Hematology Profile of Female Guinea Pig (*Cavia porcellus* (Linnaeus, 1758)) with Diet Variations

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ABSTRACT
Guinea pigs (*Cavia porcellus* (Linnaeus, 1758)) is a rodent other than rats and mice that are commonly used in biomedical research because of its physiological similarity to humans. To meet the requirement as a standard laboratory animal, the care management of GP needs to be controlled, one of which is by diet. The standard diet for GP is still limited in Indonesia, therefore GP is fed with a rabbit diet. According to references, inappropriate diet affects hematology profile. This research will be carried out to compare hematology profiles in GP fed with standard and nonstandard diets. Nine female GP were assigned into three groups, given a variety of diets based on the research design: Group BV (NS-NS-S), BVL (NS-S-S), and BL (S-S-S). The research lasted for 84 days, and blood was collected every 28 days. Hematology data profiled using fully automatic hematology analyzer Sysmex XP-100 with the following parameters: RBC, HGB, HCT, MCV, MCH, MCHC, WBC, %LYM, %NEU, %MXD, #LYM, #NEU, #MXD, N/L, PLT, PDW, MPV, P-LCR dan PCT. Cell morphology was observed through smear slide, stained by Giemsa 3%. There were significant ($p<0,05$) decrease in RBC, HGB, HCT, and MCHC. There were also significant ($p<0,05$) increases in MCV value, WBC, NEUT#, PLT, and PCT. The significant changes especially happen in the BL group. Based on the result, it can be concluded that the diet alteration to the standard diet changes the value of RBC, HGB, HCT, MCV, MCHC, WBC, NEUT#, PLT, MPV, and PCT. However, the change was ranging in the normal value, so the nonstandard diet was still safe to be given with vegetable supplementary every day.

Keywords: *Cavia porcellus, Diet type, Guinea pigs, Hematology, Laboratory animal.*

1. INTRODUCTION

Guinea pig (*Cavia porcellus* (Linnaeus, 1758)) is a small, tailless species of the Order Rodentia. The guinea pig is one of the laboratory animals that is often used in biomedical research as a test material for biochemistry, physiology, and pharmacology because it has several physiological similarities with humans. The guinea pigs used in research must be properly maintained so that they can show representative results and can be used as a reference for further research. One of the attempts to maintain a good guinea pig condition is to meet nutrient needs through a daily diet [1][2].

Diet is one of the important things to maintain good body condition. Diet feeding aims to fulfill energy needs, growth, body regulation, and protection [3]. A diet that has been formulated according to the needs of a particular species is called a standard diet. The standard diet aims to maintain nutrient balance, growth, and reproduction rates in animals, especially laboratory animals. In addition, a standard diet also reduces the variation of intrinsic factors that may be obtained from natural diets. Each species of animal has different nutritional requirements so that different standard diets are provided. However, some animals are not fed by their appropriate standard diet [4]. Most laboratory guinea pigs in Indonesia are still fed with a rabbit diet. This happens

because the standard diet for guinea pigs in Indonesia is still rarely found and has not been commonly used.

An inappropriate diet (nonstandard diet) can cause a lack of nutrient intake, causing several physiological disorders. Vitamin C deficiency can cause scurvy in guinea pigs which is indicated by coarse hair, limp, anorexia, slow wound healing, and joint swelling. Vitamin D deficiency causes fibrous osteodystrophy characterized by weight loss, hypersaliva, lethargy, and difficulty moving [5]. Deficiency of essential fatty acids can cause sores around the neck and ears, hair loss on the ventral surface, dermatitis, and stunted growth. Protein deficiency exhibits symptoms similar to Kwashiorkor syndrome including edema of the face and arms [6].

One of the parameters that can indicate the presence of physiological disorders is the hematology profile. Changes in normal values in the hematology profile indicate a physiological disorder that can be caused by disease, nutritional disorders, or stress due to external environmental conditions [7]. The research by Spittler *et al.* [8] showed that hematology profile is sex-dependent. The research about the hematology profile of male guinea pigs was done by Rachid [9]. Therefore, it is necessary to examine the hematology profile in female guinea pigs as an initial diagnosis of possible differences of disturbances in physiological conditions in female and male guinea pigs due to inappropriate feeding.

2. METHODOLOGY

This research has fulfilled the ethical eligibility requirements through ethical clearance approved by the Research Ethics Commission of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta with the registration number 0016/EC-FKH/Eks./2020. The guinea pigs care management and the data collecting were done by following methods.

2.1. Experimental Animals

Guinea pigs (*Cavia porcellus*) were kept in the *"Animal House"* of the Faculty of Biology, Universitas Gadjah Mada for 84 days. The guinea pigs were kept in standard pen-type, rust-resistant metal grid cages which could be disassembled. The base of the cages is a vinyl carpet plus wood shaving as bedding. The cages were equipped with enrichment in the form of tunnels for hiding places and shelters to support their normal activities.

The guinea pigs were divided into three groups of cages, containing three animals each. The guinea pigs were given nonstandard diet which was locally available rabbit pellets (Vittamaxx®) and standard diet which was guinea pig pellets imported from the United States (LabDiet5025®). Drinking water was taken from the tap water (Toya Gama).

Bodyweight and body temperature were calculated every week as basic physiological parameters. In addition, guinea pigs were also given vegetables such as cabbage, carrots, mustard greens, spinach, cucumber, kale, and timothy hay as much as 100 grams/cage every day.

2.2. Research Design

Before this research was done, all the guinea pigs were given fully non-standard diet. Nine female guinea pigs were assigned into three groups, which are BV, BVL, and BL. Changes in the diet were carried out every 28 days. Pellets were given as much as 150 grams/cage every day based on the research design (Figure 1). Drinking water was given as much as 250 mL/day in a rodentic bottle.

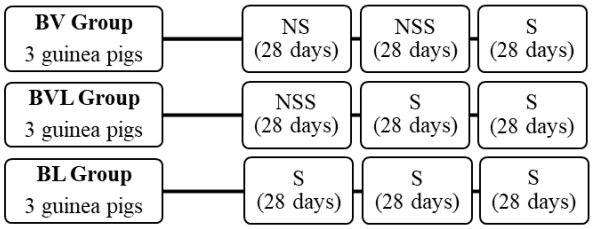


Figure 1. Research Design**.** Diet replaced every 28 days in the form of nonstandard diet (NS), a 1:1 mixture of standard and nonstandard diet (NSS), and standard diet (S). The diet given in BV group changed gradually, BVL group had longer standard diet duration after gradually changes, and BL group was changed directly without being given a mixture.

2.3. Hematology Profile

Hematology profile was observed every 28 days on D-0, D-28, D-56, and D-84. Blood samples were collected through the posterior limb by cutting the nail ±2 mm from the visible blood vessels after it was sterilized using 70% ethanol. Blood samples were collected in microtubes coated with EDTA with the help of microhematocrit. The blood was observed through a hematology analyzer (Sysmex XP100®) with the following parameters: erythrocyte profiles, leukocyte profile, and platelet profiles.

Erythrocyte profiles examined were total erythrocyte (RBC), hemoglobin level (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), RBC distribution width by coefficient variation (RDW-SD), and RBC distribution width by standard deviation (RDW-CV).

Leukocyte profiles examined were total leukocyte (WBC), total lymphocyte (#LYM), lymphocyte presentation (%LYM), total neutrophil (#NEU), neutrophil presentation (%NEU), total mixed cell

(#MXD), mixed cell presentation (%MXD), and neutrophil/lymphocyte ratio (N/L).

Platelet profiles examined were total platelet (PLT), platelet distribution width (PDW), mean platelet volume (MPV), platelet-larger cell ratio (P-LCR), and plateletcrit (PCT).

2.4. Thin Blood Smear Preparation

The method of making and staining thin blood smears was carried out based on research by Salnus & Arwie [10] with modification. The blood sample was smeared thinly on a fat-free slide. Fixation was carried out using methanol for five minutes and stained with Giemsa 3% for the next 30 minutes. Then, the blood smear was rinsed with aquadest. The morphology of blood cell was observed using a binocular microscope.

2.5. Data Analysis

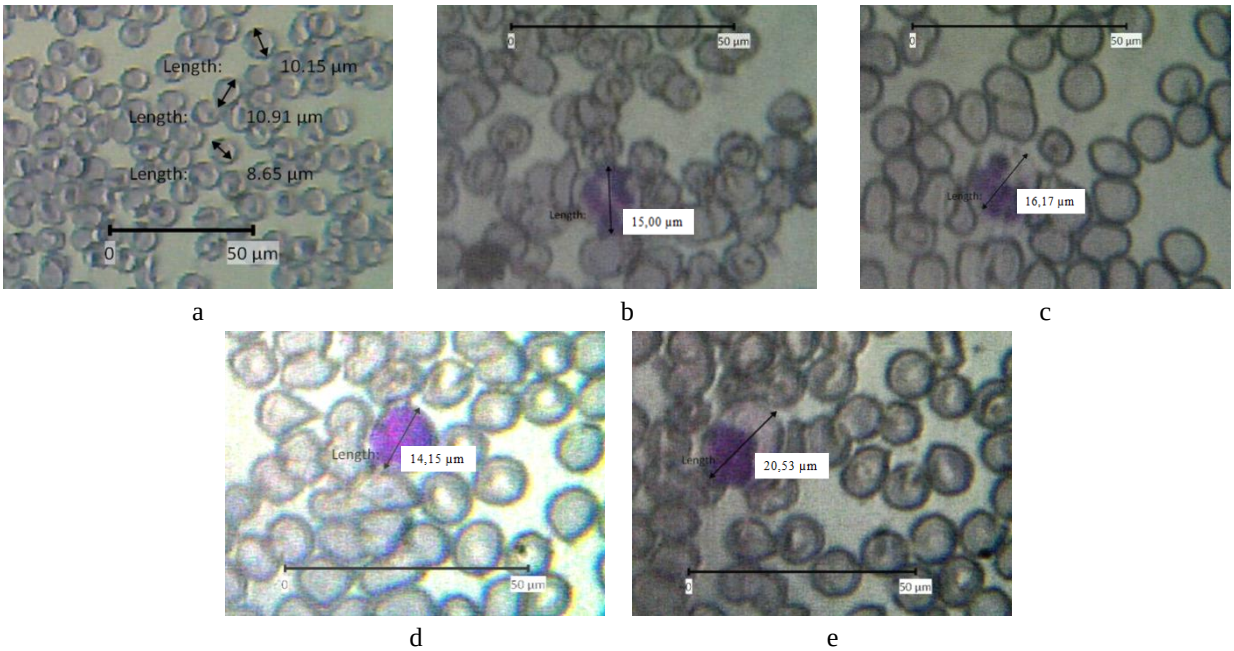
Quantitative data (body weight, body temperature, and hematology profile) were tabulated using Microsoft®Excel® and analyzed using descriptive statistics one-way Anova ($p<0,05$) followed by Duncan for the significant test. The results were visualized in the form of a line graph and compared with the baseline values for each variable arranged based on the lowest to highest values of the animal population on day 0. Microscopic images of blood cells data (erythrocytes and leukocytes) were displayed as representative images. The cells diameter was measured using Image Raster®.

3. RESULT AND DISCUSSION

The data collected from this research was examined right after and the result was presented as a picture. The result was discussed in each point below.

3.1. Blood Cell Morphology

The blood cell morphology was observed from the thin smear preparation by microscope. This observation was done to obtain each cell morphology without counting the cells. Total of each cell was examined using Hematology Analyzer. Erythrocytes in guinea pigs tend to be larger than erythrocytes in another laboratory animals such as rabbits (6.7–6.9 µm) [6] and mice (5.8 µm) [11]. The average diameter in normal guinea pigs is 6.6-7.9 µm [12]. Like the other mammals, guinea pig erythrocytes are biconcave. The observed diameters of erythrocyte were variated from 8.65-10.91 µm. This variation can occur because the guinea pig erythrocytes are moderate anisocytosis [13].



a b c

d e

Figure 2. Blood cell morphology of female guinea pig which are erythrocyte (a), eosinophil (b), neutrophil (c), lymphocyte (d), and monocyte (e)

Eosinophils have a segmented nucleus that forms two to three lobes, less than the neutrophil's lobes [12]. The basic protein in the eosinophil granules binds to the eosin dye in Giemsa and it turns dark pink. The diameter of the eosinophils observed was 15 µm which was still in the normal size (10-15 µm [12]). Neutrophils can be found with five or more nucleus segmentation [12]. The cytoplasm of neutrophils shows a lighter pink than eosinophils caused by the neutral protein in neutrophil granules binds both types of dyes [14][12]. The observed neutrophil diameter was 16.17 µm, larger than its normal size (10-12 µm [12]). The larger size of neutrophils can indicate that they were reactive. Reactive neutrophil increases the segmentation of the nucleus so that the cell

grows larger. In addition, the presence of vacuolization and granulation caused by toxic substances also affects the morphology of neutrophils [15].

Monocytes have a lower nucleus: cytoplasm ratio than lymphocytes so that lymphocytes have less cytoplasm. The nucleus of monocytes is more like oval and amoeboid than the nucleus of lymphocytes [16]. The observed diameter of the monocyte was 19.21 µm and the lymphocyte was 13.79 µm. In general, the size of a normal small lymphocyte is the same as the erythrocyte, while a large lymphocyte can be up to twice size of small lymphocyte. Monocytes sizes are slightly larger than normal large lymphocytes [12].

The morphology of erythrocytes and four types of lymphocytes (monocytes, lymphocytes, neutrophils, eosinophils) was observed through the thin blood smear shown in Figure 2. Morphological observations can be done to detect various diseases related to blood cell abnormalities [17]. Several blood abnormalities may occur in guinea pigs with inadequate nutrient intake. Research by de Oliveira & Saldanha [18] shows that protein and lipid deficiency can cause abnormalities in erythrocyte morphology because lipids and proteins are the main components of cell membranes. Some cases of erythrocyte abnormalities can occur due to gene coding disorders that are caused by protein deficiency. The thin blood smears preparation also complements the examination result that cannot be displayed through hematology analyzer, such as the presence of platelet aggregation. The low platelet count through a hematology analyzer examination can be caused by a large amount of aggregation that occurs around the wound in the blood collecting site [19]. Therefore, observation of platelet aggregation through thin blood smear preparations can support the hematology profile data.

3.2. Erythrocyte Profile

The female guinea pig erythrocyte profile was shown in Figure 3. Each graph was provided with a baseline showing the normal range for each parameter (grey area). An increase or decrease in the hematology profile that exceeds normal range indicates a physiological disorder. Most of the variable shows a normal range, but some are passing the normal range.

Erythrocytes or red blood cells are one of the blood components that play a role in oxygen delivery throughout the body. Almost 98% of the cytoplasmic protein in erythrocytes is hemoglobin that makes a red color. It plays an important role in the oxygen and carbon dioxide binding of the cell [20]. Hematocrit shows the percentage of total erythrocytes to the total blood volume, so that the RBC value is related to the HCT value. Therefore, the hemoglobin level and the percentage of hematocrit are linear to the total erythrocytes.

These three variables showed a decreasing trend for 84 days, especially in the BL group. Based on the Anova test, there was no significant difference ($p>0.05$) when guinea pigs were given the diet changes gradually, shown in the BV and BVL groups. The BL group showed a significant difference ($p<0.05$) which indicated that changes in the diet directly, without being given a 1:1 mixture (NSS) was affecting the total erythrocytes and hemoglobin levels.

Guinea pigs are the type of animals that are difficult to receive changes in diet, so they tend to refuse when given a new type of diet [2]. Diet refusal can disturb erythropoiesis due to nutrient deficiencies, especially vitamin B12, folic acid, and iron. Lacking components that support erythropoiesis will cause a lower erythrocyte production [21]. Beside that, protein intake also affects erythrocyte production. Standard diet is providing higher protein content than nonstandard diet (Table 1). Inadequate protein intake from the nonstandard diet can inhibit the hematopoiesis cycle, especially in the G0/G1 hematopoietic stem cell cycle. Protein malnutrition interferes with endothelial cells that play a role in hematopoietic stem cell differentiation. Providing the right amount of protein needs will reduce the hematopoietic disturbances [22]. If the value continuously decreases and surpasses the normal range, guinea pigs may have anemia due to the lack of RBC production. The severity level of anemia can be detected according to the hematocrit percentage. Lower hematocrit value indicates severe anemia due to the lack of total circulating erythrocytes [23].

Despite the decrease, these three variables of the BV, BVL, and BL groups did not show a significant difference ($p>0.05$) between the last day (day-84). This indicates that the three various diet change steps in 84 days did not affect the erythrocyte production. Beside that, the BVL group had a potential to increase the total erythrocyte, hemoglobin level, and hematocrit percentage (Figure 2a, 2b, 2c). But it is still necessary to do further research on standard diet feeding in a longer time.

MCV values were all elevating for 84 days and negatively correlated to the total erythrocytes. This condition shows that only a small amount of erythrocytes produced but in a large size, known as macrocytic anemia where the size and thickness of erythrocytes exceed normal level. The graph shows that the diet changes were increasing the MCV value. Based on the Anova test, all the groups showed a significant difference ($p<0.05$) during the research. The BV, BVL, and BL groups showed a significant difference between day-0 and day-84 and each was not significantly different ($p>0.05$) by the last day. Similar to the previous parameter, changes in the diet were affecting the MCV value. Despite the increase, the MCV value was still in the baseline and the normal range that is 86.1-95.9 fL [12]. According to

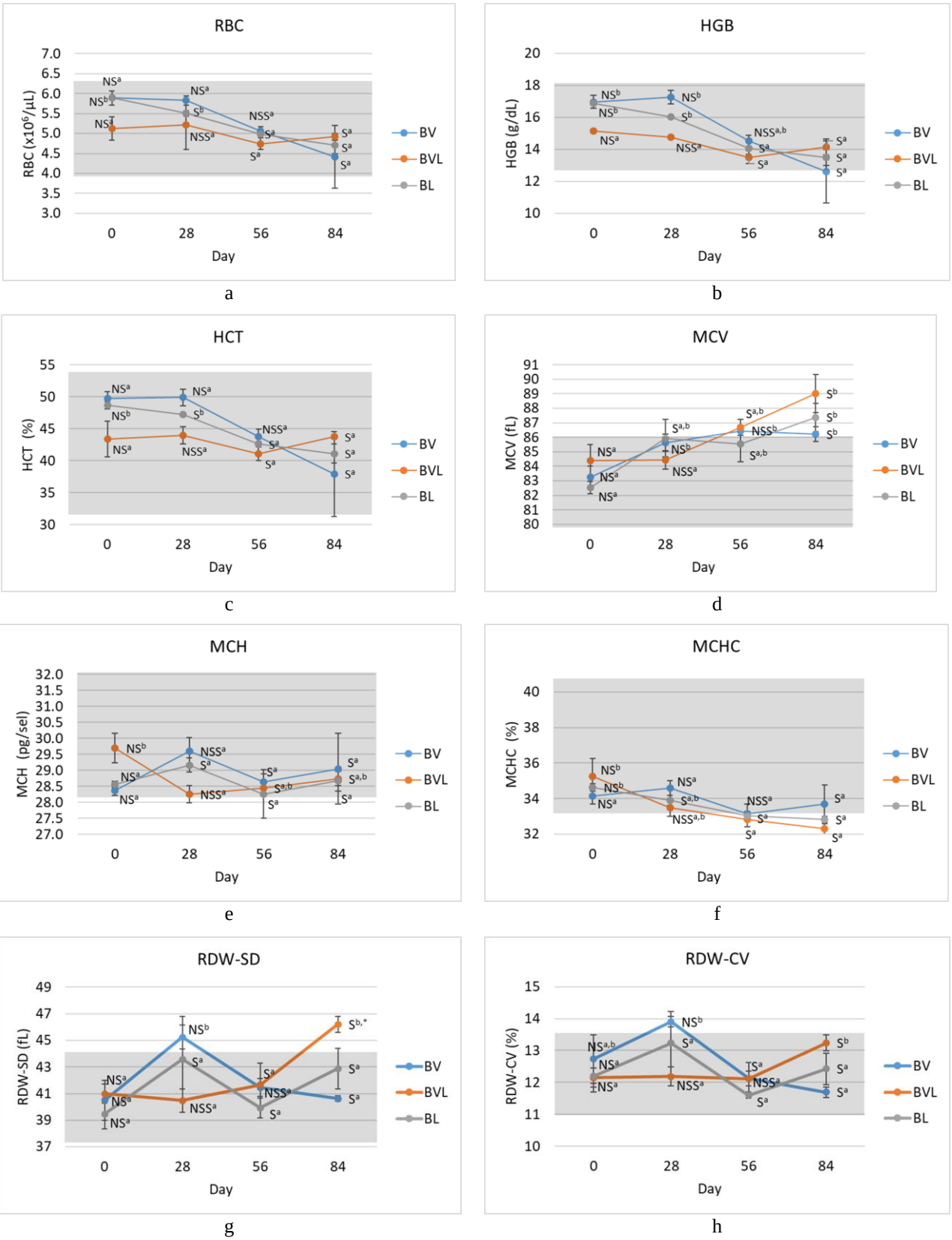


Figure 3. Erythrocyte profile of female guinea pigs with diet variations presented in total red blood cell (a), hemoglobin (b), hematocrit (c), mean corpuscular volume (d), mean corpuscular hemoglobin (e), mean corpuscular hemoglobin concentration (f), red cell distribution width by standard deviation (g) and by coefficient correlation (h). Statistically comparison between days marked by [a/b]; between groups marked by [*]

research [24], elevated MCV values also occur when the plasma concentration increases so that erythrocytes were swelling as the blood samples diluted. Erythrocyte's volume can also increase if the blood sample is left too long in a room temperature [25].

Although the size of erythrocytes was increasing, the mass of hemoglobin in each cell tended to be the same and did not change. MCH values only showed a significant decrease ($p > 0.05$) in the BVL group when they were given a mixture pellet (NSS). Even so, MCH value was fluctuating within the normal range based on the baseline. All the MCH values showed a stable number after being given a standard diet.

MCHC values showed a downward trend for 84 days, especially in the BVL group. The BVL and BL groups showed a significant decrease in the day-56 and day-84. The decrease in MCHC value indicates that the hemoglobin concentration contained in each cell is low due to the large cell. When the erythrocytes enlarged as shown by the MCV value, there was much cytoplasm and the hemoglobin mass was constant so that the hemoglobin concentration went lower. This condition can also be observed through thin blood smear, where erythrocytes appear more discolored due to the lack of hemoglobin content [26].

The examination of RDW values is divided into two types which are RDW-SD (RBC Distribution Width by Standard Deviation) and RDW-CV (RBC Distribution Width by Coefficient Variation) [26]. The RDW-SD value is the standard deviation of each size of erythrocytes so that it is shown in fL units [27]. Meanwhile, the RDW-CV value indicates the percentage of the erythrocyte standard deviation ratio (RDW-SD) to the mean erythrocyte volume (MCV) [28]. Several studies mentioned that the MCV value is also influenced by age. Therefore, the RDW-SD parameter is more widely used to reduce the influence factor of MCV [29]. The RDW value in the BV and BL groups were fluctuating within the normal range, but there is a significant ($p>0,05$) increase of RDW in the BV group (day-28) and BVL group (day-84). This elevated RDW value indicated that large size erythrocytes were produced with many size variations (macrocytic anisocytosis anemia).

Guinea pigs that experienced anemia with a low total erythrocyte and a high rate of erythrocyte destruction have a higher possibility to get more anisocytosis erythrocytes. In addition, low erythropoietin can also affect the formation of abnormal red blood cells [30][31]. The RDW values of the BV and BL groups at the end of the research (day-84) showed significantly different ($p<0.05$) from the BVL group. This indicates that giving prolonged standard diet after going through a gradual change can increase erythrocyte size variation.

3.3. Leukocyte Profile

The female guinea pig leukocyte profile (Figure 4) presented the total and differential leukocyte counts as well as the N/L ratio. Each type of leukocyte has different roles in the immune system, so the differential leukocyte count can be used as indication of guinea pigs physiological condition. The total circulating leukocytes may vary, depending on various conditions such as age and gender. Moreover, the value of each parameter is also affected by stress conditions due to external disturbances or formation abnormalities [14].

In this research, each leukocyte parameter was in the normal range based on the baseline. Examination of the total leukocyte count showed a significant increase ($p>0.05$) on the last day of the BVL group. The elevated total leukocyte count of BVL group was also followed by total neutrophils and lymphocytes. Although the increase only occurred in the BVL group, the total leukocyte count on the last day (day-84) did not show a significant difference ($p<0.05$) between groups. Lymphocyte total and percentage were relatively stable within the normal range. Based on the Anova test, there was no significant difference ($p<0.05$) tested, so the change from nonstandard diet to standard diet did not affect the number and percentage of lymphocytes. Prolonged standard diet after being given a mixed diet led to increase in total neutrophils, as shown in Figure 4d (day-84). This number also tested significantly different ($p>0.05$) with day-0 that may be caused by the diet content. One of the content differences between standard and nonstandard diets is vitamin C which is known to support the immune system. Vitamin C supports the neutrophil through the activity of enzyme cofactors and antioxidants. Fulfilling the vitamin C need can also reduce the level of neutrophil apoptosis [32]. The reduced rate of neutrophil apoptosis may increase the total neutrophils and consequently elevate the percentage. In contrast to nonstandard diets that did not contain vitamin C, standard diet supplemented with vitamin C was able to increase total neutrophils of female guinea pigs.

Mixed cell (MXD) is a leukocyte parameter that combines total eosinophils, basophils, and monocytes because of their small amount in blood circulation, which are 0-7% eosinophils, 0-0.8% basophils, and 1-2.6% monocytes [12]. Therefore, the obtained total cell was calculated as one parameter by the hematology analyzer. Examination of mixed cell total and percentage showed normal values based on the baseline. The results of the Anova test showed that there was no significant difference ($p>0.05$) in 84 days. There was also no significant difference ($p>0.05$) between each group on the last day of the research (day-84), when all were given a standard diet. This shows that the change from nonstandard diet to standard diet did not affect mixed cell total and percentage.

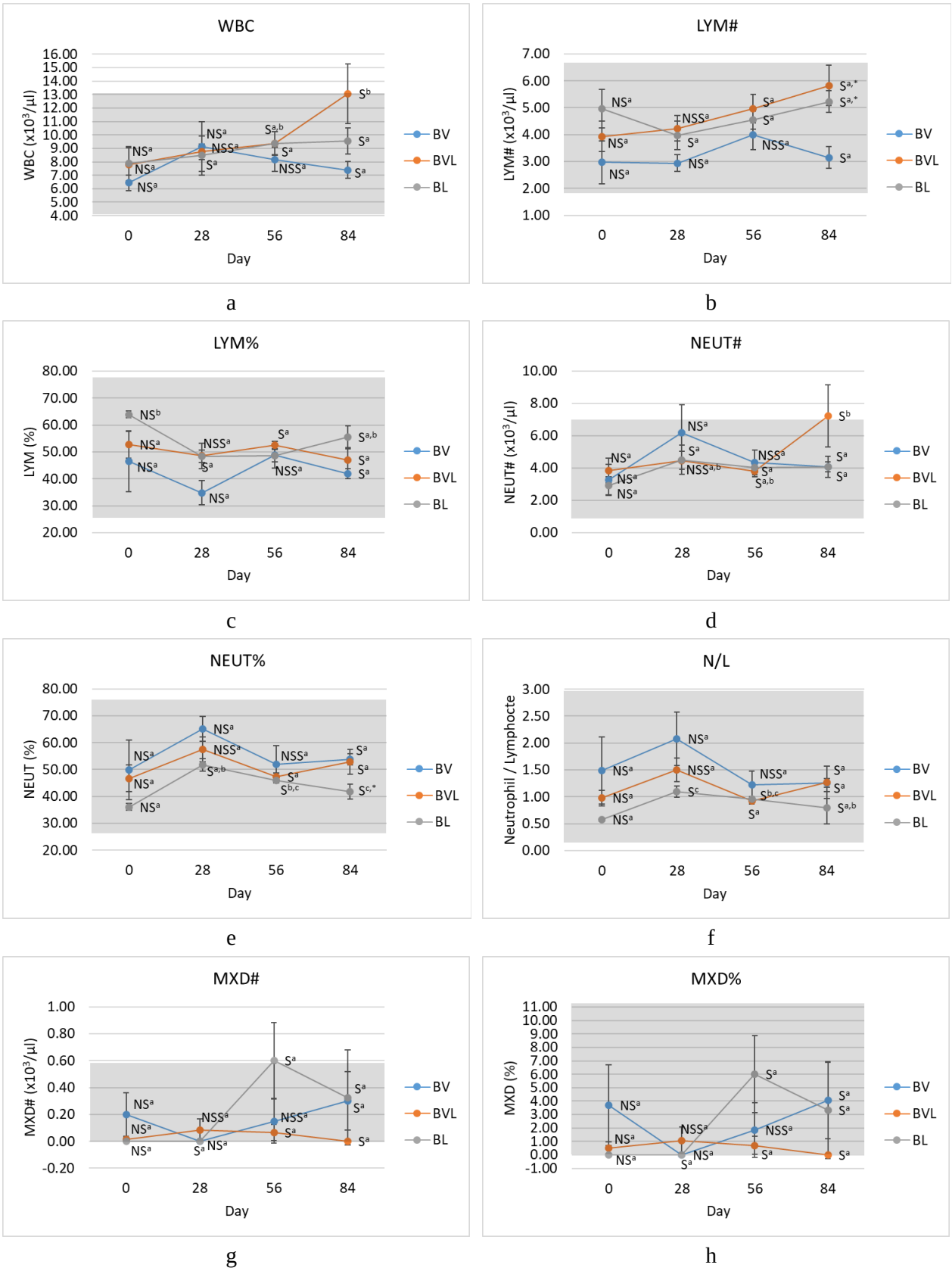


Figure 4. Leukocyte profile of female guinea pigs with diet variations presented in total white blood cell (a), total lymphocyte (b), lymphocyte precentage (c), total neutrophil (d), neutrophil percentage (e), neutrophil/lymphocyte ratio (f), total mixed cell (g), and mixed cell percentage (h). Statistically comparison between days marked by [a/b/c]; between groups marked by [*]

The N/L values were all increasing during the first 28 days, then decreased steadily thereafter. The BVL group showed an increase of N/L values by the last 28 days due to the elevated total neutrophils. Although the value seemed to fluctuate, there was no significant difference ($p>0.05$) tested by Anova between each group by the end of research (day-84). This condition showed that the diet change did not affect the N/L ratio. The BV group showed the highest average N/L value caused by high total neutrophils and low total lymphocytes so that made a high N/L ratio.

3.4. Platelet Profile

The female guinea pig platelet profile was shown in Figure 5. In general, all platelet profile variables were in the normal conditions. All the groups showed that the platelet counts were increasing significantly ($p<0.05$) based on the Anova test between day 0 and day 84. Even so, the difference was not significant ($p>0.05$) between day-56 and day-84. From this result, standard diet was able to significantly increase the platelet count and stabilizing value.

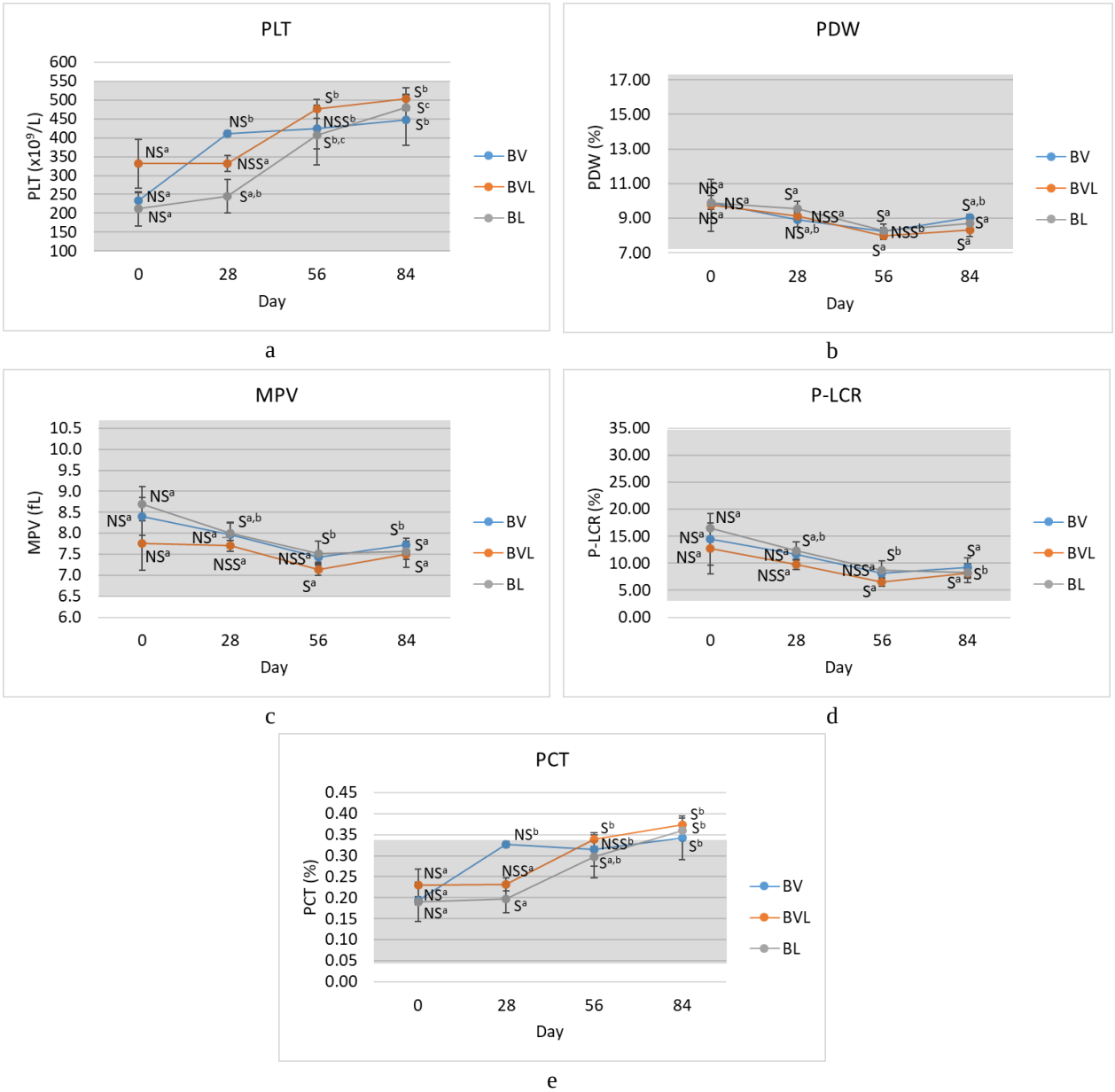


Figure 5. Platelet profile of female guinea pigs with diet variations presented in total platelet (a), platelet distribution width (b), mean platelet volume (c), platelet-larger cell ratio (d), and plateletcrit (e). Statistically comparison between days marked by [a/b/c]; between groups marked by [*]

In addition, it did not take long period for the total platelet to increase after being given a standard diet. However, further research still needs to be done to determine the effect of standard feeding in the long term

because increased total platelets that exceed normal value can cause thrombocytosis [33].

Research by Sahin *et al*. [34] showed that vitamin B12 and folic acid deficiency were able to reduce total platelets. Therefore, fulfilling the needs of vitamin B12 and folic acid through a standard diet helped improve the platelet count. Under these conditions, a standard diet can be given continuously to keep a stable platelet count. This elevated total platelet will consequently increase the plateletcrit value, because it represents the total platelet percentage to the blood volume. By the normal conditions, the total platelet is maintained to stay in a normal amount through regeneration and elimination mechanisms so that the platelet volume remains constant [35]. Therefore, plateletcrit showed a linear result to the total platelet (Figure 5).

Three platelet indices (PDW, MPV, and P-LCR) are variables associated with platelet shape and size variation. The PDW value generally increases when platelets are activated which causes morphological changes. Morphological changes in platelets can range from a rounded form to pseudopodia [36]. The level of anisocytosis in platelets can be known through PDW examination [35]. Large platelet sizes can be detected through MPV examination. The more platelets with large sizes were found, the higher the MPV value would be. When platelet production is low, the formed platelets tend to have a larger size and are more active so which will elevate the MPV value [35]. Heterogeneity of platelets is also related to Platelet-Larger Cell Ratio (P-LCR). The P-LCR value indicates the presence of large platelets with more than 12 fL in volume, expressed in percent units [35].

As shown in Figure 5., the MPV value was decreasing when the platelet count was increasing. This indicates that platelets more platelets produced, the less big-sized platelet formed. The PDW and P-LCR values showed only a slight decrease which indicated that the platelets were produced in a homogeneous form. In all three groups, the significant changes only happened in the BL group. The three platelet indices in the BL group were significantly different on day-0 with day-28, 56, and 84. However, the changes in values were still within the normal range and showed a more stable value so the diet change did not affect the platelet size and morphology.

Based on the hematology examination, it could be known that there were significant changes ($p<0.05$) in several variables during the research (84 days). Although there was a significant change, all the values were still within the normal range. The complementary supplements which are vegetables may keep the hematology value stable. Various vegetables were given every day in the form of cabbage, carrots, mustard greens, spinach, cucumber, kale, and timothy hay. Vitamin C contained in this natural diet was able to help to fulfill the vitamin C needs. Cabbage and kale have high vitamin C content, as much as 32.6 mg/89 g cabbage and 80.4 mg /67 g kale [37][38]. Research in 2019 [39] showed that guinea pigs could be fed by rabbit diet. In addition, sufficient supplemental vitamin C still need to be given every day since there was no significant change in diet consumption, body weight, and morphometric variables between the group fed by guinea pig pellets and group fed by rabbit pellets with sufficient supplemental vitamin C.

3.5. Body Weight and Temperature

Bodyweight and temperature were examined as the basic physiological parameters of the guinea pigs. The result (Figure 6) showed that bodyweight of the BVL groups slowly decreasing when they were given a mixed diet (NSS) until the first standard diet period (week 1-8). However, bodyweight was rising again after prolonged standard diet period. As mentioned before, changes in the diet may be causing weight loss due to eat refusal by the guinea pig. Even only a slight change in the diet will cause complete rejection of the new feed/drink [2].

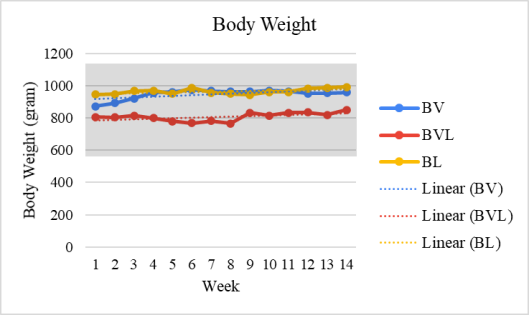


Figure 6. Bodyweight of female guinea pigs with diet variation measured every week.

Standard diet (LabDiet) has a larger pellet size than nonstandard diet (Vittamaxx) and has a slightly different color (Figure 7). Guinea pigs are unable to adapt quickly to changes in type, appearance, and percentage of diet and drink. Even different brands of pellets may also cause guinea pigs to refuse to eat [40]. Therefore, diet changes need to be done very gradually so that it can be well received and met their nutritional needs. One method that can be done is by mixing with the new diet. After

Figure 7. LabDiet and Vittamaxx pellet form. LabDiet pellets have a bigger size and brighter color than Vittamaxx pellet.

adapting for some time, the guinea pigs' weight back increase and became more stable.

Based on the results, the average body weight of guinea pigs increased after being given standard feed. This can happen because the nutrient content in standard diet is available on the right amount for guinea pigs need. The comparison of the nonstandard diet (Vittamaxx) and standard diet (LabDiet) composition was shown in Table 1.

Table 1. Composition comparison between Vittamaxx and LabDiet

Composition	Vittamaxx	LabDiet
Protein	15,0 %	18,0 %
Fat	2,5 %	4,0 %
Fiber	16,0 %	16,0 %
Moisture	10,0 %	10,0 %
Calcium	0,9 %	1,1 %
Phosphor	0,7 %	0,6 %
Vitamin C	-	0,5 mg/gram
Energy	270 kcal/100g	352 kcal/100g

Guinea pigs need protein intake approximately 18–20% to support their growth and development. The standard diet (LabDiet) which contains 18% protein is able to supply the right amount compared to the nonstandard diet (Vittamaxx) which contains only 15% protein. The other nutrient that has a major role for growth and development is fat. Providing enough fatty acid has been proven to increase body weight. Guinea pigs need fat intake in the form of n-6 fatty acid (omega-6) as much as 1,33– 4,0%. Even 0,24% of fatty acid is enough to support the growth, the higher amount can also help to prevent dermatitis. However, excessive fatty acid

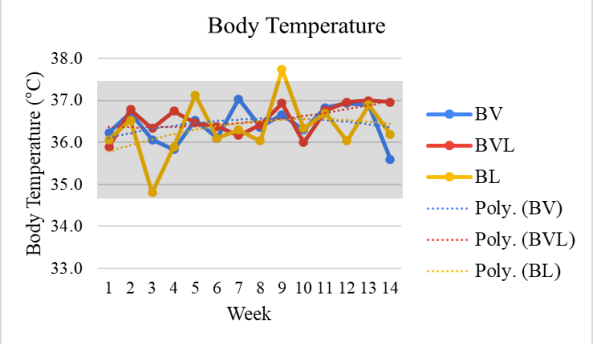


Figure 8. Body temperature of female guinea pigs with diet variation measured every week.

intake may decrease body weight [41][2]. The higher amount of fat contained in the standard diet (LabDiet) can support the growth better than the nonstandard diet (Vittamaxx).

According to Noonan [1] and Pignon & Mayer [5], the guinea pig's rectal temperature ranged from 37.2–39.5 °C. In this research, the guinea pig body temperature was lower than the normal temperature in the reference, which was 34.8–37.7 °C. Different physiological conditions depend on various internal and external factors. Baseline differences in body temperature can be affected by various internal factors such as age, circadian rhythm, metabolism, and ovulation cycles as well as external factors such as temperature (demographic factors), humidity, and examination time [42].

Figure 8 showed that the three groups had a fluctuating body temperature during the research. According to Garami & Székely [43], low food intake can reduce the basal metabolic rate that will lower the body temperature. Therefore, the rejection of a new diet could affect body temperature. The BV and BVL groups showed more stable body temperature due to gradual changes. The BL group showed a decrease in body temperature in the third week. This is caused by the standard diet that was directly given, so that the guinea pig unable to adapt to their new diet.

Based on this research, can be concluded that the diet change from nonstandard to standard diet had significant ($p<0,05$) result only in several parameters which are total erythrocyte (RBC), hemoglobin level (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), total leukocyte (WBC), total neutrophil (NEUT#), total platelet (PLT), and plateletcrit (PCT). However, the change was ranging in the normal value, so the nonstandard diet was still safe to be given with vegetable supplementary every day. But, if the diet still needs to be replaced, it must be done gradually to give the guinea pigs time for adapting to the new diet.

AUTHORS' CONTRIBUTIONS

NNR: Collecting data, data analysis, visualization, manuscript writing, and editing. AAAR: Collecting data, data analysis. LF: Research and article conceptualization, methodology, reviewer, and supervisor.

ACKNOWLEDGMENTS

Author would like to express big thanks to the following people who were contributing to any aspects for their willingness to help, continuously supporting, and all the encouragement, especially to Mrs. Laksmindra Fitria as author's undergraduate thesis supervisor for all the time spent and helpful advice for this research.

REFERENCES

[1] D. Noonan, The guinea pig (*Cavia porcellus*), ANZCCART News, 1994, 7(3), pp. 1–7.

[2] A. Witkowska, J. Price, C. Hughes, D. Smith, K. White, A. Alibhai, C.S. Rutland, The effects of diet on anatomy, physiology, and health in the guinea pig, Journal of Animal Health and Behavioural Science, 2017, 1(1), pp. 1–6.

[3] S.R. Mudambi, M.V. Rajagopal, Fundamentals of foods, nutrition, and diet therapy, 5th ed, New Age International (P) Ltd., Pub, New Delhi, 2007, p. 4.

[4] D.E. Barnard, S.M. Lewis, B.B. Teter, J.E. Thigpen, Open- and closed- formula laboratory animal diets and their importance to research, Journal of the American Association for Laboratory Animal Science, 2009, 48(6), pp. 709–713. PMID: 19930817.

[5] C. Pignon, J. Mayer, Guinea Pigs, in: K.E. Quesenberry, C.J. Orcutt, C. Mans, J.W. Carpenter, Ferrets, Rabbits, and Rodents, 4th ed, W.B. Saunders, Philadelphia, 2020, pp. 270–275.

[6] D.M. Moore, K. Zimmerman, S.A. Smith, Hematological assessment in pet rabbits, blood sample collection and blood cell identification, Veterinary Clinics: Exotic Animal Practice, 2015, 18, pp. 9–19. DOI: https://doi.org/10.1016/j.cvex.2014.09.003.

[7] N.N. Etim, M.E. Williams, U. Akpabio, E.E.A. Offiong, Haematological parameters and factors affecting their values, Agricultural Science, 2014, 2(1), pp. 37–47. DOI: https://doi.org/10.12735/as.v2i1p37.

[8] A.P. Spittler, M.F. Afzali, S.B. Bork, L.H. Burton, L.B. Radakovich, C.A. Seebart, A.R. Moore, K.S. Santangelo, Age- and sex-associated differences in hematology and biochemistry parameters of Dunkin Hartley guinea pigs (*Cavia porcellus*), PLOS ONE, 2021, 16(7), pp. 1–17. DOI: https://doi.org/10.1371/journal.pone.0253794.

[9] A.A.A. Rachid, Profil hematologis marmut (*Cavia porcellus* Linnaeus, 1758) jantan dengan pemberian pakan standar dan nonstandard, Thesis, Universitas Gadjah Mada, Yogyakarta, 2019.

[10] S. Salnus, D. Arwie, Ekstrak antosianin dari ubi ungu (*Ipomoea batatas* L.) sebagai pewarna alami pada sediaan apusan darah tepi, Jurnal Media Analis Kesehatan, 2020, 11(2), pp. 96–103.

[11] T. Fukuda, E. Asou, K. Nogi, K. Goto, Evaluation of mouse red blood cell and platelet counting with an automated hematology analyzer, The Journal of Veterinary Medical Science, 2017, 79(10), pp. 1707–1711. DOI: https://doi.org/10.1292/jvms.17-0387.

[12] K. Zimmerman, D.M. Moore, S.A. Smith, Hematological assessment in pet guinea pigs (*Cavia porcellus*), blood sample collection and blood cell identification, Veterinary Clinics of North America: Exotic Animal Practice, 18 (2015), pp. 33–40. DOI: https://doi.org/10.1016/j.cvex.2014.09.002.

[13] K. Zimmerman, M.D. Moore, S.A. Smith, Hematology of the Guinea Pig, in: D.J. Weiss, K.J. Wardrop, O.W. Schalm, Schalm's Veterinary Hematology, 6th edition, Ames (IA), Wiley-Blackwell, 2010, pp. 893–898.

[14] R.A. Omman, A.R. Kini, Leukocyte Development, Kinetics, and Function, in: E.M. Keohane, C.N. Otto, J.M. Walenga, Rodak's Hematology Clinical Principle and Applications, 6th ed, Elsevier, Canada, 2016, pp. 117–118.

[15] D. Xu, Clinical applications of leukocyte morphological parameters, International Journal of Pathology and Clinical Research, 2015, 1(1), pp. 1–4. ISSN: 2469-5807.

[16] S.C. Genzer, T. Huynh, J.D. Coleman-McCray, J.R. Harmon, S.R. Welch, J.R. Spengler, Hematology and clinical chemistry reference intervals for inbred strain 13/N guinea pigs (*Cavia porcellus*), Journal of the American Association for Laboratory Animal Science, 2019, 58(3), pp. 293–303. DOI: https://doi.org/10.30802/AALAS-JAALAS-18-000118.

[17] A.S. Adewoyin, B. Nwogoh, Peripheral blood film - a review, Annals of Ibadan Postgraduate Medicine, 2014, 12(2), pp. 71–79. PMID: 25960697.

[18] S. de Oliveira, C. Saldanha, An overview about erythrocyte membrane, Clinical Hemorheology and Microcirculation, 2010, 44(2010), pp. 63–74. DOI: https://doi.org/10.3233/CH-2010-1253.

[19] I. Yavasoglu, B. Acar, G. Kadikoylu, Z. Bolaman, Platelet aggregation tests are affected in pseudo thrombocytopenia, Labmedicine, 2010, 41(8), pp. 483–485. DOI: https://doi.org/10.1309/LM9UXAORTFONZ6U5.

[20] E. M. Keohane, An Overview of Clinical Laboratory Hematology, in: E.M. Keohane, C.N. Otto, J.M. Walenga, Rodak's Hematology Clinical Principle and Applications, 6th ed, 2016, Elsevier. Canada. pp. 3–4.

[21] M. Koury, P. Ponka, New insight to erythropoiesis: the roles of folate, vitamin B12, and iron, Annual Review of Nutrition, 2004, 24, pp. 105–131. DOI: https://doi.org/10.1146/annurev.nutr.24.012003.132306.

[22] A.A. Hastreiter, G.G. dos Santos, E.W.C. Santos, E.N. Makiyama, P. Borelli, R.A. Fock, Protein malnutrition impairs bone marrow endothelial cells affecting hematopoiesis, Clinical Nutrition, 39(5), pp. 1551–1559. DOI: https://doi.org/10.1016/j.clnu.2019.06.021

[23] T.D. Johnson-Wembley, D.Y. Graham, Diagnosis and management of iron deficiency anemia in the 21st century, Therapeutic Advances in Gastroenterology, 2011, 4(3), pp. 177–184. DOI: https://doi.org/10.1177/1756283X11398736.

[24] C.A. Moore, A. Adil, Macrocytic Anemia. StatPearls, Treasure Island (FL), 2020.

[25] S. Batool, R. Iqbal, Macrocytic anemia: a review, Journal of Entomology and Zoology Studies, 2016, 4(5), pp. 544–547. ISSN: 2320-7078.

[26] B. Ciesla, Hematology in Practice. F.A. Davis Company. Philadelphia, PA, 2007, pp. 51–55.

[27] F.A. Caporal, S.R. Comar, Evaluation of RDW-CV, RDW-SD, and MATH-1SD for the detection of erythrocyte anisocytosis observed by optical microscopy, The Jornal Brasileiro de Patologia e Medicina Laboratorial, 2013, 45(5), pp. 324–331. DOI: https://doi.org/10.1590/S1676-24442013000500005.

[28] C. Fava, F. Cattazzo, Z. Hu, G. Lippi, M. Montagnana, The role of red blood cell distribution width (RDW) in cardiovascular risk assessment: useful or hype?, Annals of Translational Medicine, 2019, 7(20), pp. 581–590. DOI: https://doi.org/10.21037/atm.2019.09.58.

[29] J.J.M.L. Hoffman, K.C.A.M. Nabbe, N.M.A. van den Broek, Effect of age and gender on reference intervals of red blood cell distribution width (RDW) and mean red cell volume (MCV), Clinical Chemistry and Laboratory Medicine, 2015, 53(12), pp. 2015–2019. DOI: https://doi.org/10.1515/cclm-2015-0155.

[30] M. E. Emans, K. van der Putten, K.L. van Rooijen, R.J. Kraaijenhagen, D. Swinkels, W.W. van Solinge, M.J. Cramer, P.A.F.M. Doevendans, B. Braam, C.A.J.M. and Gaillard, Determinants of red cell distribution width (RDW) in cardiorenal patients: RDW is not related to erythropoietin resistance, Journal of Cardiac Failure, 2012, 17(8), pp. 626–633. DOI: https://doi.org/10.1016/j.cardfail.2011.04.009.

[31] C. P. Titcomb, Red cell distribution width (RDW): an underappreciated marker for increased mortality, On the Risk, 2017, 33(1), pp. 30–46.

[32] M. Liugan, A.C. Carr, Vitamin C and Neutrophil Function: Findings from Randomized Controlled Trials, Nutrients, 2019, 11(2102), pp. 1–16. DOI: https://doi.org/10.3390/nu11092102.

[33] K. Jimenez, F. Leitner, A. Leitner, G. Scharbert, P. Schwabls, A. Kramer, A. Krnjic, J. Friske, T. Helbich, R. Evstatiev, V. Khare, C. Gasche, Iron deficiency-induced thrombocytosis increases thrombotic tendency in rats, Haematologica, 2021, 106(3), pp. 782–794. DOI: https://doi.org/10.3324/haematol.2019.245092.

[34] K. Şahin, M. Elevli, C. Coşkun, M. Koldaş, The effects of vitamin B12 and folic acid deficiency on hemogram parameters in children, Medical Science and Discovery, 2019, 6(9), pp. 186–191. DOI: https://doi.org/10.36472/msd.v6i9.290.

[35] Y.U. Budak, M. Polat, K. Huysal, the use of platelet indices, plateletcrit, mean platelet volume, and platelet distribution width in emergency non-traumatic abdominal surgery: a systematic review, Biochemia Medica, 2016, 26(2), pp. 179–193. DOI: https://doi.org/10.11613/BM.2016.020.

[36] E. Vagdatli, E. Gounari, E. Lazaridou, E. Katsibourlia, F. Tsikopoulou, I. Labrianou, Platelet distribution width: a simple, practical, and specific marker of activation of coagulation, HIPPOKRATIA, 2010, 14(1), pp. 28–32. PMID: 20411056.

[37] Nutrition Data, Cabbage, raw Nutrition Facts & Calories, 2021, https://nutritiondata.self.com/

[38] Nutrition Data, Kale, raw Nutrition Facts & Calories, 2021, https://nutritiondata.self.com/

[39] F. Trejo-Sánchez, G. Mendoza-Martínez, F. Plata-Pérez, J. Martínez-García, O.A. Villarreal-Espino-Barros, Growth of guinea pigs (*Cavia porcellus*) with feed forrabbits and supplementation of vitamin C, Revista MVZ Córdoba, 2019, 24(3), pp. 7286–7290. DOI: https://doi.org/10.21897/rmvz.1384.

[40] K.E. Quesenberry, T.M. Donnelly, C. Mans, Biology, Husbandry, and Clinical Techniques of Guinea Pigs and Chinchillas, in: K. Quesenberry, J. Carpenter, Ferrets, Rabbits, and Rodents, 3rd ed, W.B. Saunders, Philadelphia, 2011, pp. 279–280.

[41] National Research Council (US) Subcommittee on Laboratory Animal Nutrition, Nutrient Requirements of Laboratory Animals, 4th Revised ed, National Academies Press, Washington, DC, 1995, https://www.ncbi.nlm.nih.gov/books/NBK231932/

[42] Z. Obermeyer, J.K. Samra, S. Mullainathan, Individual differences in normal body temperature:

longitudinal big data analysis of patient records, BMJ, 2017, 359: j5468. DOI: https://doi.org/10.1136/bmj.j5468.

[43] A. Garami, M. Székely, Body temperature: Its regulation in framework of energy balance, Temperature, Austin, 2014, 1(1), pp. 28–29. DOI: https://doi.org/10.4161/temp.29060.