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The Effects of Food Deprivation on Hematological Indices and Blood Indicators of Liver Function in *Oxyleotris marmorata*

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Abstract*—Oxyleotris marmorata*** is considered as undomesticated fish, and its culture occasionally faces a problem of food deprivation. The present study aims to evaluate alteration of hematological indices, blood chemical associated with liver function during 4 weeks of fasting. A non-linear relationships between fasting days and hematological parameters (red blood cell number; $y = -0.002x^2 + 0.041x + 1.249$; R^2=0.915, P<0.05, hemoglobin; $y = -0.002x^2 + 0.030x + 3.470$; R^2=0.460, P>0.05), mean corpuscular volume; $y = -0.180x^2 + 2.183x + 149.61$; R^2=0.732, P>0.05, mean corpuscular hemoglobin; $y = -0.041x^2 + 0.862x + 29.864$; R^2=0.818, P>0.05 and mean corpuscular hemoglobin concentration; $y = -0.044x^2 + 0.711x + 21.580$; R^2=0.730, P>0.05) were demonstrated. Significant change in hematocrit (Ht) during fasting period was observed. Ht elevated sharply increase at the first weeks of fasting period. Higher Ht also was detected during week 2-4 of fasting time. The significant reduction of hepatosomatic index was observed ($y = -0.007x^2 - 0.096x + 1.414$; R^2=0.968, P<0.05). Moreover, alteration of enzyme associated with liver function was evaluated during 4 weeks of fasting (alkalin phosphatase; $y = -0.026x^2 - 0.935x + 12.188$; R^2=0.737, P>0.05, serum glutamic oxaloacetic transaminase; $y = 0.005x^2 – 0.201x^2 + 1.297x + 33.256$; R^2=1, P<0.01, serum glutamic pyruvic transaminase; $y = 0.007x^2 – 0.274x^2 + 2.277x + 25.257$; R^2=0.807, P>0.05). Taken together, prolonged fasting has deleterious effects on hematological indices, liver mass and enzyme associated in liver function. The marked adverse effects occurred after the first week of fasting state.

***Keywords*—**food deprivation, *Oxyleotris marmorata*, hematology, alkaline phosphatase, SGOT, SGPT

I. Introduction

FISH is an animal that withstand a variety of food deprivation periods. Wild fish undergo period of food deprivation in their lifetime due to the seasonal fluctuation of food availability and migration trip. Atlantic salmon and eel swim long distances for mating without eating, reviewed in [1]. It is worth to understand the allocation process of storage energy utilization for vital biological processes, maturation of gonad, and extreme exercise. In aquaculture system, although food is available, several fish also undergo periods of starvation such as during the stressful conditions such as fluctuation of water quality and disease outbreak. Culture of undomesticated fish occasionally faces a problem of starvation during capture, transportation, and acclimation to farm condition. Therefore, investigation biological responses to food deprivation in fish would provide valid information for comparative physiology of food deprivation.

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Hematological indices are able to use as indicator to asses nutrition status [2-4]. Food deprivation was reported to affect alteration of hematological indices, and the alteration depended on period of starvation [5-6]. Liver is the main organ that responsible to allocate the nutrient storage for vital process during starvation period. Effect of food deprivation was found in the relative liver size [4-8]. However, the significant change in liver size was not observed in [9]. In salmonid, food deprivation affected the changes in several enzymes associated including alkaline phosphatase, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT). In addition, the alteration of these enzymes varied depending on the species and rearing condition [10].

Marbled sleeper, *Oxyleotris marmorata*, is an economically important freshwater fish in Southeast Asia. It is considered as undomesticated fish and strict carnivores. Its culture faces problems of the collection of juvenile fish from river basin, transportation, and acclimation to farm condition. During production process, it occasionally withstands a starvation period. Therefore, the study on the effects of food deprivation on the health status interpretation by hematological indices and enzyme associated with liver function would provide valid biological data for its aquaculture. The aim of the present study is to investigate the effect of food deprivation on the alteration of hematological indices and blood chemical related to liver function and liver morphology.

II. Materials and Methods

A. Experimental design, fish and culturing system

Marbled sleeper were collected from Chao Praya River basins. After fish were transferred to Suranaree University of Technology Farm, they were acclimated hatchery condition to concrete pond (2 W x 2 L x 1 H m) under continuous conditions for two weeks. Fish were fed ad libitum on fresh teleost meat once daily. Before the beginning the fasting experiment, twelve fish (50-100 g) were again acclimatize to aquarium (80 L) by randomly selected to each aquarium. A flow-through water change system was implemented by replacing one-half of water with dechlorinated water every three days. The temperature in the culture system was maintained at ambient temperature (28-30 °C). Fish were fed on fresh teleost meat once daily, and only fish that accepted the giving food for one month were used in this experiment. Six aquaria (replications) of fish that eat fresh teleost meat normally were used to test the validity of results.

B. Fish sampling

Fish samplings for hematological and blood chemical analyses were conducted at 0, 3, 7, 14, 21, 28 days of food deprivation. On the sampling days, the fish were not fed for

18 h prior blood sampling. Two representative fish were selected and anaesthetized with 2-phenoxyethanol (0.35 ml L^{-1}). Blood sample was collected by a hypodermic syringe from the caudal vein and divided into two sets. One set was added to the tube containing 1.0 % (v/v) of 15 % Na_2EDTA, and the other was allowed to clotted at room temperature for 1 h. The serum was collected by centrifuging the clotted blood at 5,000 rpm for 10 min at room temperature and stored at -80 ^{o}C for further analysis. After blood sampling, liver was dissected from each fish, and the hepatosomatic index was determined

C. Haematological assays

Immediately after blood sampling, EDTA blood was used to analyze haematological parameters. The hematocrit (HT) was determined in duplicate by centrifuging the whole blood for 5 min by microhematocrit centrifugation and then measuring the packed cell volume (PCV). The hemoglobin [Hb] was determined by the photometrical cyanohaemoglobin method use. The red blood cell count (RBC) was determined optically with a Neubauer chamber using a 1:200 dilution of the blood sample in Grower's solution [11]. In addition, the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated from the Ht, [Hb], and RBC individually, as described in Voigt [11].

D. Blood chemistry analysis

The serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were measured using Reitman & Frankel's colorimetric mehod [12]. In addition, alkaline phosphatase was examined using PMP method [13].

E. Statistical analysis

The analyses were carried out using SPSS for Windows, version 10 (SPSS Inc., Chicago, IL). All data were analysed by one-way analysis of variance (ANOVA). Data were compared using Duncan's multiple-range test when F values were significant (P<0.05). Regression analysis of the fasting time (x) and each hematological and blood chemical parameters together with goodness of fit (R^2) were conducted. Differences was declared to be significant when the values was < 0.05 (P<0.05) throughout this study.

III. Result

The changes is hematological parameters during starving period were shown in Fig. 1. Although there was no significant differences in RBC and [Hb] during fasting period, RBC and [Hb] decreased at the beginning of fasting (3 days) and then increased during 7-14 days of fasting days (Fig. 1A-B). RBC and [Hb] decreased again during 21-28 fasting days. In addition, a significant non-linear relationship between fasting time and RBC was observed ($y=-0.002x^2+0.041x+1.249$; R^2=0.915, P<0.05). Significant changes in Ht was detected during experimental period (Fig. 1C) (P<0.05). Ht decreased at the early fasting time and then increased after the first week of fasting. Ht appeared to decrease gradually after the second week of fasting. There were no significant changes in MCV, MCH and MCHC during the 4 weeks of fasting period (Fig. 1D-F). Nevertheless, MCV and MCH tended to reduced at the early fasting time (P=0.080 and P=0.067, respectively). MCV and MCH appeared to increase at 28 days after fasting. MCHC tended to gradually decreased during 4 weeks of fasting (P=0.258).

Liver is the one of most important organ for starvation response to food deprivation period. In this study, we evaluate change in liver weight and several enzymes that indicating the function of liver. Means HSI decreased progressively with a significant trend ($y = 0.007x^2 – 0.096x + 1.414$; R^2=0.968, P<0.05) during fasting period (P<0.05) (Fig. 2). Alkaline phosphatase significantly decreased during after 1-4 weeks of fasting time (P<0.05); however, no significant trend was observed (Fig. 3). SGOT drastically decreased after 1-4 week of fasting time with a significant trend ($y = 0.005x^3 – 0.201 x^2 + 1.297x + 33.256$; R^2=1, P<0.01) (Fig. 4). SGPT increased during the first week of fasting time; however, no significant differences were observed (P>0.05) (Fig. 5).

IV. Discussion

Carnivorous fish naturally withstand fasting state during waiting for the next meal. Although food is available, a number of cultured fish which are still undomesticated often experience fasting state during acclimating process. *O. marmorata* is an example of fish which is considered an undomesticated and strict carnivore. The present study demonstrated the effects of fasting state on hematological and blood chemical related to liver function in *O. marmorata*, providing valid information on the response to starvation in fish culture.

Hematological indices are among the pathophysiological values used to interpret fish health status. Numerous studies have discussed the potential use of hematological values for the health status of fish [2-4]. Fasting state affected to decrease RBC which drastically reduced after four weeks of fasting. Similarly, in *Prochilodus lineatus*, gradual decrease in RBC was observed during the five weeks of fasting, and the main reduction in RBC occurred after five weeks of fasting Phase [5]. However, no significant change in RBC was observed in channel catfish at 2 and 4 weeks of food deprivation [6]. Nevertheless, elevation of RBC was reported in wolf [14]. The effect of starvation on [Hb] varies across studies. The present study showed the non-significant decreased trend of [Hb]. Our present results were similar to that of the study in fasted largemouth bass (*Micropterus salmoides*) [4]. Channel catfish showed no significant changed in [Hb] after 2 fasting weeks; however, [Hb] was significant increased after 4 fasting weeks [6]. The increase in [Hb] was also reveaed in wolf [14]. The increase in the Ht during 1 and 2 weeks of fasting was found in this study and *P. lineatus* [5].

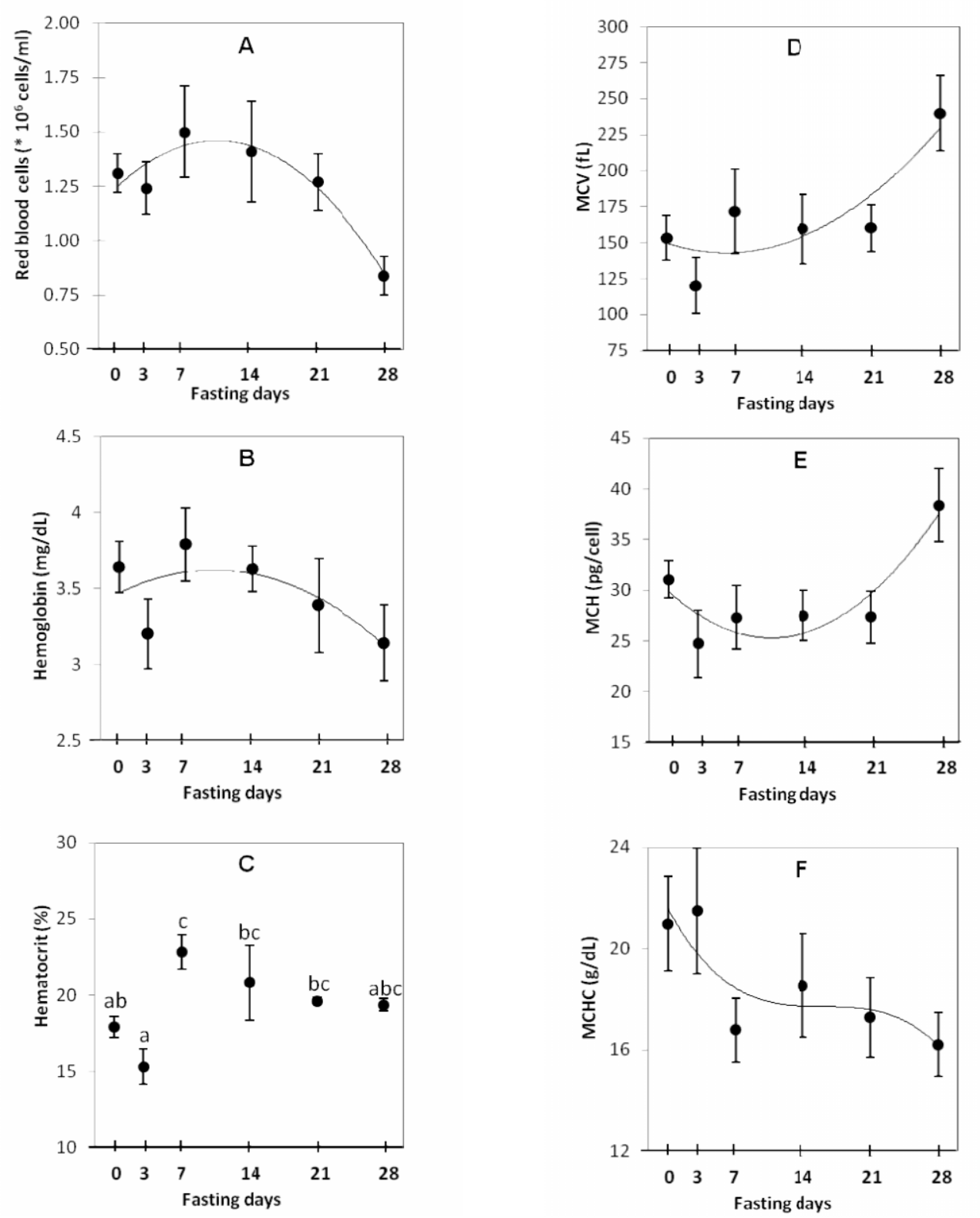

Fig. 1 Alteration of hematological parameters during fasting period. (A) Red blood cell number (RBC), (B) Hemoglobin, (C) Hematocrit, (D) Mean corpuscular volume (MCV), (E) Mean corpuscular hemoglobin (MCH), (F) mean corpuscular hemoglobin concentration (MCHC). A non-linear relationship was observed between fasting days and RBC ($y = -0.002x^2 + 0.041x + 1.249$; R^2=0.915, P<0.05), hemoglobin ($y = -0.002x^2 + 0.030x + 3.470$; R^2=0.460, P>0.05) (B), MCV ($y = -0.180x^2 + 2.183x + 149.61$; R^2=0.732, P>0.05), MCH ($y = -0.041x^2 + 0.862x + 29.864$; R^2=0.818, P>0.05), or MCHC ($y = -0.044x^2 + 0.711x + 21.580$; R^2=0.730, P>0.05). Values are means $\pm$ SEM, n = 6 (0, 3, 7, 14 days), 4 (21, 28 days). Different letters indicate significant difference (P<0.05)

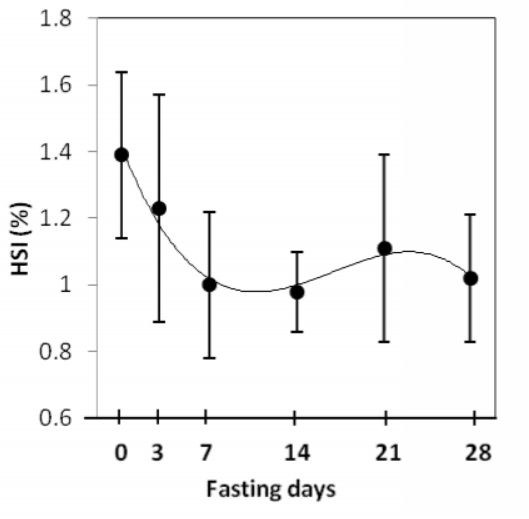

Fig. 2 Alteration of Hepatosomatic index (HSI) during fasting period. A non-linear relationship was observed between fasting days and HSI ($y = -0.007x^2 - 0.096x + 1.414$; R^2=0.968, P<0.05). Values are means ± SEM, n = 6 (0, 3, 7, 14 days), 4 (21, 28 days)

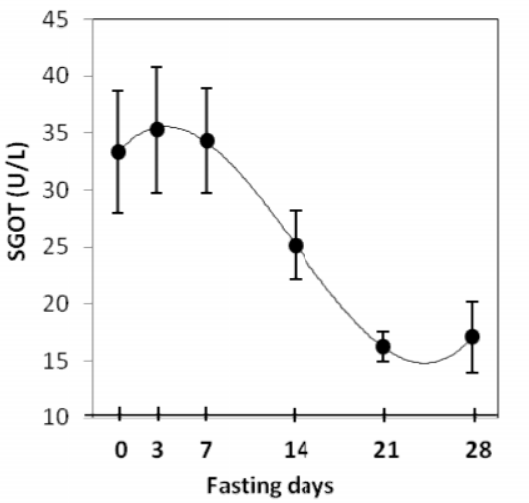

Fig. 4 Alteration of serum glutamic oxaloacetic transaminase (SGOT) during fasting period. A non-linear relationship was observed between fasting days and SGOT ($y = 0.005x^2 – 0.201x^2 + 1.297x + 33.256$; R^2=1, P<0.01). Values are means ± SEM, n = 6 (0, 3, 7, 14 days), 4 (21, 28 days)

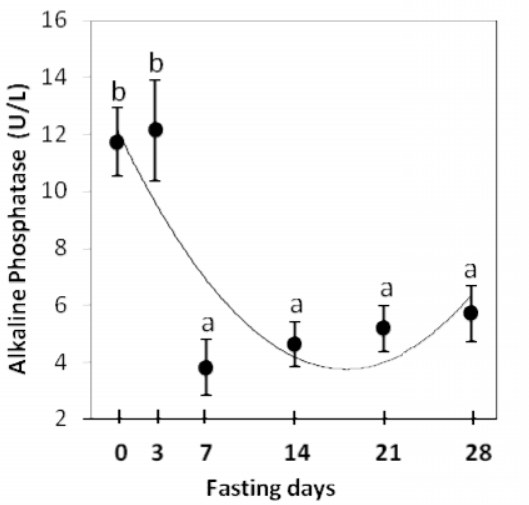

Fig. 3 Alteration of alkaline phosphatase during fasting days. A non-linear relationship was observed between fasting days and alkaline phosphatase ($y = -0.026x^2 - 0.935x + 12.188$; R^2=0.737, P>0.05). Values are means ± SEM, n = 6 (0, 3, 7, 14 days), 4 (21, 28 days). Different letters indicate significant difference (P<0.05)

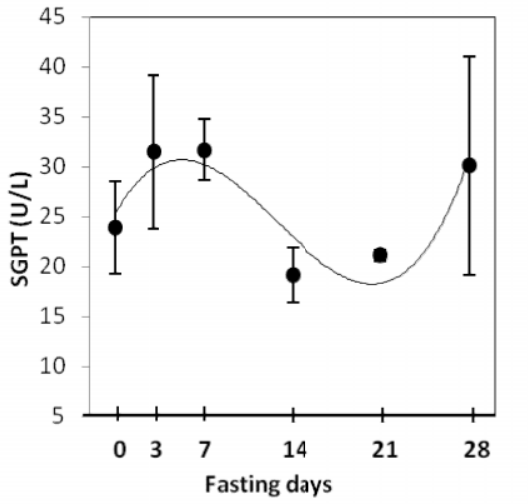

Fig. 5 Alteration of serum glutamic pyruvic transaminase (SGPT) during fasting period. A non-linear relationship was observed between fasting days and SGOT ($y = 0.007x^2 – 0.274x^2 + 2.277x + 25.257$; R^2=0.807, P>0.05). Values are means ± SEM, n = 6 (0, 3, 7, 14 days), 4 (21, 28 days)

It has been demonstrated that hematological values respond to a stressful condition in humans. A stressful condition elevated hemoconcentration [15]. Hemoconcentration relates to a mechanism of red blood cell swelling in which fluid moves from plasma into red blood cells. In addition, the release of red blood cells from the producing organ increases hemoconcentration. Hemoconcentration is also due to reduced plasma volume with normal red blood cell mass. Swelling of erythrocytes was induced by adrenergic stimulation in nucleated red blood cell [16]. In this study, an increase in the Ht percentage and MCV with decreasing MCHC indicated that the hemoconcentration was caused by red blood cells swelling. Contradictory, Ht was not altered by fasting state in largemouth bass and channel catfish [4,6]. Liver functions for food metabolism as storage of macro-and micro-nutrient. Consequently, liver is the main organ to ameliorate the stress-mediated adverse effects in vertebrates. Although HSI did not significantly differ at various analyzed fasting time, a significant reduction trend was observed during fasting period. The effect of food deprivation on the reduction in liver mass was reported in a number of fish including *P. leneatus*, channel catfish, gilthead seabream, red porgy, and largemouth bass [4-7]. Moreover, in tench, the reduction of HSI causing by fasting for a week was increased after refeeding for 2 days [17]. On the other hand, *Dentex dentex* deprived nof food for 5 weeks did not showed significant decrease in HSI [9]. Therefore, the response of starvation would vary among fish. We further evaluated the changes in enzyme associated with liver function. Metabolic alteration affected the changes in SGOT and SGPT in human [18]. In contrast, fasting did not affect SGOT, SGPT and alkaline phosphatse in wolf [14]. The present result showed that SGOT and SGPT increase gradual during the first week of fasting, thereafter, they markedly decreased following week 2-4. Additionally, the significant reduction was detected only SGOT. Similar fasting effect on SGPT and SGOT was demonstrated in Atlantic salmonin [19]. The significant reduction in alkaline phosphatase was observed after the first week of fasting which was consistent to the report in juvenile salmonids [10]. Furthermore, food deprivation had deleterious effects on the reduction in the intestinal alkaline phosphatase in European glass eels [20-21].

In conclusion, prolonged fasting has deleterious effects on hematological indices, liver mass and enzyme associated in liver function. The marked adverse effects occurred after the first week of fasting state.

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