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Effect of Water Intake on Sprague-Dawley Rat Off Spring's Linear Growth

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Abstract: The present study was designed to analyze the effect of water intake rat offspring's body length and IGF-1 hormone level of rats An experimental animal study using randomized block design was applied to pregnant female of rats with gestation age ±22 days. Body length and IGF-1 hormone level of the offspring were measured at the age of five weeks after born. The results showed the mean offspring's body length at birth was 44.22±1.99, 46.20±3.93, 50.69±3.14, 52.17±2.48, 52.05±2.67 and 52.48±2.27 mm for water intake level of 1, 3, 6, 9, 12 and 15 ml, respectively. The result of ANOVA test showed that water intake level significantly and positively affects body length with coeficient of determination of 76.33%; but not significant for IGF-1 hormone level. This implies that chronic dehydration during pregnancy of rat affects offspring's linear growth.

Key words: Water intake, pregnancy, offspring's length, hormone growth

INTRODUCTION

Water is an essential component for living, without it human can survive for only a few days. The human body composes 55-75% of water (Popkin, 2013).

Water is one of the macronutrients it has several functions, such as involving in metabolism and transportation of nutrients, controlling body temperature, as material to form the cell (Santoso *et al*., 2014).

Child's growth process takes place in cells, organs and body and it can be divided into three stages: hyperplasia-increase in cell number, hyperplasia and hypertrophy-increase in cell number and size or maturity and hypertrophy-increase in cell size or maturity. A linier growth failure or stunting effected by quantity and quality of nutrient intake and growth hormone level (Peyreigne, 2001; Peter *et al*., 2003). One of the growth hormones is insulin-like growth factor (IGF-1). As a nutrient water might play a role in the production of cells and IGF-1 and so on linier growth.

Stunting is prevalence among Indonesian children. The Basic Health Survey 2013 of the Ministry of Health showed 37.2% of children under-five years in Indonesia were stunting (Balitbangkes, 2013). Mean while,The Indonesian Regional Hydration Study (THIRST) showed 46.1% of Indonesians suffered from mild dehydration (Hardinsyah and Dodik Briawan, 2012). The hormone consists of IGF-1 binding proteins (IGFBPs) and IGF receptor (IGFR) affecting fetus' amino acid metabolism to skeletal and muscle formation (Peter, 1986) in proliferation and differentiation process, IGF-1 and growth hormone (GH) form chondroitin.

Up till now, there is no study on the effect of water intake level or dehydration on linier growth failure. Therefore, this study aimed at analyzing the effect of water intake level on rat offspring's body length and IGF-1 hormone level.

MATERIALS AND METHODS

Design: This study was conducted on March-May 2014 (three months) in Rat Experiment Laboratory of Faculty of Veterinary Medicine, Bogor Agricultural University. Considering safety, ethics and to ensure water intake control, the present experimental study used Sprague-Dawley rats and applied a randomized design on six groups of rats. A linier model for this study design is as follows:

$$Y_{ij} = \mu + \beta_j + \in_{ij}$$

Where:

Y_{ij} = j^{th} observation of the i^{th} treatment
μ = population mean
β_j = effect on the treatments group number
$\in_{ij}$ = sampling error effect of i^{th} treatment on j^{th} group
i = 1,2,..6 (treatments)
j = 1,2,...5 (replications)

Number of replication was determined by the following formula (Montgomery, 2001):

$$(i-1)(j-1) \geq 15$$
$$(6-1)(j-1) \geq 15$$
$$j = 4$$

where, i is number of treatment and j is number of replication. This study used six treatments, as shown in

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Table 1. By applying the six treatments into the formula, therefore j is five replications.

This study was carried out on 30 adult female rats aged 16 weeks. Vulva monitoring was performed daily to determine rat's pregnancy condition. The treatment was started since the conception uptill delivery infants (the mean pregnancy period was 22.39±0.43 days), which is equal to nine months of human pregnancy period (Peters, 1986).

Main materials for this study were gallon commercial mineral water. The equipments used were digital camera, microscope, surgical instruments, glove, mask, glasswares, test tubes, freezer, eppendorf tubs, micropipette, clinical centrifuge, vortex, analytical balance, scale and ELISA reader. Rat's blood plasma hematocrit was measured in Yasa Pharmacy Laboratory and IGF-1 hormone was measured in Primate and Animal Laboratory of Bogor Agricultural University.

The body weight of each of the pregnant rats was measured weekly uptill birth (four times). After birth, the weight of each of infant rats was measured weekly uptill weaning period (5 weeks). At the age of five weeks, body length, hematocrite and IGF-1 hormone levels of the offsprings were measured. Weight measurement was conducted using digital scales during pregnancy and after rat reached one month in age; while body length was measured using ruler and JM64 image software program.

Hematocrite levels was measured automatically by Mindray machine. IGF-1 was measured by Enzyme-Linked Immunosorbent Assay (ELISA) technique. A color-based measurement was carried out qualitatively by measuring optical density (OD) on ELISA reader (Burgess, 1995). The effects of water intake on linier growth and IGF-1 hormone were analyzed statistically using logistic regressions and Analysis of Variance (ANOVA).

RESULTS

Result of ANOVA test on effect of treatment on body length generated determinant coefficient value of 0.763 or 76.33%. This shows that 76.33% of rat infant body length is affected by several water intake levels recording in Table 2. Hydration level is positively and significantly associated to rat infant body length by p-value of $0.001<\alpha$ (5%).

Hematocrit test on blood sample was carried out to determine dehydration level as the test can determine dehydration level in both animal and human (Shirreffs, 2003). Chronic dehydration The level of hematocrit was showed in Table 3.

Result of hematocrit test shows severely-dehydrated rats (water intake of 1 and 3 ml/day) has hematocrit level of 55.8-68.6% or out of the normal level.

Result of ANOVA test of the effect of hydration on IGF-1 hormone acquired determinant coefficient (R^2) value of 0.474 or 47.4%. It means that 47.4% of IGF-1 hormone is affected by level of hydration treatments and the other 52.6% affected by other variables. Hydration level does not give significant effect, or in other words, there is no significant difference for dosage and IGF-1 level by p-value of $0.157>\alpha$ (5%).

Table 1: Number of treatment

Treat-ment	Water intake volume (ml)	Daily water intake frequency	No. of treatment replication (rat)
1	1	1 x 1 ml	5
2	3	3 x 1 ml	5
3	6	3 x 2 ml	5
4	9	3 x 3 ml	5
5	12	4 x 3 ml	5
6	15	5 x 3 ml	5
Total number of rat			30

Table 2: Mean body length of offsprings rats at age of five weeks

Dosage	Number	Mean body length of rats at age of five weeks (mm)
1 ml	16	44.22±1.99
3 ml	38	46.20±3.93
6 ml	39	50.69±3.14
9 ml	41	52.17±2.48
12 ml	38	52.05±2.67
15 ml	35	52.48±2.27

Table 3: Mean level of hematocrit of the offspring rats at age of five weeks

Treatment	Mean level of hematocrit
1 ml	68.6±2.5
3 ml	55.8±4.2
6 ml	46.2±3.2
9 ml	43.6±1.1
12 ml	41.8±2.6
15 ml	44±1.6

DISCUSSION

This study showed that the water intake levels of pregnant rats affects on the body length of their offsprings; but not on IGF-1 hormone level of their offsprings.

Good hydration is essential for health and wellness. Every cell in the human body requires water. Hydration is central to the most basic physiological functions such as regulating blood pressure and body temperature, hydration and digestion. Hydration in the body is important for transporting carbohydrates, vitamins, minerals and other important nutrients and oxygen to the cells. The cells then produce energy for the body to function. Furthermore, hydration facilitates disposal of the waste products of metabolism, enabling the right cellular chemical function (Coyle, 2007).

A chronic dehydrated animal used its homeostatic mechanism by taking fluids from body organs to be survive (Hafez and Dyer, 1968). This may caused cell formation and growth failures, including a linier growth.According to Peyreigne (2001) and Peter *et al.* (2003), a linier growth failure or stunting effected by

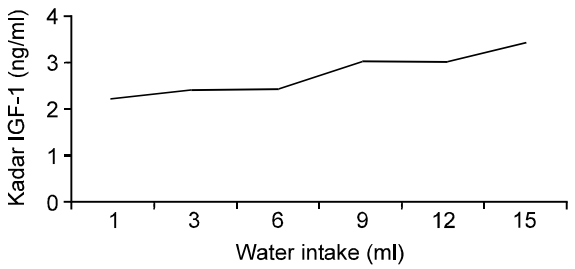

Fig. 1: IGF-1 hormone level by water intake level

quantity and quality of nutrient intake and growth hormone level. In this study, the IGF-1 hormone level was not effected by the water intake level. This implied that the linier growth failure in these rats was effected by water intake level, not by IGF-1 hormone level. In this case water play important role in the formation of cell and the size of cell, which is in line with Peter *et al.* (2003) finding that nutrient intake effects the formation and the size of cell. Poor nutrition during late pregnancy causes deficiency in fetus' muscle and liver glycogen as fetus' energy source at birth. Such deficiency is the cause of fetus death or growth failure before or at birth (Hafez and Dyer, 1968). Limitation in food and drink intake amount also affects organogenesis, neurogenesis and skeletogenesis. Dehydration during pregnancy can lead to increased risk of oligohydramnios, i.e., deficiency of amniotic fluid surrounding fetus (Shumway *et al.*, 1999). Adequate hydration is especially important during pregnancy and after birth to meet the physiological changes. Water is needed to form amniotic fluid that surrounds the baby, support the increase in blood plasma volume and produce breast milk (NHC, 2010).

A lower hematocrit level is and indicator of dehydration, (Campbell *et al.*, 2004). Hematocrit level increases along with pregnancy; the higher hematocrit level leads to more coagulated blood (Mary *et al.*, 1993). Increase in plasma volume is shown by increase in percentage of hematocrit level during pregnancy. Normal rat blood's hematocrit level is 39-53% (Aboderin and Oyetayo, 2006). High hematocrit level during pregnancy can lead to intrauterine growth retardation (IUGR) and low body weight (Masoomeh *et al.*, 2012). Besides, dehydration conected to the amniotic fluid contains carbohydrates, proteins and peptides, pyruvic acids, electrolytes, enzymes and hormones, including IGF-1 (Mark *et al.*, 2005). This fluid is a regulatory pathway of proteins or peptides for fetus growth (Xing-Long *et al.*, 2009).

In addition, hematocrit level is also adversely associated with hemoglobin level; increase in hematocrit level leads to decrease in hemoglobin and ferritin levels which eventually leads to low infant weight (Lao *et al.*, 2000). A low ferritin level causes anemia in pregnancy and it leads to decrease in folic acid uptake for fetus and infant death (Lindsay, 2000). Greater number of red blood cells or hematocrit causes thicker blood and higher blood viscosity. Thicker blood may limit the delivery of oxygen, especially in the capillaries at the tissue and organ levels (Burn *et al.*, 2010).

IGF-1 hormone level below a normal range indicates growth hormone (GH) deficiency. According to Pangkahila (2007) GH deficiency might caused by several factors such as age, nutrient deficiency, liver disease, uncontrolled diabetes mellitus and hypothyroid. The limitation of diet, such as nutrient deficiency in young rat decreases its GH but not IGF-1, because the young rat has greater capacity to synthesize proteins (Klatz and Carol, 1997). This is one plausible explanation that in this study, the IGF-1 hormone level was not effected by the water intake level.

The above explanations support the result of this study that the hydration status or water intake level during pregnancy effects the linier growth of the offsprings and do not effect the IGF-1 hormone level. This study showed that adequate water intake during pregnancy is important to avoid stunting infant. However further studies in human are required.

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