk or fatty foods (WHO, 2015) but previous studies have shown that the administration of DHP drugs together with 200mL of milk (containing 6.4 grams of fat) did not improve pharmacokinetic parameters (including absorption of DHP drugs) when compared with the control group (Annerberg *et al*., 2011). Giving goat milk with DHP drugs might inhibit the bioavailability of the drug because goat milk contains higher calcium and magnesium than cow milk (Park, 2010). Calcium and magnesium have divalent ionic bonds which may inhibit drug absorption (Bushra *et al*., 2011). On the other hand, administration of DHP with goat milk yogurt can decrease the parasitemia index better than goat milk, making it a more favorable option for malaria adjuvant therapy. The decrease in parasitemia index is due to the content of lactic acid bacteria in goat milk yogurt which may affect intestinal microbiota profile. In accordance with previous studies, intestinal microbiota influenced the pathogenesis of malaria and altering the intestinal bacterial community affected the parasitic load and death

risk after *Plasmodium* infection. Providing yogurt helped modulate the intestinal microbiota so that it can reduce the burden of parasites (Villarino *et al*., 2016).

Yogurt is made by fermentation that causes the release of bioactive peptides from the major protein of milk. The fermentation process produced antioxidant peptides consisting of 5-11 hydrophobic amino acids which include proline, histidine, tyrosine or tryptophan. These amino acids prevent the formation of free radicals and inhibit the process of lipid peroxidation. Lactic acid bacteria exhibit antioxidant activity through suppressing the accumulation of ROS during the food digestion process and destroying superoxide anions and hydrogen peroxide (Gjorgievski, 2015).

Giving goat milk yogurt was proven to help reduce the parasitemia index in our study. One potential mechanism to explain this finding is that intestinal microbiota influenced the severity of malaria by giving a direct effect on the parasite itself, where the products of intestinal microbiota inhibit the growth of parasites. Another potential mechanism is that intestinal microbiota may have modulated the host immune response to *Plasmodium*. Previous researches showed that intestinal microbiota may send signals for monocytes/macrophages to respond to and control the infection (Denny, 2018).

An important mechanism of yogurt during *Plasmodium* infection is through the ability of intestinal microbiota to express the glycemic molecule in the form of α-gal (Gal1 α1-3Galβ1-4GlnAc-R). The α-gal antibody binds to the surface of the sporozoite, induces lysis order and prevents the spread of *Plasmodium* to liver cells. The administration of yogurt can trigger an α-gal response to inhibit the transmission of *Plasmodium* sporozoite (Burgess *et al*., 2017). The decreased parasitemia index after malaria inoculation implied that diet (intake) played an important role in shaping the composition and activity of intestinal microbiota. Based on previous studies, *Lactobacillus* and *Bifidobacterium* have a protective role by modulating the burden of parasites and reducing the severity of malaria. Therefore, the administration of yogurt containing *Lactobacillus* bacteria can cause a decrease in parasitemia. Changes in intestinal microbiota can prevent malaria severity and accelerate the healing process of malaria. These results also support the possibility that altering the intestinal microbiota profile by giving goat milk yogurt cannot prevent malaria but has the potential to control the severity of malaria in humans (Shasteen *et al*., 2015).

5. Conclusion

The addition of yogurt and DHP resulted in higher parasitemia index in malaria-infected mice in comparison with DHP-only treatment, but the mechanism is unknown. The addition of goat milk yogurt is more effective in lowering the parasitemia index than goat milk only. The administration of goat milk yogurt at a dose of 0.5 mL/20g BW can be considered an additional therapy in the treatment of malaria.

Conflict of interest

The authors declared that they have no competing interests.

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References

Alyaqoubi, S., Abdullah, A. and Addai, Z.R. (2014). Antioxidant activity of goat milk from three different locations in Malaysia. *AIP Conference Proceedings,* 1614, 198. https://doi.org/10.1063/1.4895195

Alyaqoubi, S., Abdullah, A., Samudi, M., Abdullah, N., Radhi, Z. and Al-ghazali, M. (2014). Effect of Different Factors on Goat milk Antioxidant Activity Ministry of Regional Municipalities and Water Resource. *International Journal of Chem Tech Research,* 6(5), 974-4290. http:// sphinxsai.com/2014/vol6pt5/9/(3191-3196)S-2014.pdf.

Alyaqoubi, S., Abdullah, A., Samudi, M., Abdullah, N., Addai, Z.R. and Al-ghazali, M. (2015). Physicochemical properties and antioxidant activity of milk samples collected from five goat breeds in Malaysia. *Advance Journal of Food Science and Technology,* 7(4), 235-241. https://doi.org/10.19026/ ajfst.7.1301

Annerberg, A., Lwin, K.M., Lindegardh, N., Khrutsawadchai, S., Ashley, E., Day, N.P., Singhasivanon, P., Tarning, J., White, N.J. and Nosten, F. (2011). Small Amount of Fat Does Not Affect Piperaquine Exposure in Patients with Malaria. *Antimicrobial Agents and Chemotherapy,* 55, 3971–3976. https://doi.org/10.1128/AAC.00279-11

Ariani, Anjani, G., Achasan, M.A.S. and Djamiatun, K. (2018). Tepung ulat sagu (*Rhyinchophorus ferrugineus*) imunomodulator Nitric Oxide (NO) sirkulasi mencit terapi antimalaria standar. *Jurnal Gizi Indonesia*, 6(2), 131-138. [In Bahasa

Indonesia]. https://doi.org/10.14710/jgi.6.2.131-138

Aristya, A.L., Legowo, A.M. and Al-baarri, A.N. (2013). Karakteristik fisik, kimia, dan mikrobiologis kefir susu kambing dengan penambahan jenis dan konsentrasi gula yang berbeda. *Jurnal Aplikasi Teknologi Pangan,* 2(3), 139-143. [In Bahasa Indonesia].

Banjare, K., Kumar, M., Kumar, R. and Kartikyen, S. (2017). Perspective role of goat milk and products : A review. *International Journal of Chemical Studies,* 5(4), 1328-1338.

Burgess, S.L., Gilchrist, C.A., Lynn, T.C. and Petri, W.A. (2017). Parasitic Protozoa and Interactions with the Host Intestinal Microbiota. *Infection and Immunity,* 85, 1-12. https://doi.org/10.1128/IAI.00101-17.

Bushra, R., Aslam, N. and Khan, Y.A. (2011). Food-Drug Interactions. *Oman Medical Journal,* 26(2), 77-83. https://doi.org/10.5001/omj.2011.21

Denny, J.E. (2018). Characterizing the gut microbiota during plasmodium infection and antimalarial treatment. USA: University of Louisville, PhD Thesis. https://doi.org/10.18297/etd/3109

Douglas, N.M., Ansley, N.M., Angus, B.J., Nosten, F. and Price, R.N. (2010). Artemisinin combination therapy for vivax malaria?. *Lancet Infectious Diseases,* 10(6), 405–416. https://doi.org/10.1016/S1473-3099(10)70079-7

El-Dein, N.A., El-Deen, N.M.A., Tolba, M.S., El-Shatoury, H.E., Awad, A.G., Ibrahim, K.M. and Mohamed, A.F. (2017). Probiotic Properties and Bile Salt Hydrolase Activity of Some Isolated Lactic Acid Bacteria. *Egyptian Journal of Microbiology,* 52 (1), 87-100. https://doi.org/10.21608/ejm.2017.1336.1025

Emmanuel, A.N., Oliver, N.O. and Angela, U.N. (2016). *Using Plant Materials for Treatment of Malaria in Imo State, Nigeria*. *American Journal of Life Science Researches,* 4(2), 67-71. https://doi.org/10.20286/ajlsr-040287

Fabbri, C., De-Cássia, M.N.R. and Lalwani, P. (2013). Lipid peroxidation and antioxidant enzymes activity in Plasmodium vivax malaria patients evolving with cholestatic jaundice. *Malaria Journal,* 12(1), 315. https://doi.org/10.1186/1475-2875-12-315

Fardet, A. and Rock, E. (2017). In vitro and in vivo antioxidant potential of milks, yoghurts, fermented milks and cheeses: a narrative review of evidence. *Nutrition Research Reviews,* 31(1), 1-19. https://doi.org/10.1017/S0954422417000191

Gahruie, Hashem, H., Eskandari, Hadi, M., Mesbahi, G. and Hanifpour, A.M. (2015). Scientific and Technical Aspects of Yogurt Fortification: A Review. *Food Science and Human Wellness,* (4), 1-8. https://doi.org/10.1016/j.fshw.2015.03.002

Gjorgievski, N., Ansley, N.M., Angus, B.J., Nosten, F. and Price, R.N. (2015). Determination of The Antioxidant Activity in Yogurt. *Journal of Hygienic Engineering and Design,* 8, 88-92.

Gomes, Q.B.A., Da-Silva, L.F.D. and Gomes, Q.A.R. (2015). N-acetyl cysteine and mushroom *Agaricus sylvaticus* supplementation decreased parasitaemia and pulmonary oxidative stress in a mice model of malaria. *Malar Journal,* 14(1), 1-12. https://doi.org/10.1186/s12936-015-0717-0

Holvik, S.L. (2013). Introduction Macronutrient Nutritional Composition of Goat milk. Happy Days Dairies*,* p. 1-12. Canada: Happy Days Diaries

Isah, M.B. and Ibrahim, M.A. (2014). The role of antioxidants treatment on the pathogenesis of malarial infections: A review. *Parasitology Research,* 113(3), 801-809. https://doi.org/10.1007/s00436-014-3804-1

Khalid, M., Alam, R., Khan, S. and Prakash, V. (2013). Oxidative Stress Marker and Antioxidant Status in Falciparum Malaria in Relation to the Intensity of Parasitaemia. *International Journal of Biological and Medical Research,* 3(1), 3469-3471.

Lad, S.S., Aparnathi, D.K., Mehta, B. and Velpula, S. (2017). Goat milk in Human Nutrition and Health – A Review. *International Journal of Current Microbiology and Applied Sciences,* 6(5), 1781-1792. https://doi.org/10.20546/ijcmas.2017.605.194

Mahdi, C., Untari, H., Padaga, M.C. and Raharjo, S.J. (2018). The characterization of bioactive peptide of goat milk fermented to activities as anti-hypercholesterolemia. *International Food Research Journal*, 25(1), 17-23

Mala, M., Imam, M. and Hassan, K. (2016). Interaction between parasite and vector for Malaria disease transmission-a review on Malaria. *Progressive Agriculture*, 27(2), 168-174. https://doi.org/10.3329/pa.v27i2.29327

Moreno-Fernandez, J., Alférez, M.J.M., López-Aliaga, I. and Javier, D. C. (2019). Protective effects of fermented goat milk on genomic stability, oxidative stress and inflammatory signalling in testis during anaemia recovery. *Science Report,* 9, 2232. https://doi.org/10.1038/s41598-018-37649-6

Mota, M.M. and Mello-Vierra, J. (2019). Parasitism: Anopheles Mosquitoes and Plasmodium Parasites Share Resources. *Current Biology*, 29(3), 632-634. https://doi.org/10.1016/j.cub.2019.05.030

Muniandy, P., Shori, A.B. and Baba, A.S. (2016).

Influence of green, white and black tea addition on the antioxidant activity of probiotic yogurt during refrigerated storage. *Food Packgaing and Shelf Life,* 8, 1-8. https://doi.org/10.1016/j.fpsl.2016.02.002

Nakagawa, H. and Miyazaki, T. (2017). Beneficial effects of antioxidative lactic acid bacteria. *AIMS Microbiology,* 3(1), 1-7. https://doi.org/10.3934/microbiol.2017.1.1

Ngatidjan. (2006). Petunjuk Laboratorium Metode Laboratorium Dalam Toksikologi. Yogyakarta: Fakultas Kedokteran Universitas Gadjah Mada. [In Bahasa Indonesia].

Nguyen, L. and Hwang, E.S. (2016). Quality characteristics and antioxidant activity of yogurt supplemented with aronia (*Aronia melanocarpa*) juice. *Preventive Nutrition and Food Science,* 21(4), 330-337. https://doi.org/10.3746/pnf.2016.21.4.330

Okpe, O., Habila, N., Ikwebe, J., Upev, V.A., Okoduwa, S.I.R. and Isaac, O.T. (2016). Antimalarial Potential of *Carica papaya* and *Vernonia amygdalina* in Mice Infected with *Plasmodium berghei*. *Journal of Tropical Medicine,* 2016*,* 8738972. https://doi.org/10.1155/2016/8738972

Park, Y.W. (2010). Goat milk: Composition, Characteristics. In Pond, W.G. and Bell, N. (Eds.) Encyclopedia of Animal Science. 2nd ed. Boca Raton, FL: CRC Press.

Percário, S., Moreira, D.R., Gomes, B.A.Q. and Ferreira, M.E.S. (2012). Oxidative Stress in Malaria Review. *International Journal of Molecular Sciences,* 13(12), 16346-16372. https://doi.org/10.3390/ijms131216346

Polak-Berecka, M., Wasko, A., Szwajgier, D. and Choma, A. (2013). Bifidogenic and antioxidant activity of exopolysaccharides produced by *Lactobacillus rhamnosus* E/N cultivated on different carbon sources. *Journal of Microbiology,* 62, 181–189. https://doi.org/10.33073/pjm-2013-023

Queiroz, V.V.O., Assis, A.N.O. and Junior, H.C.R. (2013). Protective effect human lactoferrin in the gastrointestinal tract. *Revista Paulista de Pediatria*, 31(1), 90-95. https://doi.org/10.1590/S0103-05822013000100015

Sabeena, F.K.H., Baron, C.P., Nielsen, N.S. and Jacobsen, C. (2010). Antioxidant activity of yoghurt peptides: Part 1-in vitro assays and evaluation in ω-3 enriched milk. *Food Chemistry*, 123(4), 1081–1089. https://doi.org/10.1016/j.foodchem.2010.05.067

Shasteen, M.A., Lebeis, W.S. and Schmidt, N. (2015). Lessening Malaria Parasite Burden with Yogurt. Microbiology Publications and Other Works. Retrieved from Tennessee Research and Creative Exchange (TRACE) website: http://trace.tennessee.edu/utk_micrpubs/74

Shu, G., Shi, X., Chen, L., Kou, J., Meng, J. and Chen, H. (2018). Antioxidant Peptides from Goat milk Fermented by *Lactobacillus casei* L61: Preparation, Optimization, and Stability Evaluation in Simulated Gastrointestinal Fluid. *Nutrients,* 10(6), 797**.** https://doi.org/10.3390/nu10060797

Subdit Malaria Direktorat KR. (2018). Buku Saku Tatalaksana Malaria 2018. Indonesia: Kemenkes RI. [In Bahasa Indonesia].

Tafor, D., Djunaidi, A., Wasityastuti, W. and Sholikhah, N.E. (2013). Tumor Necrosis Factor-Alpha (TNF-Alpha) and Intercellular Adhesion Molecule-1 (ICAM-1) Expression of *Plasmodium berghei* Infected Swiss Mice Treated with Red Fruit (*Pandanus conoideus* Lam) Ethanol Extract. *Tropical Medicine Journal*, 3(2), 155-165.

Torres, K., Bachman, C.M., Delahunt, C.B., Jhonatan, A.B., Freddy, A., Dionicia, G.V., Stephane, P., Courosh, M., Shawn, K. McG., Clay, M.T., Travis, O., Liming, H., Mayoore, S.J., Victoria, M.H. and David, B. (2018). Automated microscopy for routine malaria diagnosis: a field comparison on Giemsa-stained blood films in Peru. *Malaria Journal,* 17**,** 339. https://doi.org/10.1186/s12936-018-2493-0

Tyagi, A.G., Tyagi, R.A., Choudhary, P.R. and Shekhawat, J.S. (2017). Study of antioxidant status in malaria patients. *International Journal of Research in Medical Sciences,* 5(4), 1649-1654. https://doi.org/10.18203/2320-6012.ijrms20171281

Villarino, F.N., Lecleir, R.G., Denny, E.J., Dearth, P.S., Harding, L.C., Sloan, S.S., Jennifer, L.G., Shawn, R.C., Steve,n W.W. and Nathan, W.S. (2016). Composition of the gut microbiota modulates the severity of malaria. *Proceedings of the National Academy of Sciences,* 113(8), 2235–2240. https://doi.org/10.1073/pnas.1504887113

WHO. (2015). Guidelines for the treatment of malaria. 3rd ed. Geneva: WHO.

Yangilar, F. (2013). As a Potentially Functional Food: Goats' Milk and Products. *Journal of Food and Nutrition Research,* 1(4), 68-81. doi:10.12691/jfnr-1-4-6

Zapata, C.R., Singh, A., Pezeshki, A., Nibber, T. and Chelikani, K.P. (2017). Whey Protein Components - Lactalbumin and Lactoferrin - Improve Energy Balance and Metabolism. *Scientific Reports,* 7, 9917. https://doi.org/10.1038/s41598-017-09781-2

