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Submission date: 06-Aug-2021 09:56PM (UTC+0700)

Submission ID: 1628450897

File name: Fitra_2021_IOP_Conf._Ser._Earth_Environ._Sci._788_012186.pdf (506.01K)

Word count: 4647

Character count: 24898

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4

To cite this article: D Fitra *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **788** 012186

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Plasma metabolites of Kampung chicken (*Gallus gallus domesticus*) in peatland free-range system with differences of vegetation

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Abstract. Vegetation in free range rearing systems can be obtained from grass or legumes. The aims of this study was to investigate the effect of different vegetation on free range systems in peatlands on plasma metabolites of kampung chickens, including concentration of cholesterol (CHO), triglycerides (TG), and glucose (GLU). This experimental study used two hundred and ten kampung chickens with a completely randomized design of 4 treatments with 5 replications. Each paddock was placed in a portable housing. Twelve kampung chickens aged 5-12 weeks were reared in different types of vegetation with a density of 6.67 m²/head. The treatment without vegetation was reared in the postal housing. At the end of the study, blood was collected from the jugularis vein to observe plasma metabolites. The results showed that the maintenance system and differences in vegetation types had a significant effect (P<0.05) on CHO, TG and GLU. The highest TG was in the *Axonopus compressus* treatment, GLU was in the *Indigofera zollingeriana* treatment. while the lowest CHO was in the control or without vegetation. The conclusion of this study was the paddock planted with *Indigofera zollingeriana* can reduce CHO levels of kampung chickens in addition to control treatment.

1. Introduction

The high conversion of agricultural land in Indonesia to residential land, industry, property and commercial roads will pose a threat to the livestock business. At least there have been a decrease in paddy fields area by 12.41% during 2014–2018 [1]. The increasingly limited mineral ²⁷ will force farmers to be able to farming on marginal land, including on peatlands. Indonesia ¹³ has the largest area of peat in the tropical zone. It is estimated reaches 21 million ha or represents 70% of the peat area in Southeast Asia or 50% of the world's tropical peatlands. Riau Province has an area of 4,044 million ha



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or equivalent to 45% of the land area of Riau Province and 56% of the area of peatlands on the Sumatera island [2].

In 2017, The directorate general of animal husbandry and animal health of the Indonesian Ministry of Agriculture (2018) [3] reported that the population of native chickens (including kampung chickens) was around 299,701,400. The number increased about 1.82% from 2016. On the other hand, the native chicken population is very low when compared to the population of broilers and laying hens commercial. As a result, consumption of native chicken is only able to contribute about 11.9% in national poultry meat consumption. This condition is alarming if the government still considers kampung chicken only a complement in the poultry industry. Even though kampung chickens have many advantages, high adaptability to the environment genetically [4], resistant to disease, especially those caused by *Salmonella* sp [5], and lower contains cholesterol [6,7]. The existence of kampung chickens will be threatened with extinction if it continues to be consumed without any effort to increase population.

One of the alternative cultivation systems to eliminate land limitations and the problem of intensive care patterns in poultry production is the free-range maintenance system [8]. In this cultivation system, chickens are raised freely in the fields and consume natural feed so that the resulting products are healthier than chickens that are kept in cages intensively [9], and then chickens reared in a free-range system will naturally express instincts [10]. Pavlovski et al (2009) reported that natural maintenance in a free-range system produces chickens with a higher welfare level so that they can produce better product quality. Free range in developed countries has many rules regarding animal welfare [11]. Bailey et al (2010) stated that native chickens kept in free cages to be limited in number, had more room to move and perch completely and were able to meet feed requirements [12].

The free-range system is also a solution to address the large consumer demand for natural products (organic poultry) and free from antibiotic residues resulting from the intensive maintenance system using conventional battery cages. The placement of chickens in a very limited space and the provision of commercial feed containing high agrichemicals. The maintenance in the pasture land must be stress free, not dense, get natural feed from seeds and insects and get plenty of fresh air and sunlight [13]. On the other hand, the freedom of chicken activity and the opportunity for chickens to obtain forage from their environment are expected to improve the quality of life for chickens raised in a free-range system [14].

Different types of forage cause differences in nutritional value and food chemical substances in chickens because each type of forage contains different nutritional elements. The difference in freedom of activity and access to forage has an impact on production performance which is also described by physiological parameters. One of the physiological parameters that reflect the condition of poultry is the blood plasma profile. Blood is a component that has a very important function in the physiological regulation of the body and the most important transport medium in almost all body functions [15]. Hematological studies can help in understanding the relationship between physiological characteristics, especially the circulatory system, and environmental aspects. Blood is a good parameter for assessing the physiological status of an animal, with an idea that animal with a good blood composition condition can be said to be in a good performance condition [16].

Nowadays, the aspect of meat quality is the main concern of consumers. especially for consumers who have middle and upper income, they are better off choosing meat with low cholesterol even though it costs more [17]. Cholesterol and triglyceride are believed to be factors causing coronary heart disease, so that consumers are starting to be selective in consuming fatty foods, especially in some animal protein and their products [18]. Good and healthy food products from poultry for consumption are the responsibility of all parties from upstream to downstream of the production system.

The provision of feed ingredients containing omega-3 can reduce concentration of cholesterol and triglycerides and increase the elasticity of blood vessels, preventing the formation of harmful fats that stick to the arteries [19]. Blood glucose levels are also important to observe, because blood glucose

levels are regulated so that they are always in a stable condition in the body through a process of homeostasis [20], this process involves other sources of glucose in the body such as glycogen, fatty acids, and amino acid. Low glucose levels indicate that livestock are not getting the appropriate feed [21].

However, few studies have investigated the advantages and disadvantages of free range system with different vegetation on plasma metabolite of kampung chickens. Therefore, a study was conducted aimed at examining the impact of different forages on the free range maintenance system on the health level of chickens which was described by the blood plasma status of kampung chickens.

2. Materials and methods

2.1. Bird and experimental design

All animal experiments were approved by the Institution Animal Care and Use Committee of IPB University (permit number: 170–2019 IPB). All protocols were carried out in accordance with relevant regulations issued by this committee. The study was conducted at Teaching Farm Faculty of Agriculture and Animal Science State Islamic University of Sultan Syarif Kasim Riau, Pekanbaru. A total of 300 one-day-old kampung chickens obtained from local breeders UD. Nata Hatchery, Medan, Indonesia were selected as the experimental populations and raised in postal housing within the same brood house until 42 days of age. The experiment was performed on day 42 until 84 (5–12 weeks), and 210 chickens with similar average body weight were randomly divided into treatment. Body weight of chicken was used 455.72 ± 37.38 . This research was an experimental study using a completely randomized design. The treatment was a kampung chicken free-range system with different vegetation, consisting of: T1= conventional cage (without vegetation/control), T2= Paddock *Brachiaria decumbens* (PBD), T3= Paddock *Axonopus compressus* (PAC), T4= Paddock *Indigofera zollingeriana* (PIZ). Each vegetation treatment included 5 replicates with 12 birds in each replicate and control consist 30 birds.

The equipment used in this study were 5 units of cage with the size $100\text{cm} \times 100\text{cm} \times 60\text{cm}$ in a postal cage for control treatment. Meanwhile, in the free-range system used 15 units of portable cages measuring 2 m^2 , equipped with a feeder and drinker. In addition, each the free-range system was equipped with a paddock area measuring $4\text{m} \times 20\text{m}$, which was fenced with a netting barrier. Portable cage and paddock densities refer to the Australian Code of Practice, with a maximum of 30 kg/m and 1,500 birds/ha (SCARM, 2002). So that the density of the portable cage is 6 birds/ m^2 , while the paddock has a density of 6.67 m^2/bird . The indoor area for control was covered with wood shavings and was cleaned weekly.

The experimental paddock used was peatland that had been planted with different types of vegetation according to treatment. The treatment paddock consisted of 15 units, each of which was planted with *Brachiaria decumbens* (grass), *Axonopus compressus* (grass) and *Indigofera zollingeriana* (legume). Each treatment consisted of 5 paddocks as replicates with the same size and have be filled with 12 chickens. Each paddock is given a mesh fence around it to prevent the chicken from moving to the other paddock. Before the paddock was used, the growing vegetation was cut to get a uniform growth rate.

During the brooding period the first 42 days of chickens were kept in postal housing and had been given feed and drink adlibitum. The chickens used previously were weighed to obtain a uniform body weight and then completely randomized. while during the treatment the chickens were only given drinking water adlibitum. Every day the chickens were released to the paddock except for control treatment from 08.00 am to 17.00 pm. after that the chickens are put into the cage at night to avoid predators.

The feed used during brooding was broiler chicken starter period produced by PT. Charoen Pokphand Indonesia, Medan, Indonesi, code 311–VIVO® with a maximum crude protein content of 22%. After the treatment was started, the chickens were still fed during the outdoor adaptation period. During the 1

week adaptation period, feeding was reduced from 100%, 50% until not given at all. The feed given was commercial native chicken feed with code N-582® CP 16–17.5 %. Assuming the chicken has received feed from forage grazing. Whereas in the control treatment, feed and drink were given ad libitum as well as in the forage difference treatment.

2.2. Sample collection and analytical determination

At 84 days of age, prior to slaughter, all birds were individually weighted after being fasted for 12 hr, and then 128 birds with similar body weight were selected from each replicate for blood sample collection. Blood samples (3 mL/chicken) were taken from the brachial vein and stored in anticoagulant vacuum tubes for determining plasmas. The blood samples were transported to our laboratory (stored in ice) and immediately centrifuged at 5,000 rpm for 15 min [22]. Then the plasma obtained and analyzed for concentration of Cholesterol (CHO), Triglycerides (TG), and Glucose (GLU) using microlab-300 machine.

The data were analyzed using the SPSS (Statistical Product and Service Solutions, version 16.0). Before data processing was carried out, all raw data was tested to eliminate outlier data ($P < 0.05$), then continued with data analysis using a completely randomized design (CRD) and if the treatment showed a significant effect on the measured variables, then continued with the LSD test [23]. Data shown are mean \pm standard deviation.

3. Result and discussion

Blood biochemical content in this study for all variables showed a statistical difference ($P < 0.05$), especially the difference was very visible between cage treatment and free range system. This is generally due to differences in maintenance systems resulting in different amounts of physical activity and freedom.

3.1. Cholesterol (CHO)

Concentration of cholesterol of kampung chickens reared in the paddock of peatlands was different in vegetation presented in table 1.

Table 1. Average blood cholesterol (mg/dl) of kampung chickens.

Treatment	Cholesterol (CHO)
Control	131.11 \pm 26.86 ^a
PBD	182.30 \pm 36.43 ^{bc}
PAC	230.90 \pm 43.08 ^c
PIZ	149.88 \pm 18.99 ^{ab}

Superscript letters indicate differences ($P < 0.05$) in columns.
 Control: conventional cage (without vegetation), PBD: paddock *Brachiaria decumbens*, PAC: Paddock *Axonopus compressus*, PIZ: Paddock *Indigofera zollingeriana*.

The treatment of differences in vegetation on the free range of native chickens significantly effect on cholesterol content. The cholesterol levels in results of this study don't show a regular trend. The lowest was in the control treatment or conventional cage 131.11 mg/dL, while for the free range system the highest was the axonopus compressus treatment (230.90 mg/dL) and the lowest was the indigofera zollingeriana treatment (149.88 mg/dL). This finding is different from what was obtained by Erwan et al (2017) that the cholesterol levels in broiler chickens range from 18–194 mg/dL [22]. However, the cholesterol levels in this result study were still in the normal range. According Basmacioglu and Ergul (2005) stated chicken cholesterol in the range 87–192 mg/dl [24]. However, chicken blood cholesterol

levels in this study were still lower than the results of Abdel-Fattah et al (2008) study which used citric acid 1.5% (316.83 mg/dL) and control (393.33 mg/dL) [25]. Cholesterol is synthesized from fats consumed and endogenously synthesized within the cells. A high level of cholesterol is an indication of a high risk to cardiovascular disease.

Indigofera zollingeriana treatment was able to produce the lowest cholesterol content compared to other vegetation. This is presumably because indigofera, apart from having a very high protein content, also contains xanthophyll and β -carotene of 507.6 mg/kg [26]. The findings of the current study agrees with reports that the β -carotene diet reduces serum cholesterol [27]. Other earlier report agree with the finding of this study that the carotenoids diet reduces total cholesterol in chickens [28]. Furthermore, the absence or presence of cholesterolaemic effects of dietary components in an animal depends on various factors such as breed, sex and age, and also on the composition of the feed [29].

3.2. Triglycerides (TG)

Concentration of Triglycerides of kampung chickens reared in the paddock of peatlands was different in vegetation presented in table 2.

Table 2. Average blood Triglycerides (mg/dL) of kampung chickens.

Treatment	Triglycerides (TG)
Control	38.22±9.43 ^a
PBD	45.88±12.56 ^{ab}
PAC	52.78±11.52 ^b
PIZ	49.00±3.70 ^{ab}

Superscript letters indicate differences ($P < 0.05$) in columns.

Control: conventional cage (without vegetation), PBD: paddock *Brachiaria decumbens*, PAC: Paddock *Axonopus compressus*, PIZ: Paddock *Indigofera zollingeriana*.

The average blood triglycerides of kampung chickens with different vegetation in the free range paddock ranged from 38.22 to 52.78 mg/dL. This result was lower than Hasanudin et al (2013) where layer chickens given lime juice have triglycerides ranging from 87.50 to 129.17 mg/dL [30]. The results of the analysis of variance show the effect of lime juice. Erwan et al (2014) reported that the blood triglycerides level in layer chicks was 17-78 mg/dL and 140-57 mg/dL in broiler chicks [31].

Treatment of differences in vegetation with conventional cage had a significant effect ($P < 0.05$) on blood triglycerides of kampung chicken blood. Although it does not represent a unidirectional trend, the treatment of paddock with *Axonopus compressus* grass vegetation produced the highest triglyceride value, namely 52.7 mg/dL. Triglyceride values are still within normal limits, namely <150 mg/dL [24].

The levels of triglycerides are almost the same in each different treatment of vegetation due to the function of triglycerides to meet energy needs in the body. Tornheim and Ruderman (2011) state that energy needs in the body can be met by utilizing triglycerides in fat tissue. When the need for energy in the body increases, the body will synthesize enough triglycerides (in the form of fatty acids) which are stored in fatty tissue, where fatty acids are re esterified to provide fuel for all cells in the body that require energy [32].

3.3. Glucose (GLU)

Concentration of Glucose of kampung chickens reared in the paddock of peatlands was different in vegetation presented in table 3.

Table 3. Average blood glucose (mg/dL) of kampung chickens.

Treatment	Glucose (GLU)
Control	272.69±27.66 ^{ab}
PBD	211.20±36.23 ^a
PAC	243.50±99.22 ^{ab}
PIZ	285.76±42.50 ^b

Note : data are mean ± standard deviation
 Superscript letters indicate differences ($P < 0.05$) in columns.
 Control: conventional cage (without vegetation), PBD: paddock *Brachiaria decumbens*, PAC: Paddock *Axonopus compressus*, PIZ: Paddock *Indigofera zollingeriana*.

Regulation of blood glucose levels is closely related to the function of several hormones, especially insulin and glucagon hormones [33]. Blood glucose is a source of energy for the body which is obtained after glucose is converted into ATP (Adenosine Triphosphate). Blood glucose is obtained from food sources which mainly come from carbohydrates and other food sources such as protein and fat [34].

The average blood glucose content (mg/dl) of kampung chickens with differences in vegetation in the free range paddock ranged from 211.20 to 285.76 mg/dL. This value is relatively the same as the glucose levels in broiler chickens, namely 230–370 mg/dL [35]. Suchy et al (2004) conducted a study on layer hens Moravian BSL strains aged 25–50 weeks to obtain glucose levels in the range of 234–252 mg/dl [36]. This is because the maintenance environment, namely THI and the feed given is relatively the same. The results of the analysis of variance show treatment of differences in vegetation with conventional cage had a significant effect ($P < 0.05$) on blood glucose of kampung chickens. Although it does not represent a unidirectional trend.

The low blood glucose content indicate livestock divisit energy, and vice versa [37]. Zahra (2014) states that cells that lack energy will synthesize glycogen, which is called the glycogenolysis process, and in a body that is deficient in glucose, cells synthesize energy stores in the form of fat, which is called the lipolysis process [38]. In this study, the levels of blood glucose content in all treatments were still within reasonable limits, so that energy sufficiency was obtained. The trend of increasing glucose values was in line with number treatment, as for the high glucose was *Indigofera zollingeriana*.

4. Conclusion

Treatment of vegetation differences in free range on peatlands can affect cholesterol, triglycerides and glucose content in blood kampung chickens. The best treatment that can reduce cholesterol levels in the blood of kampung chickens aged 42–84 days is a paddock planted with *Indigofera zollingeriana*.

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