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Impact of Glutathione Administration on Antioxidant Levels and Ileum Histologic of Growth Phase of Cihateup Duck in Extensively Maintained

A. Mushawwir[1], N. Suwarno[1], and H. N. Aritonang[2]

[1]Animal Science Faculty, University of Padjadjaran
[2]Magister Program in Animal Science, Animal Science Faculty, University of Padjadjaran
Jl. Raya Sumedang KM. 21 Bandung-Sumedang, Jatinangor Sumedang, West Java, Indonesia
Corresponding Author: mushawwir@unpad.ac.id

ABSTRACT

This research has been carried out for a month using eighty three-month-old female Cihateup ducks and has been used to study the influence of glutathione on the endogen antioxidant response and histological illeum. The separation of glutathione was carried out using the distillation technique. Each experimental group consisted of 20 ducks, each treated with G0 = without glutathione; G1= Administration of glutathione 150 µL/head; G2 = Administration of glutathione 175 µL/head; G3= Administration of glutathione 175 µL/head. Tissue and blood sampling was collected at the end of the study by EDTA tube and a fixative solution, and tissue preparations were made using the Mallory-Asan technique and analyzed using a binocular microscope. Antioxidant levels were measured using a spectrophotometer technique based on the instructions of the Randox Kit. The results showed that glutathione affected ($P<0.05$) morphometric ileum (villi, Peyeri's plaque and goblet cells) and increased with increasing glutathione level and endogenous antioxidant response. It was concluded that glutathione is able to stimulate protein and lipid anabolism, as well as hormonal signals related to ileal tissue growth and antioxidants.

Keywords : additive, physiologic, duck

INTRODUCTION

The wealth of Indonesian local livestock commodities must be developed in order to have high productivity. One of these commodities is ducks. At present, the determination of the Cihateup local duck line has been carried out. Livestock productivity is only determined by 30% if the genetic aspect and the remaining 70% is determined by the environmental aspect, although the two cannot play a role independently, the interaction of the two is always needed.

The environmental aspects that are generally a problem for local poultry are feed and the physical environment. It is known that Indonesia as a tropical country has a high environmental temperature (ranging from 25 – 35^0C) even in certain areas it can exceed 35^0C. Temperatures above the comfort zone for laying ducks (19 - 22^0C) cause oxidative stress (Mushawwir et al., 2010; Siregar et al., 2020; Adriani et al., 2020, 2021) which has an impact on not achieving optimum production.

Cihateup ducks generally spread in West Java, including in the lowlands such as Cirebon, Indramayu, Subang, lowlands of Garut and Tasikmalaya, Kuningan. In addition to the high temperature, another problem that causes the duck's productivity is not optimal is the maintenance system which is generally still traditional (extensive). Although this system has the advantage of minimizing feed costs. However, this maintenance system is at risk of infection with aflatoxins and pathogenic microbes. Both of these conditions (temperature and maintenance system) can trigger increased damage to intestinal tissue (ileum) (Allen et al., 2015; Fabris et al., 2017, Mushawwir et al., 2011, 2021[a,b,c,d]).

The study reports on environmental impacts on the histological condition of the ileum are still inadequate, especially related to efforts to overcome them using natural materials. One of the natural ingredients that can be used to overcome these environmental impacts is glutathione.

Several previous studies have shown that several micronutrients such as amino acids from natural ingredients can act as effective additives, induce immune production, improve metabolism (Xu et al., 2015; Mushawwir et al., 2018, 2020[d]; Nurmalia et al., 2020); reduce cell death (Loyau et al., 2014; Mushawwir et al., 2020[a,c,d]). Amino acids are widely used independently (Tian et al., 2015). There are still very few reports of the use of amino acids synthesized into glutathione. Related to this phenomenon, researchers are

interested in exploring the effect of fed glutathione on the morphometric ileum and the endogenous antioxidant response of cihateup ducks in reared traditionally.

MATERIALS AND METHOD

Animal and Experiment Design

Eighty female Cihateup ducks, aged 3 months with a body weight of 1476 ± 42.39 g, were used in this study. The experimental ducks were divided into four treatment groups, consisted of 20 ducks, severally.

Each experimental duck was given a wing tag coded for the treatment group (G0, G1, G2 and G3). Ducks are allowed to move freely (playing, eating and drinking) according to the maintenance technique, which is extensive. This experiment was conducted in one of the duck breeders in Subang Regency, West Java, for one month. Tissue samples were analyzed at the Laboratory of Animal Structure, Department of Biology, Faculty of Mathematics and Natural Sciences and at the Laboratory of Physiology and Biochemistry, Faculty of Animal Science, Padjadjaran University.

Glutathione Assembly and Treatment Method

The glutathione used in this study was the result of chemical synthesis based on national competitive basic research (Mushawwir et al., 2020). Glutathione synthesis includes binding of the first amino acid C terminal (loading resin), coupling of the second amino acid (Fmoc-Cys-OH), coupling of the third amino acid (Fmoc Glu-O(tBu), release of tripeptides from the resin which is characterized by a change in resin color to the resin was filtered then the filtrate was concentrated using a rotary evaporator, then the solids (glutathione) were dried using a freeze dryer, tested for purity by analytical RP-HPLC, purified with semi-preparative RP-MPLC and preparative RP-HPLC, then the purification results were tested again for purity with RP - Analytical HPLC, then the purified peptides were characterized using TOF-MS and NMR

The characteristics of the results of glutathione synthesis are shown in Table 1 below:

Table 1. Characteristics of glutathione synthesis results

Characteristics	Value
Density in solution	50.000 ppm
Viscosity	86%
pH	6,5

The treatment of glutathione solution was carried out with the following doses:

Group G0 = Without glutathione
Group G1 = Administration of glutathione 150 µL/head
Group G2 = Administration of glutathione 175 µL/head
Group G3 = Administration of glutathione 175 µL/head

The administration of glutathione to each experimental duck was carried out every morning (hours 06.00 – 07.00) for one month, before the cattle were active, eating and drinking. The technique of administering glutathione to ducks was carried out using a force-feeding technique, using a micropipette.

Tissue, Blood Collection, and sample Analysis

Tissue samples were collected at the end of the experiment, 10 experimental ducks were randomly selected in each experimental group, respectively. The selected ducks were cut and immediately separated from the intestinal tissue (illeum) while dripping with physiological NaCl solution, the illeum was put into a sample bottle that already contained a fixative solution, immediately.

The Mallory-Asan staining technique was used to analyze ileal tissue samples. The reagent solutions used included 95% ethanol, Aniline oil, Aquades, each as solution A. Solution B, consisting of Glacial Acetic Acid, Azocarmine G Cl n.50085, Aquades), and the other reagent included solution C and D. Determination of histological parameters (illium morphometrics, plaque Peyeri and goblet cells) were performed using a microscope.

The spectrophotometer technique, based on the instructions in the Randox kit, has been applied to determine the level of antioxidant response. For this purpose, the blood has been centrifuged to obtain the liquid plasma.

Data Analysis

The data were analyzed using the semi qualitative of Kruskal Wallis comparison test (Suwarno and Mushawwir, 2019). The analysis was carried out with the SPSS IBM 21 software application.

RESULT AND DISCUSSION

The endogenous antioxidant response of Cihateup ducks based on the results of the study is shown in Table 2.

Table 2. Levels of Endogenous Antioxidants in Cihatep Ducks with Glutathione Administration

Antioxidant	Glutathione Levels			
	GO	G1	G2	G3
Glutathione peroxidase, nmol.mg-1	3.21±0.31[a]	5.55±1.23[b]	6.66±1.12[b]	8.94±2.33[c]
Glutathione reductase, nmol.mg-1	3.22±1.42[a]	7.31±1.12[b]	8.11±1.42[b]	10.53±1.31[c]
Total Antioxidant Status, nmol.mg-1	10.13±0.41[a]	14.11±0.42[b]	15.53±1.22[b]	18.41±0.21[c]

[a,b] Response means followed by different notations on the same line show significant differences ($p<0.05$); Average±Standard deviation

The results of Kruskal Wallis' analysis showed that there was a significant difference ($P<0.05$) in the antioxidant levels of the duck group without glutathione administration and the group of ducks with glutathione administration. The antioxidant levels of the ducks that were given glutathione appeared to be higher than those without glutathione.

These results can explain that the biomolecules contained in blood plasma which are amphiphatic causes the formation of a very beneficial interaction of glutathione which carries charged S and O atoms, thereby increasing the electrostatic interaction pattern. Na et al. (2020); Mushawwir et al. (2021[a,e] and 2020[b,c,e]); Tanuwiria et al. (2020[a]), informed the entanglement of charged electrons on their ability to maintain the structure of amino acids, glucose and glycogen as well as fatty acids and sterols. This biomolecule fact, seems to be able to prevent tissue damage as a result of metabolism and heat, as well as increase the body's fluid capabilities. This condition directly supports cell growth to produce antioxidants (Mushawwir et al., 2021[c,d]).

The histology of the ileum based on the results of the study showed that the administration of glutathione appeared to have a significant effect ($P<0.05$) on the ileum morphometrics of Cihateup ducks in the growth phase, except for the diameter of goblet cells. Illeum morphometrics based on the Mallory-Asan staining technique showed an increase in ileal dimensions and goblet cell density, along with an increase in the level of glutathione administration (Table 3).

Table 3. Histology of the Cihateup Duck Illeum Growth Phase Traditionally Maintained by Giving Glutathione

Histologik	Glutathione Levels			
	G0	G1	G2	G3
Number of Villi*	7±1,21[a]	10±2,12[a]	14±1,14[b]	16±1,15[c]
Length of Villi (μm)	233±16,15[a]	271±9,24[b]	351±11,15[c]	411±10,22[d]
Number of Plaque Peyeri*	4±1,20[a]	5±1,21[a]	8±0,21[b]	11±1,11[c]
Height of Plaque Peyeri (μm)	21±2,31[a]	34±2,12[b]	37±2,31[c]	41±1,08[d]
Number of Goblet cells#	15±1,22[a]	45±2,43[b]	55±2,34[c]	76±3,11[d]
Diameter of Goblet Cells (μm)	5±2,30[a]	7±1,53[a]	7±2,61[a]	7±2,21[a]

* Per field 10x Objects; # Per field 100x Objects; [a,b] Response means followed by different notations on the same line show significant differences ($p<0.05$); Average±Standard deviation

The results of Kruskal Wallis analysis showed that there were significant differences ($P<0.05$) in the number of villi, villi length, number of Peyeri's plaques, Peyeri's plaque

height and number of goblet cells, between the duks groups. In this study, the higher the level of glutathione administration, the greater the morphometric dimensions of the ileum. Several previous studies that have been informed have shown similar symptoms to the kidneys (Ansar et al., 2014; Eyng et al., 2015), namely decreasing damage to kidney cells and being able to cope with oxidation caused by free radicals thereby reducing apoptosis (cell death) and tissue damage, and increase the response of immunity. The same additive effect has also been represented by Mushawwir et al. (2021[e]) and Hermawan et al. (2017), increased utilization of nutrients and decreased enzyme levels in the liver (AST and ALT). This enzyme indicated an increase in healthy of duck liver cells.

The growth of ileal villi (shows in Table 3), both in number and in length, showed a significantly higher difference ($P<0.05$) with increasing levels of glutathione administration. The growth of these villi is stimulated due to the presence of glutamyl cysteine protein, which plays a role in increasing protein anabolism. Ammer et al., (2018); Kamil et al. (2020); Carrol et al. (2016) and Dinana et al. (2019) have indicate that naturally, glutathione contains high levels of glutamyl and cysteine.

Regarding cell growth, Gehrke et al. (2013); Loyau et al. (2014) and Kharazi et al. (2022), suggested that lipid synthesis and amino acid metabolism increase sharply during growth, as well as increase the utilization of nutrients Gray et al., 2015; Ippolito et al., 2014; Mushawwir et al., 2020[a,d,e] and Istvan et al., 2020; Rahmania et al., 2022). This situation can be supported by the provision of additives such as essential oils. The content of essential amino acids in glutathione prevents oxidative stress for ducks that are extensively reared, so that muscle tissue can grow faster. The results of other studies show that high tissue growth is the impact of increasing antioxidants and conversely reducing free radicals, the risk of cell inflammation also decreases (Dinana et al., 2019; Siregar et al., 2020; Mushawwir et al., 2021[b,c] and Tanuwiria et al., 2022), decreased free radicals from oxidative stress (Tian et al., 2015; Roland et al., 2016); also due to decreased apoptosis or cell death so that tissue metabolism becomes optimal (Xu et al., 2015; Sang-Ho et al., 2018; Jiwandini et al., 2020).

The results also showed that the growth of Peyeri's plaques and goblet cells (Table 3) seemed to increase with increasing glutathione levels. It is known that Peyeri's plaques are lymph nodes containing T cells and B lymphocytes, as immune compounds. Glutathione contains allyl and sulfide groups (Allen et al., 2015; Khan et al., 2015; Adriani et al., 2015). The increase in activities of antioxidant is also increased by the administration of glutathione in experiment groups (Adriani et al., 2018, 2020; Mushawwir et al., 2021[a,b]). Increased glutathione stimulates the differentiation of white blood cells and avoid proinflammation molecules.

The same thing has also been shown by Borek (2001), also reported that glutation and some natural additives are effective in inhibiting gene mutation, and intensify DNA repair. The results of other studies, Amagase et al. (2001) reported an increase in the bioavailability of nutrients in the gut. Decreased cells damage and gene mutations support for growing of Peyeri plaques.

CONCLUSION

Glutathione as an additive is able to stimulate anabolic pathways, related to the growth of ileal tissue. This effect leads to increase growth of villi, Peyeri's plaques and ileal goblet cells. Glutathione is also able to increase the endogenous antioxidant response.

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