Comparison of Rat Cecum Appearances Fed with Different Fat Sources Using Smartphone-Based Image Analysis

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Abstract. The aim of the study was to compare the rat cecum color and/appearances which fed for 13 weeks with high-fat diet (HFD) which three different fat sources (beef tallow, corn oil, and palm oil) were used. Fresh cecum was photograph during the necropsy using smartphone. The images were analyzed using photoshop software to get the L^*, a^*, and b^* values. The results showed that no significant differences between the three groups in the color of cecum (the means of L^*, a^*, and b^* values), although we can see that the appearances from beef tallow and corn oil groups were slightly darker that palm oil group.

Keywords: rat · cecum · palm oil · appearance · color · smartphone

1 Introduction

Dietary guidelines since decades ago recommend limiting the amount of total fat intake to maintain overall health. At the same time, the level of available evidence regarding the relationship between fat intake and risk of diabetes and metabolic syndrome is still insufficient [1]. Recently, a study showed that increased fat intake reduced mortality and was not associated with cardiovascular disease [2] nor incidence of type 2 diabetes [3]. Dietary intake of saturated fatty acids, which has long been identified as unhealthy, is not associated with the risk of insulin resistance or cardiovascular disease [4–6]. Currently, high-fat diet was still believed to be related to the incidence of colon cancer [7].

Palm oil is widely used oil in food processing industry and has become the most important vegetable oil in the world [8]. The fatty acid composition of palm oil is quite different from that of other vegetable oil, which mainly composed palmitic acid (44%) dan oleic acid (39%), with total saturated fatty acids and unsaturated fatty acids similar in percentage (49% and 49%) [9]. Although the consumption of palm oil often caused concern because its composition, currently there is no scientific evidence (experimental or epidemiological) to warrant associating palm oil consumption with a higher cancer incidence or mortality risk in human [10]. Nevertheless, the health benefit of red palm oil in animal and human studies have been reviewed, these including improvement

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cardioprotective effects in ischemic heart disease, antiatherogenic, antihemorrhagic, antihypertensive, and anticancer properties [11].

Animal models (rat and/or mouse) has been widely used in studies involving high-fat diet, including the organ-specific pathologies associated with exposure to a maternal high-fat diet [12]. Occasionally, study protocols are received which require the necropsy of large numbers of animals in a short period of time. Sometimes, differences in diet could affect the shape, size, color, and appearance of the rodent's organs [13], including cecum [14]. Therefore, since necropsy is an unrepeatable procedure, the photographic documentation should be accurate and detailed without much delay in the execution time of the necropsy. With its portability, connectivity, and their constantly improving image performance [15, 16], the smartphone cameras can be used for this purposed.

The effects of the type of fat sources in the diet to the appearance and color of rat organ is a question that remains to be answered, defining whether it will give the same appearance or not. The implementation of smartphone-based image analysis during the necropsy of laboratory animals provides its own advantages, with simple and reliable results. The present study was conducted to compare the appearance and color indexes of selected organ of rats fed with 3 different types of fat sources (beef tallow, corn oil, and refined bleached deodorized palm oil (RBDPO)) using smartphone-based image analysis.

2 Materials and Methods

2.1 Animal and Experimental Protocols

Male Sprague Dawley rats (3 weeks old) were obtained from The Center for Food and Nutrition Studies Laboratory Animal Center, Universitas Gadjah Mada and housed in individual cages in a room at 25–26 °C and 50–60% relative humidity, with a 12 h light-dark cycle. The rats were fed a pellet diet for 1 week before commencement of the experimental diet, and were assigned randomly to one of three groups. Animal care maintained according to the "Guide for the Care and Use of Laboratory Animals" established by Universitas Gadjah Mada and approved by the Medical and Health Research Ethic Committee of Universitas Gadjah Mada. Rats were divided into 3 groups ($n = 3$), and fed by the following experimental diets (AIN-93): a beef tallow diet containing 20% casein and 20% beef tallow, corn oil diet containing 20% casein and 20% corn oil, and palm oil diet containing 20% casein and 20% RBDPO. All the diets were completely consumed everyday until next day 12:00 a.m. Thirteen weeks after feeding the diets, animals were killed by cervical dislocation with euthanasia of diethyl ether.

2.2 Smartphone Setup

A smartphone (Model iPhone 11 Pro Max, Apple Inc., California USA) was used by holding (handheld) it vertically just above the rat organ at a distance about 20 cm. The main camera (wide angle) and native camera application were used for image acquisition. The camera settings as follows: camera capture most compatible format, ISO auto, shutter speed auto, aperture lens auto, white balance auto, exposure program normal, metering mode pattern, no flash, daylight conditions, resolution 4032x3024 pixels, color space RGB, with the images saved in jpeg.

2.3 Image Analysis

The Adobe Photoshop CC 2019 Software for Mac (Adobe Systems Inc., San Jose, California) was use to obtain the color parameters from the digital images of rat organ surface. The images were open in the photoshop with image mode Lab Color chosen. The software was capable of displaying the color parameters *L* *, *a* *, *b* *, a model commonly used in food research [17]. The measurement or color analysis of the sample was based on what has been suggested by Papadakis *et al*. [18]. One of the tools in the Photoshop used in this research was Magic Wand Tool and Quick Selection Tool. With these tools, we can choose the field of images that we want to be analyzed further. We can select the dark or bright surface part of the sample with the Magic Wand Tool, for which we can then measure *L* *, *a* *, *b* *, and the precentage of the color surface differences. The selected image range was controlled with the tolerance value of the Magic Wand Tool, which is used for this research value 50.

2.4 Statistical Analysis

To compare mean values, one-way ANOVA was used for color index and surface appearance. The Tukey-Kramer post hoc test was conducted when a significant effect was found by one-way ANOVA. Data analysis was performed using SPSS Statistics for Mac (version 26.0; IBM SPSS Statistics). Differences were considered as differently significant at $P < 0.05$.

3 Results and Discussions

The appearance of fresh cecum in three groups of rats fed with high-fat diet which different in fat sources were shown in Figs. 1, 2, and 3. Although the color of the cecum from beef tallow and corn oil groups looked darker compared palm oil group, no significant differences were observed in *L**, *a**, and *b** mean values (Table 1).

The cecum in rats is blind sac-like organ located at the junction of the ileum and the proximal colon [19]. The cecum provides storage food (especially fiber-rich plant) while bacteria break down the cellulose. Therefore, usually it is relatively large and almost

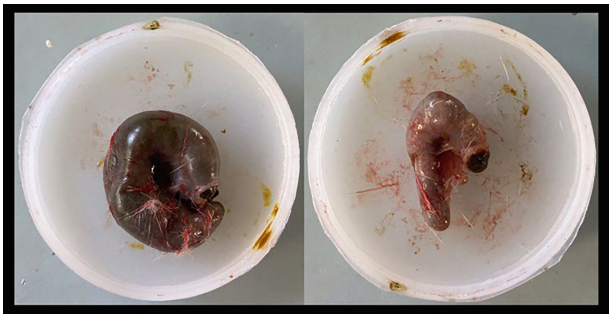

Fig. 1. The appearances of the cecum in Sprague Dawley rats fed with 20% beef tallow diet for 13 weeks

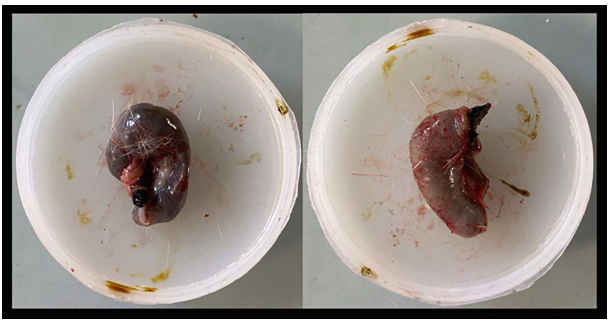

Fig. 2. The appearances of the cecum in Sprague Dawley rats fed with 20% corn oil diet for 13 weeks

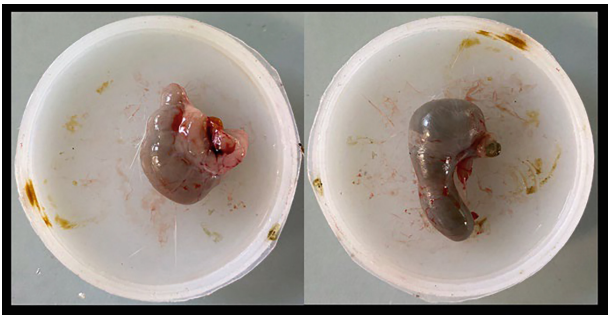

Fig. 3. The appearances of the cecum in Sprague Dawley rats fed with 20% palm oil (RBDPO) diet for 13 weeks

Table 1. The L^*, a^*, and b^* mean values of the cecum from three groups of rats fed with beef tallow, corn oil, and palm oil (means ± SE)

Value	Beef Tallow	Corn Oil	Palm Oil
L^*	29.87 ± 4.07	24.60 ± 3.22	38.21 ± 4.39
a^*	14.38 ± 4.00	13.41 ± 3.21	13.30 ± 4.15
b^*	12.64 ± 3.10	9.52 ± 3.14	13.36 ± 1.73

always filled with the dietary content. The study that discuss the correlation between diet and cecum color and/or appearances still limited. Because the rat cecum is the main site of fermentations, generally the study observed the relationship between diet and the parameters of cecal digestion, for example: short-chain fatty acids and/or bile acids production, absorption of nutrients, and bacterial microflora. It would be interesting to study further whether there are any relationships between cecum color or appearances with those parameters of cecal digestion. The cecum image resulted from our study (Fig. 4) could be useful for that purpose.

Fig. 4. The cecum image after we applied magic wand tool and/or quick selection tool in photoshop for the lab color analysis (A: beef tallow; B: corn oil; and C: palm oil)

4 Conclusion

Generally, color and appearance of the cecum from rats with beef tallow and corn oil diets slightly darker, if we compared from rats with palm oil (RBDPO) diet. The use of smartphone image analysis can provide a favourable assessment, with fast and easy to implemented, especially for the documentation of the rats organ during necropsy.

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