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Article

Physiological Organ Profile of Broilers Added Fermented Extract of Bitter Leaves *(Vernonia amydalina)* in Drinking Water

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Abstract. The use of AGP (Antibiotic Growth Promoter) in several countries has been widely banned in broiler cultivation, thus triggering experts to discover the use of medicinal plants as antibiotics and natural broiler growth promoters. One of the active substances that function as AGP is flavonoids. Bitter leaves are one of the medicinal plants containing saponins, tannins and flavonoids which have functions as antibacterial, antifungal, antidiabetic, antiplasmodial and anticarcinogenic. The aim of the study was to determine the effect of adding fermented Bitter leaf extract (*Vernonia amygdalina*) to drinking water on the physiological organs of broilers, and to determine the correct dose of adding fermented Bitter leaf extract to drinking water on physiological organs of broilers. The research results obtained were the addition of fermented extract of Bitter leaves (*Vernonia amygdalina*) in drinking water had no significant effect (P>0.05) on the percentage of broiler heart, liver and spleen. The addition of fermented Bitter leaf extract (*Vernonia amygdalina*) up to 15% in drinking water can be tolerated against the heart, liver and spleen in broiler drinking water. The addition of fermented Bitter leaf extract has a good effect on internal organs, namely the heart, liver and spleen.

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1. Introduction

The broiler industry faces a constant challenge in developing management strategies to improve chicken performance while reducing product safety issues. Meat products that are produced in the short term to meet the needs of animal protein are broilers. The resulting broilers must be healthy and free of antibiotics and hormones which is a concern when using growth promoter (AGP) antibiotics[1-2]. This antibiotic is given to eliminate harmful bacteria in the digestive tract to achieve better body weight and feed conversion. The use of antibiotics as an additional ration supplement is used to stimulate cattle growth and to protect the immune system, but the disadvantage of continuous use of antibiotics is that residues are formed in chickens and humans, which is why their use is prohibited.

Some breeders (96.97%) use commercial feed that contains AGP (Antibiotic Growth Promoter), which is not recommended by the government [3], while according the addition of AGP can increase growth by around 3.8–11.1% and improve FCR by 3.9–8.2% [4]. According to [5] the use of AGP can increase body weight gain by 3.9% and feed efficiency (FCR) by 2.9%. Its use in feed affects the resistance of bacteria in the body and continues in humans [6]. Antibiotic growth promoter (AGP) has been banned for use in Indonesia since January 1 2018, besides that due to the adverse effects of giving these antibiotics to consumers, thus giving birth to Minister of Agriculture No 14/2017 concerning the prohibition of the use of antibiotics especially AGP (antibiotic growth promoter) in the ration.

Even though the law has been issued, until now feed additive antibiotics have not been completely eliminated. This is because, if AGP is immediately eliminated, then the poultry industry can experience a crisis. The crisis will have an impact on increased feed conversion and high depletion. Since the ban on the use of AGP in many countries, there has been a growing use of medicinal plants as antibiotics and natural growth promoters in poultry rations [2]. One of the active substances that function as AGP is flavonoids.

Flavonoids are compounds that are widely recommended as substitutes for AGP, because of their ability as antibacterial, antioxidant, anti-inflammatory and hepaprotective compounds [7]. Flavonoids inhibit bacterial growth which causes damage to the permeability of the bacterial cell wall, microsomes and lysosomes [8]. Saponins have a working mechanism that results in damage to cell membranes so that various important components are released from inside the bacterial cell, namely proteins, nucleic acids and nucleotides [9]. Tannin is a component of a very complex organic substance consisting of phenolic compounds which are difficult to separate and difficult to crystallize, precipitating proteins from their solution and combining with these proteins [10]. One of the medicinal plants containing saponins, tannins and flavonoids is bitter leaves (*Vernonia amygdalina*).

Bitter leaves (*Vernonia amygdalina*) have been known by the people of Southern Nigeria as traditional medicine [11]. Bioactivity of *V. amygdalina* can be used as an antibacterial, antifungal, antidiabetic, antiplasmodial, anticarcinogenic, and others. Components in bitter leaves that are believed to be responsible for their antibacterial potential are flavonoids, tannins, saponins and alkaloids [12]. Bitter leaves have potential natural antioxidant agents which are used as neutralcetical/functional food [13]. Furthermore, from the results of research [14], Giving 5 cc/liter of African leaf extract drinking water can increase broiler body weight gain.

Safe and natural alternative materials are currently needed as a substitute for the function of antibiotics, including herbal plants. The use of herbs as feed additives in broiler rations aims to replace the use of antibiotics as growth promoters and disease prevention in poultry so that livestock and humans can avoid antibiotic residue and bacterial resistance.

The benefits of using herbs in rations and given in drinking water are as feed additives which have a positive impact on increasing the growth and health of livestock in the presence of active compounds contained in them. Nutrients that will be absorbed by the chicken's body can launch metabolic processes in the broiler's body which can be seen from the profile of the physiological organs of the

broiler. In addition, the use of herbs is relatively cheaper compared to antibiotics, so the use of herbs at this time must be further improved and in the future in a modern way and have a longer shelf life.

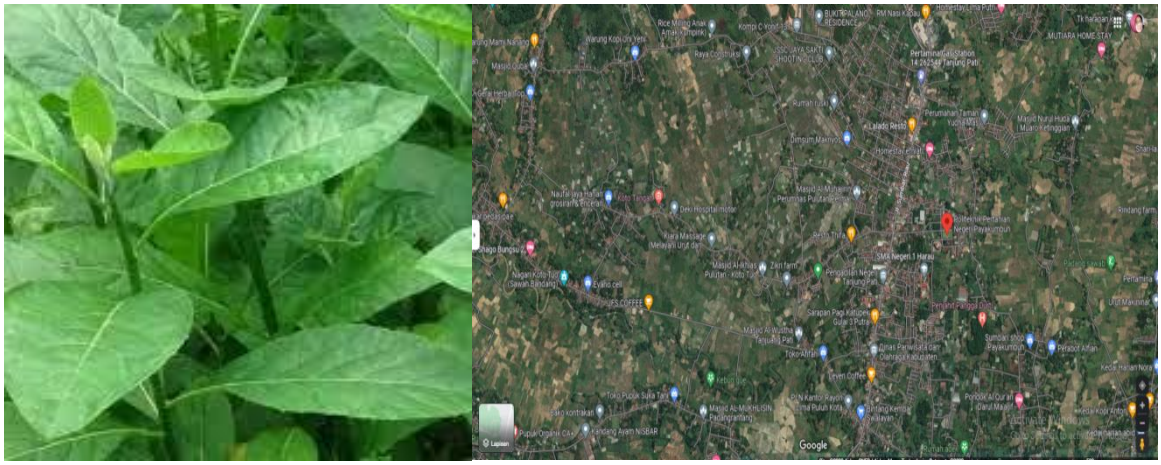

Figure 1. Bitter leaf plant and research locations in Payakumbuh Agricultural Polytechnic

The aim of the study was to determine the effect of adding fermented bitter leaf extract in drinking water to the physiological organs of broilers, and to determine the appropriate dosage of adding fermented bitter leaf extract in drinking water to the profile of broiler physiological organs.

2. Experimental Section

2.1. Materials

This study used 200 DOC without sex separation. Livestock slaughtered and weighed for liver, heart and spleen organs were 2 heads per unit treatment cage, fresh and healthy bitter leaves. The ration used was a commercial ration with a protein content of around 22%, an energy content of ± 3000 Kcal/kg from baseline to 4 weeks of study. Rations with code 511 issued by PT. Charoen Pokpand. Brown sugar, clean water and EM4. The cages used were cages with a size of 100 x 100 cm with a partition height of 60 cm, each unit consisting of 10 heads, totaling 20 units. Feed scales and capacity Ohaus scales, blenders, conductors.

2.2. Methods

2.2.1. The Process of Making Bitter Leaf Fermented Extract

Procedure for making fermented bitter leaf extract (Figure 2): 500 g of bitter leaves are washed, then mashed with a blender. Next put in jerrycan. 500 g of Saka diluted with 500 ml of water, 500 ml of EM4 (5%) added, then put in jerrycan. Water is added to the jerry can until the total solution is 10 liters, stirred evenly and tightly closed. The fermentation process lasts for 15 days, with the characteristic that no gas is formed.

During the fermentation process, when gas is formed, it is removed by opening the lid of the jerrycan for a while, then closing it again. The result of the process of extracting fermented bitter leaves, then filtered, so that it is in the form of a solution. Bitter leaf fermented extract is put in a container in the form of a plastic bottle and stored in an aerobic state [15]. A total of 200 DOC broilers were randomly divided into 20 experimental units, each unit consisting of 10 DOC and each individual was placed randomly in each treatment cage unit.

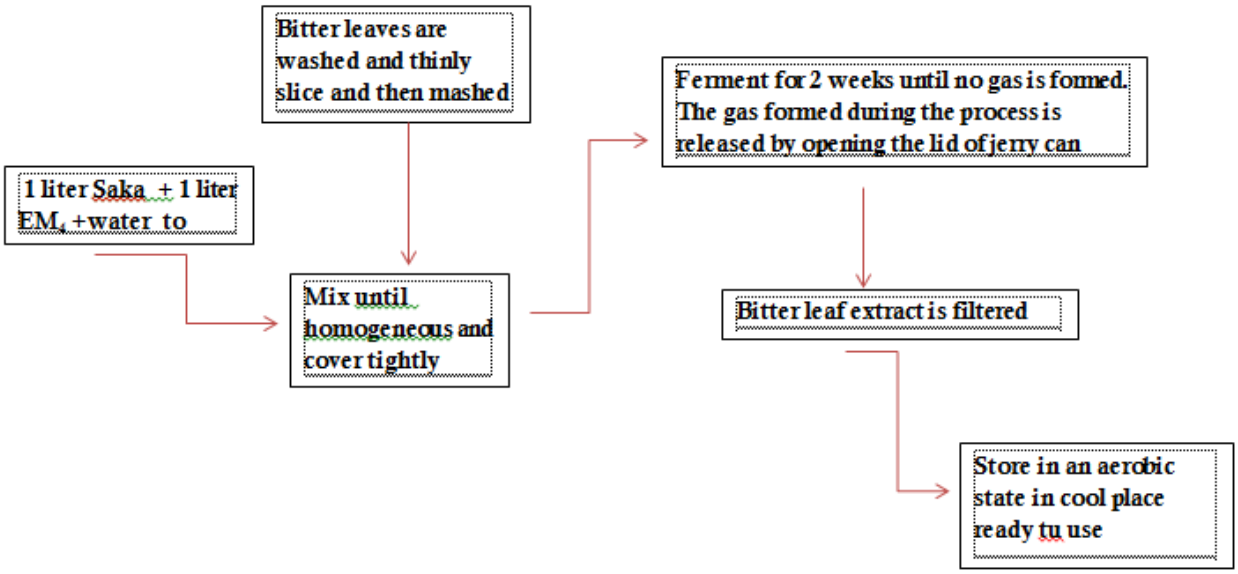

Figure 2. Chart of manufacture of fermented bitter leaf extract [15]

2.2.2. Research Design

This study used a completely randomized design with 4 treatments and 5 replications. As a treatment is fermented bitter leaf extract. The treatment of bitter leaf fermented extract is:

A = Addition of 0% fermented bitter leaf extract in drinking water
B = Addition of 5% fermented bitter leaf extract in drinking water
C = Addition of 10% fermented bitter leaf extract in drinking water
D = Addition of 15% fermented bitter leaf extract in drinking water

In order to determine the effect of the treatments, the data obtained were analyzed with variance, and continued with the DMRT test if there were differences between the treatments [16]. Parameters measured were the weight percentage of liver, spleen and heart.

Table 1. The nutritional composition of bitter leaf meal

Nutrition Composition	Bitter leaf flour
Water content (%)	5.99
Crude protein (%)	27.93
Crude fat (%)	4.15
Crude fiber (%)	10.41
Ash (%)	12.05
Ca (%)	1.43
P (%)	0.426
Total phenol	40.59 mg/g*
IC_{50}	675.06 ppm*

Description: Results of the analysis of Animal Feed and Nutrition Laboratories (2021)
* Quality labor analysis results (2021)

3. Results and Discussion

Broiler physiological organs consist of the heart, spleen and liver. The addition of fermented bitter leaf extract in drinking water to the weight of heart, liver and spleen per body weight of broilers reared for 28 days can be seen in Table 2. The percentage of hearts per broiler body weight produced by the addition of fermented bitter leaf extract sequentially with the addition of 0% fermented bitter leaf extract, 5% fermented bitter leaf extract, 10% fermented bitter leaf extract and 15% fermented bitter leaf extract was 0.721% ± 0.049, 0.718% ± 0.042, 0.712% ± 0.095, and 0.732% ± 0.0098.

While the percentage of spleen per broiler body weight produced by the addition of fermented bitter leaf extract sequentially with the addition of 0% fermented bitter leaf extract, 5% fermented bitter leaf extract, 10% fermented bitter leaf extract and 15% fermented bitter leaf extract was 0.266 ± 0.03, 0.275 ± 0.04, 0.293 ± 0.04, and 0.260 ± 0.01. Effect of the addition of fermented bitter leaf extract in drinking water on liver weight per broiler body weight sequentially with the addition of 0% , 5% fermented bitter leaf extract, 10% fermented bitter leaf extract and 15% fermented bitter leaf extract was 2.29 ± 0.184, 2.60 ± 0.194 , 2.41 ± 0.271, and 2.34 ± 0.097

Table 2. The average percentage of heart, spleen and liver in broilers aged 4 weeks

Treatment	Heart	Spleen	Liver
A	0.721 ± 0.049	0.266 ± 0.03	2.29 ± 0.184
B	0.718 ± 0.042	0.275 ± 0.04	2.60 ± 0.194
C	0.712 ± 0.095	0.293 ± 0.04	2.41 ± 0.271
D	0.732 ± 0.009	0.260 ± 0.01	2.34 ± 0.097

3.1. Heart

The effect of the addition of fermented bitter leaf extract (FBLE) on the percentage of broiler hearts 4 weeks old can be seen in Table 2 and Figure 3 . The results showed that the average broiler heart weight for each treatment ranged from (0.712 ± 0.095)% to (0.732 ± 0.0098)% as shown in Table 2 and Figure 3. The results of variance showed that the addition of fermented bitter leaf extract had no significant effect ($P > 0.05$) on broiler heart weight percentage. The results of variance showed that the addition of fermented bitter leaf extract had no significant effect ($P > 0.05$) on broiler heart weight percentage. This insignificant difference ($P > 0.05$) was caused by the addition of fermented bitter leaf extract which does not contain toxins or anti-nutritional substances so it does not cause excessive contractions in the heart muscle so that the heart muscle does not enlarge or shrink due to the effect of adding fermented leaf extract bitter.

The percentage of heart weight resulting from the addition of fermented bitter leaf extract was (0.712 ± 0.095)% to (0.732 ± 0.0098)%, the results obtained from this study were still in the normal range of heart weight percentage, namely 0.42% - 0 .70% of live weight [17]. Research result [18], by administering bitter leaf juice in drinking water, the range of heart weight percentages was obtained from 0.50 to 0.67%, while the results of this study[19] who added a mixture of *Ocimum gratissimum* and *Vernonia amygdalina* flour, the range of heart weight percentages was 0.42 - 0.74%, research result [20].

The weight of the heart given *Vernonia amygdalina* extract was 9.300 + 0.58 – 11.100 + 0.58 g. The research results obtained from several researchers by giving bitter leaf herbal feed additives, the percentage of heart weight is still in the normal weight range. This means that bitter leaves do not affect heart weight or the heart does not experience enlargement or shrinkage as a result of the addition of bitter leaves in the ration or drinking water which functions as a feed additive.

According to [21] The heart is an internal organ that is very susceptible to toxins and anti-nutritional substances. Heart enlargement can occur due to the accumulation of toxins in the heart muscle. If the blood contains poison and anti-nutrients, it will trigger excessive contractions, causing

swelling of the heart. Besides that, the factors that affect heart size are sex, age, body weight and activity of the livestock. The heavier the heart, the smoother the flow of incoming and outgoing blood, and has an impact on the metabolism in the animal's body [22]. The components of active substances contained in bitter leaf flour, miana leaves, namely flavonoids, tannins, saponins and phenols work and support each other in inhibiting microbial growth. The mechanism of action of flavonoids as antimicrobials is to form complex compounds with extracellular and dissolved proteins that damage the microbial membranes [23]. Besides that, flavonoids have phenolic compounds which are a type of acidic alcohol which is often called carbolic acid. Phenol has the ability to denature proteins and damage bacterial cell walls [24].

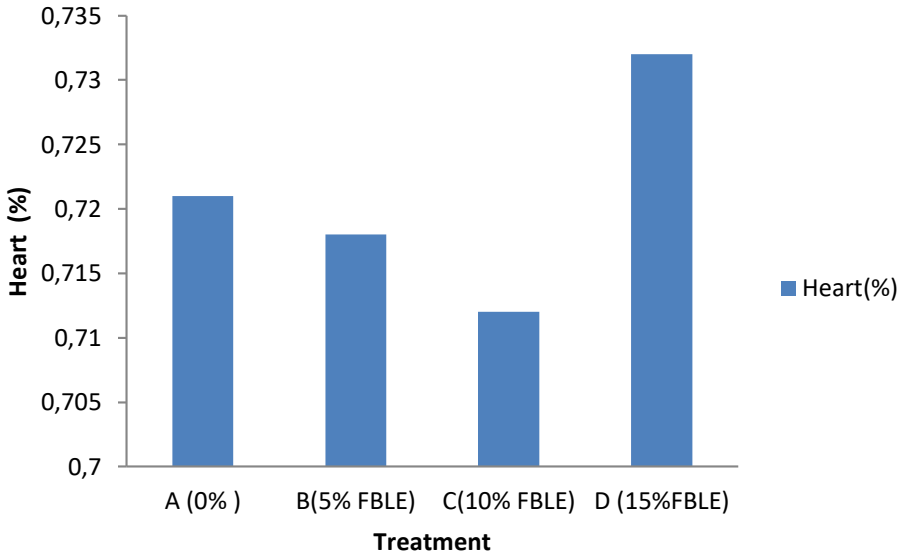

Figure 3. Graph of the effect of giving fermented bitter leaf extract on the percentage of heart of broilers

3.2. Spleen

The effect of the addition of bitter leaf fermented extract on the percentage of broiler spleen aged 4 weeks can be seen in Table 2 and Figure 4. The results showed that the average broiler spleen weight percentage for each treatment ranged from (0.260 ± 0.01)% to (0.293 ± 0 .04)% as shown in Table 2. The results of variance showed that the addition of fermented bitter leaf extract had no significant effect ($P > 0.05$) on the percentage of spleen weight per broiler live weight, this means that the addition of fermented African leaf extract did not have a negative effect on broilers.

The spleen did not experience an increase in size due to the addition of fermented bitter leaf extract. This condition is proof that anti-nutritional compounds in fermented bitter leaf extracts such as tannins and saponins do not have a toxic effect on the spleen. So that the percentage of spleen weight between control and treatment was not significantly different. This is supported by the results of research showing that fermented extracts of bitter leaves do not affect the work of the spleen and do not cause an increase in the size of the spleen [25].

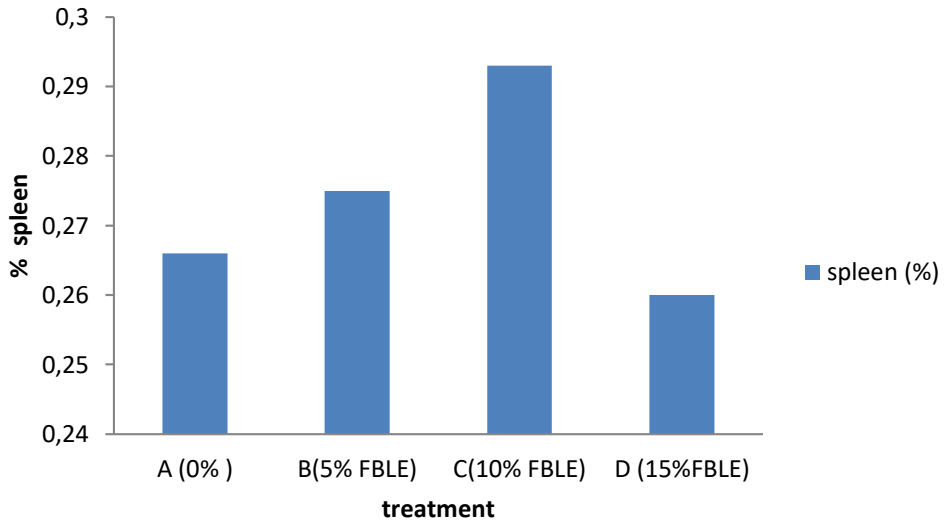

Figure 4. Graph of the effect of giving fermented bitter leaf extract on the percentage of spleen of broilers

The percentage of spleen weight resulting from the addition of fermented African leaf extract is (0.260 + 0.01)% to (0.293 + 0.04)%, these results are in accordance with the opinion [17] chicken spleen weight ranges from 1.5 to 4.5 g, while the results of the study[26], the percentage of spleen weight produced was (0.0088 + 0.01)% to (0.105 + 0.02)% given glucomannan extract from *Amorphophallus onchophyllus* Tuber. While the research results [27] with the addition of the enzyme phytase obtained the percentage of spleen is 0.083 - 0.088% of body weight. Bagus (2008) *cit.* [28] states that the spleen organ forms lymphocytes to form antibodies when food substances contain toxic, nutritional substances or disease. The activity of the spleen increases or even decreases in size because the spleen is attacked by foreign objects or disease.

One of the functions of the spleen is to form lymphocytes associated with the formation of antibodies. In addition, the spleen also stores blood, along with the liver and bone marrow which plays a role in the destruction of old erythrocytes, participates in nitrogen metabolism, especially in the formation of uric acid and forms lymphocyte cells associated with the formation of antibodies [22].

3.3. Liver

The effect of the addition of bitter leaf fermented extract on the percentage of broiler livers aged 4 weeks can be seen in Table 2 and Figure 4. The results showed that the average broiler liver weight percentage for each treatment ranged from (2.29 ± 0.184) % to (2 .60 + 0.194) % as shown in Table 2. The results of variance showed that the addition of fermented bitter leaf extract had no significant effect ($P > 0.05$) on the percentage of liver weight per broiler live weight, this absence of difference is due to the fact that the addition of fermented African leaf extract does not contain toxins or anti-nutrients, so it does not cause excessive contractions in the liver. The results of this study indicated that the addition of fermented bitter leaf extract up to 15% had no significant effect ($P<0.05$) on broiler liver weight percentage.

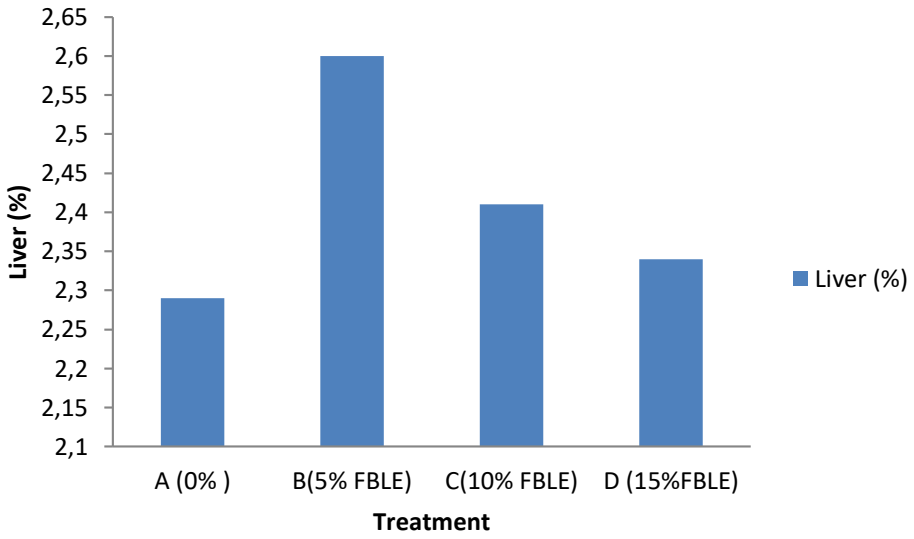

Figure 5. Graph of the effect of giving fermented bitter leaf extract on the percentage of liver of broilers

The resulting liver weight percentage ranged from (2.29 ± 0.184)% to (2.60 + 0.194)%, while from the research results [18] by giving bitter leaf juice, the percentage range of liver weight is 0.50 – 0.64%, while the research results [19] who added a mixture of *Ocimum gratissimum* and *Vernonia amygdalina* flour, the range of liver weight was 1.71 - 2.80 %, research result [20] obtained liver weight was 38.1 ± 0.58 to 44.100 ± 0.58 g by administration of African leaf extract (*Vergonia amydalina*). Research result [29] the percentage of liver weight was 2.5% - 2.77% of live weight added with the extract of the leaves of the plant in drinking water.

The results of research by several experts show that the addition of bitter leaves, both in the form of extracts and powder of bitter leaves containing tannins and saponins, is still within the safe threshold range and does not cause toxic effects, and does not affect liver activity in the detoxification process of toxic compounds. The work of this gland will increase if there are a lot of anti-nutritional compounds such as tannins and saponins in the feed, and vice versa will decrease if the amount is small. Liver weight in this study ranged from 2.29 to 2.60%. The liver weight is still in normal condition because it is still in the range between 1.7% - 2.8% of live weight [17].

4. Conclusion

The conclusions of this study are the addition of bitter leaf fermented extract in drinking water did not affect the percentage of liver, heart and spleen.The addition of fermented bitter leaf extract up to 15% in drinking water can be tolerated by broilers on the weight percentage of liver, heart and spleen. Suggestions from this study are it is recommended for breeders to add drinking water with fermented bitter leaf extract in broiler cultivation which does not negatively affect the heart, liver and spleen organs.

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