Research Article

Immune Response and Carcass Quality of Broiler Treated with Bitter Grape, Red Ginger Extract and Bee Glue: Single or Combine Application

Dwi Desmiyeni Putri[1]*, Nurhayati[1], Anggita Oktafiani[1], Bayu Febram Prasetyo[2], Vindo Rossy Pertiwi[1], Riko Noviadi[1]

[1]Department of Animal Husbandry, Politeknik Negeri Lampung, Indonesia; [2]School of Veterinary Medicine and Biomedical Sciences, IPB University, Indonesia.

Abstract | This study aims to analyze the effectiveness of bitter grape, red ginger extract, and bee glue in drinking water on broiler chickens' immunity and carcass quality. The research was carried out using a Completely Randomized Design (CRD) with 5 treatments and 5 replications. Treatment included P0 = drinking water without added extract (control), P1 = drinking water + 0.5% bitter grape extract and 0.5% bee glue extract, P2 = drinking water + 0.5% red ginger extract and 0.5% bee glue extract, P3= drinking water + 0.5% bitter grape extract, 0.5% red ginger extract and 0.5% bee glue extract, P4= drinking water + 1% bee glue extract. The immune response was assessed using the Hemagglutination Inhibition (HI) test. Variables related to carcass quality included carcass percentage, abdominal fat, blood cholesterol, and cooking loss. The results showed that the addition of bitter grape, red ginger, and bee glue extract either single or as a combination had a significant effect ($P<0.05$) on immunity, abdominal fat, carcass percentage, cooking loss, and blood cholesterol. This study concluded that administering bitter grape, red ginger, and bee glue extracts either individually, in pairs, or as a combination of all three in drinking water can enhance the immunity and carcass quality of broiler chickens.

Keywords | Broiler, Bee glue, Bitter grape, Carcass quality, Immune response, Red ginger

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***Correspondence** | Dwi Desmiyeni Putri, Department of Animal Husbandry, Politeknik Negeri Lampung, Indonesia; **Email:** desmiyenidwi@gmail.com
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INTRODUCTION

Broiler chickens are one type of livestock widely developed as a source of animal protein. In the last 50 years, genetic selection has been able to produce broiler chickens with fast growth (Tixier-Boichard, 2020; Mench *et al*., 2021), which can be harvested at 28-35 days of age with a weight of 1.0-1.2. However, this selected genotype causes the immune system of broiler chickens to tend to be lower than other chickens. This condition makes broiler chickens more susceptible to various diseases and infections. Therefore, it is very important to provide feed additives that can strengthen the body's immune system, prevent infection, and support the overall health of broiler chickens thereby increasing productivity and reducing mortality rates (Dawkins, 2017; Nuraini et al., 2021).

The application of herbal as feed additives for broiler chickens is increasingly popular as a safe and natural alternative to improve livestock health and performance. Several herbs contain bioactive compounds that have antibacterial, antiviral, and anti-inflammatory properties, which can help strengthen the immune system of broiler chickens (Wang *et al*., 2024). Apart from that, herbal

ingredients can also increase appetite, support digestion, and reduce stress in chickens, which often triggers a decline in health. The use of herbal ingredients as feed additives provides a solution that is environmentally friendly and free from synthetic chemicals, and reduces dependence on antibiotics, which is important to prevent antibiotic resistance. Thus, the use of herbal ingredients in broiler feed can enhance production efficiency, reduce mortality rates, and contribute to the production of healthier chicken meat. Commonly used feed additives include rhizome-based extracts such as bitter grape and red ginger, among others (Ayalew et al., 2022; Jachimowicz *et al*., 2022).

Bitter grape (*Tinospora crispa* (L.) Hook.f. and Thomson) is a plant from the Menispermaceae family, commonly found in the rainforests of Southeast Asia and Africa. In Indonesia, it is traditionally known as brotowali, it has been used to treat a variety of health conditions, including fever, malaria, diabetes, rheumatism, and hypertension. Phytochemical analysis shows that *T. crispa* contains various bioactive compounds, including alkaloids, flavonoids, flavone glycosides, triterpenes, diterpenes, lactones, sterols, lignans, and nucleosides. Scientific studies have confirmed various pharmacological activities of *T. crispa* extract, such as anti-inflammatory, antioxidant, immunomodulatory, cytotoxic, antimalarial, cardioprotective and antidiabetic. However, most pharmacological studies are based on crude extracts, and the specific bioactive compounds responsible for the activity have not been clearly identified. Therefore, further research is needed to convert experience-based claims on traditional uses of *T. crispa* into scientific evidence-based information. Bitter grape is widely used as a medicinal plant in Indonesia because this plant contains flavonoids, phenols, alkaloids, diterpenoids, lignins, and lactones (Harmoko and Choironi, 2016). The addition of red ginger extract (1%) and Bitter grape extract (5.12 g/kg) in drinking water, either single or in combination, had a significant effect on feed conversion ratio, body weight gain, mortality, carcass percentage, abdominal fat, and cooking loss (Shaffira *et al*., 2023).

Red ginger (*Zingiber officinale* Roxb. var Rubra) is a rhizome plant generally used as a kitchen spice and herb. Red ginger has a higher content of 6-gingerol, 8-gingerol, 10-gingerol, and 6- shogaol when compared to elephant ginger, namely 18.03, 4.09, 4.61, and 1.36 mg/g, so it is widely consumed as a medicinal ingredient. Red ginger contains volatile compounds, namely terpenoids, and non-volatile compounds consisting of gingerol, shogaol, paradol, zingerone, and their derivative compounds and flavonoid and polyphenol compounds (Ahmed *et al*., 2014). Sa'aci *et al*. (2018) stated that ginger water extract supplementation positively affected total feed consumption, final body weight, and feed conversion ratio. The ginger extract supplementation level of 127.49 mL/L maximizes daily weight gain, while the maximum FCR at the inclusion level is 88.79 mL/L through drinking water.

Furthermore, another type of rhizome that can be used to improve broiler performance is bitter grape. Herbs will be optimal if combined with animal products that have benefits, such as bee glue (propolis). Bee glue extract contains several chemical compounds, including polyphenols (flavonoids, alcohol, phenolic aldehydes, and acids), steroids, and terpenoids (Maksoud *et al*., 2023). The biological activities of bee glue include antimicrobial, antiparasitic, immunomodulatory, antiprotozoal, anti-inflammatory, and antioxidant (Magnavacca *et al*., 2022). Bee glue evidenced considerable antimicrobial activity, as it inhibited the growth of yeasts, Gram-negative and Gram-positive bacteria, being the latter the most sensitive to this beehive product's action (Silva *et al*., 2012). Bee glue is thought to exert antibacterial activity by enhancing the organism's immunity or acting directly on microorganisms (Maurício and Bankova, 2011). Single administration of red ginger extract (1 mL/L drinking water), bee glue extract (1 mL/L drinking water), and combined administration of ginger extract (0.5 mL/L drinking water) and bee glue. extract (0.5 mL/L drinking water) shows immunomodulatory properties against three common diseases, NDV, AI, and IB in broiler chickens (Maksoud *et al*., 2023). These three ingredients individually have been proven to have the potential to stimulate productivity or immunity or carcass quality of broiler chickens. There is no research has shown that the combination of these three ingredients has the potential as a feed additive that can work multi-effect in improving broiler chicken performance, including productivity, immunity, and carcass quality. This study aims to analyze the potential effects of herbal preparations of bitter grape, red ginger, and bee glue extract on immunity and broiler carcass quality.

MATERIALS AND METHODS

Preparation of red ginger and bitter grape extract

The extraction process for bitter grape and red ginger were conducted using the maceration technique. Fresh bitter grape and red ginger were first gathered and cleaned to remove any impurities, then rinsed thoroughly under running water before being drained. Afterward, they were thinly sliced and dried either by sun-drying for 2 to 7 days or using an oven at a temperature of 49–55°C for 2 days. Once dried, they were ground into a fine powder and passed through a 50-mesh sieve before being stored in a container.

Bitter grape and red ginger extracts are done using the maceration method with a ratio of 1:5, namely 50 g of bitter grape powder and red ginger powder were put into

a bottle, poured with 250 mL of 96% ethanol, closed, and left for 24 hours, shaken every hour, so that it was distributed evenly. The juice is poured out, and the dregs are squeezed and separated into another bottle. The remaining pulp was added to 250 mL of 96% ethanol and then macerated for 24 hours, stirred, and mixed; the maceration results were combined to obtain 250 mL of whole fruit juice. The bottle was closed and left for a day, and then continued to make extract concentrated using a rotary evaporator at a temperature of 55–65°C. The final product was then weighed, and its yield and characteristics were analyzed (Zulharmitta *et al.*, 2017). The bee glue extract used in this study was a commercial product, Propolis Trigona Khatulistiwa, registered with the Food and Drug Supervisory Agency under number 183621641, and containing 150 mg of bee glue extract per unit.

Research design

One hundred day-old broiler chickens with an average body weight 42.73±1.55 g/head were raised for 28 days with ad libitum access to feed and water. They were provided with BR-1® and BR-2® commercial feed from PT Japfa Comfeed. The method for incorporating bitter grape, red ginger and bee glue extracts into the drinking water is presented in Table 1.

This research was carried out using a Completely Randomized Design (CRD) consisting of 5 treatments and 5 replications, each containing 4 broilers age 7 days (Gomez and Gomez, 1995). In this study, the treatments were the administration of bitter grape, red ginger extract, and bee glue. Bitter grape, red ginger extract, and bee glue are given to broiler chickens at 7-28 days. The treatments were: P0 = drinking water without adding extract (control), P1= drinking water + 0.5% bitter grape extract and 0.5% bee glue extract, P2= drinking water + 0.5% red ginger extract and 0.5% bee glue extract, P3= drinking water + 0.5% bitter grape extract, 0.5% red ginger extract and 0.5% bee glue, P4 = drinking water + 1% bee glue. Throughout the research, the broilers were housed in cages with rice husk bedding, and the extract-enhanced water was administered daily from day 8 until the end of the study.

Immunity

The broiler chicken immunity that measured in this research was specific immunity to Newcastle Disease (ND). Immunity to certain antigens will appear after the chicken is induced by that antigen (Delves, 2024). To analyze specific immunity to ND, broiler chickens given ND vaccination according to the vaccination schedule.

The first ND vaccination on broiler chickens is carried out in the hatchery, and the second ND vaccination is carried out on day 11. To determine ND antibodies before vaccination, blood is drawn 1 day before vaccination. The blood sample used was taken from the brachial vein using a 3 mL syringe. To determine immune response to ND, blood samples were taken on days 16, 20, 24 and 27. Next, the blood samples were analyzed for antibody titers to ND using the Haemaglutination Inhibition Test (Bordoloi et al., 2021).

Carcass quality

Carcass quality was analyzed using 20 broiler chickens at the end of rearing to determine carcass percentage, abdominal fat percentage, cholesterol and cooking loss. The carcass percentage is obtained from the ratio of carcass weight to live weight multiplied by 100%. Abdominal fat percentage is calculated using a formula: (abdomen fat weight)/(live weight) x 100%. Cholesterol is carried out using a digital cholesterol measuring device (EasyTouch). Cooking loss percentage calculated by formula:

$$\frac{\textit{initial weight before steaming} - \textit{final weight after steaming}}{\textit{initial weight before steaming}} \times 100\%$$

Statistical analysis

The research data were analyzed using one-way analysis of variance (ANOVA). ANOVA applied to SPSS conforms to a Completely randomized design. The difference between treatments was analyzed using Duncan's multiple distance test $P<0.05$ using SPSS version 23.

Table 1: Study protocol for administering bitter grape, red ginger, and bee glue extracts in the drinking water of broilers.

Days	Treatment
1-6	Broilers are given water ad libitum without adding bitter grape, red ginger, and bee glue extracts
7-28	1. P0 was given drinking water ad libitum without the addition of bitter grape, red ginger, and bee glue extracts. 2. P1 was given drinking water with the addition of bitter grape extract at a dose of 0.5% and bee glue extract at 0.5%. 3. P2 was given drinking water with the addition of red ginger extract at a dose of 0.5% and bee glue extract at 0.5%. 4. P3 was given drinking water with the addition of a combination of 0.5% bitter grape extract, 0.5% red ginger extract, and 0.5% bee glue extract. 5. P4 was given drinking water with the addition of 1% bee glue extract.

RESULTS AND DISCUSSION

Immunity

In this study, the parameters observed in immunity were the Newcastle Disease antibody titer of broilers treated with bitter grape, red ginger extract, and bee glue, either alone, in a combination of 2 ingredients, or 3 ingredients in drinking water, which is presented in Figure 1. The antibody titer values for all groups showed a decrease after given all treatment; this is the opinion (Rahman *et al.*, 2017) which states that vaccinated broiler chickens will produce higher antibody titers on the next day-7 after vaccination and will experience a reduction in the number of antibody titers on the 14th day and 21st day after vaccination. Then, when the chickens were 24 and 27 days old, blood was retaken, showing that the average antibody titer value had increased. The increase in the antibody titer value that had the best results was in the chicken group treated with 1% bitter grape extract (P4), then continued with the chicken group treated with a combination of bitter grape extract, red ginger and bee glue (P3) and the chicken group treated with red ginger extract and bee glue (P2). It is estimated that in the next few days, the titer treated with bitter grape extract, red ginger extract, and bee glue extract will continue to increase to reach a titer with a protective level.

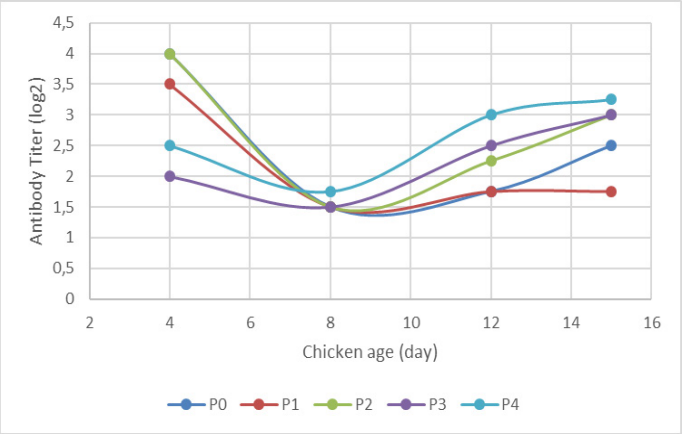

Figure 1: Antibody titers of Broiler after treatment. P0= drinking water ad libitum without the addition of bitter grape extract, red ginger extract, and propolis. P1= drinking water + 0.5% bitter grape extract and 0.5% propolis. P2= drinking water + 0.5% red ginger extract and 0.5% propolis. P3= drinking water + 0.5% bitter grape extract and 0.5% red ginger extract and 0.5% propolis. P4= drinking water + 1% propolis extract.

This increase in antibody titer showed that the addition of bee glue and red ginger extract has immunomodulatory capabilities that stimulate cells that produce antibodies to increase the quantity of antibodies in a reasonably short time. This is in line with research (Maksoud *et al.*, 2023), which reports that adding 1% bee glue extract to drinking water and a combination of 0.5% bee glue extract and ginger extract can increase chicken antibody titers. The biological activities of bee glue include antimicrobial, antiparasitic, antiprotozoal, anti-inflammatory, immunomodulatory, and antioxidant. Based on the isoprene unit, terpenoids are divided into various groups such as monoterpenoids, hemiterpenoids, sesquiterpenes, diterpenes, triterpenes, and tetraterpenoids which act as antioxidants, anticancer, antidiabetic, immunostimulant, anti-inflammatory and immunomodulatory (Paudel *et al.*, 2023).

Immunomodulatory components are from the flavonoid group. The biological activities of flavonoids include immune cells, differentiation, modulating their activation and proliferation. They can work on neutrophils by reducing protein and receptor expression on T cells, NK cells, DC, and macrophages (Gozzi-silva *et al.*, 2021). The antimicrobial and antioxidant effects of bee glue and bee pollen, the role of phenolic components such as various flavonoids, phenolic acids and their derivatives are emphasized, which can increase nutrient absorption and protect intestinal villi (Chitura *et al.*, 2022). Flavonoid compounds are polar compounds, so they will dissolve in water (Hossain *et al.*, 2021). Extracts made using the maceration method are very influential in obtaining many immunomodulatory compounds because flavonoid compounds are polar. During treatment, the immunomodulatory compound can dissolve in the broiler's water and increase the broiler's antibody titer.

The effectiveness of red ginger is due to the presence of high concentrations of polyphenols and sesquirpens (eg curcumin, salvinorin A), especially flavonoids and tannins, which are known for their capacity to donate the electrons (Rampengan *et al.*, 2024). Phenolic chemicals and are vital for counteracting free radicals and reactive oxygen species (ROS), to reduce oxidative stress, and to protect cells from damage. An important role of this antioxidant effect is to improve diseases caused by oxidative stress, such as inflammatory disorders and metabolic syndrome (Rahman *et al.*, 2021).

Broiler chicken carcass quality

Table 2 presents the effect of treatment results of bitter grape extract, red ginger, and bee glue in drinking water on carcass percentage, belly fat percentage, cholesterol levels, and cooking losses in broiler chickens. The values followed by different superscripts (a, b, c) indicate significant differences between treatments. The highest carcass percentage was observed in P4 (68.80%), followed closely by P2 (68.23%), P1 (67.96%), and P3 (66.47%). The control group (P0) had the lowest carcass percentage (57.86%). The values followed by different superscripts (a, b, c) indicate significant differences between treatments. Based on a statistical analysis of groups of chickens that were treated with bitter grape extract, red ginger, and

Table 2: Carcass, abdominal fat, cholesterol, and cooking loss of broiler chickens treated with bitter grape, red ginger and bee glue extracts either singly or in combination.

Variable	P0	P1	P2	P3	P4
Carcass (%)	57.86^{a} ±1.30	67.96^{b}±0.99	68.23^{b}±0.33	66.46^{b}±2.19	68.79^{b}±1.69
Abdominal fat (%)	1.46^{b}±0.25	0.88^{a}±0.30	1.04^{a}±0.10	0.77^{a}±0.12	0.77^{a}±0.20
Cholesterol (mg/dL)	287.75^{c}±11.24	247.75^{ab}±12.37	234.50^{ab}±14.20	265.75^{bc}±15.90	230.00^{a}±14.33
Cooking loss (%)	49.40^{c}±3.33	33.60^{b}±3.93	29.80^{ab}±2.01	29.95^{ab}±3.97	22.30^{a}±1.89

bee glue given alone, the combination of two ingredients or three ingredients showed a significant effect ($P < 0.05$) on the group of chickens that were not given additional extracts in their drinking water. (P0). The results showed that the percentage of carcasses in the group of chickens that were not given extract treatment (P0) was (57.86%), in the group of chickens that were treated with bitter grape extract and bee glue (P1) was (67.96%), in the group of chickens that were treated with bitter grape extract and bee glue (P1). Given treatment with red ginger extract and bee glue (P2) amounted to (68.23%), for the group of chickens treated with a combination of bitter grape, red ginger extract, and bee glue (P3) amounted to (66.4675%). At the same time, for the line with research, Bulkaini (2022) reported, with a maximum percentage of 84.62% and a minimum percentage of 60% and an average percentage of broiler chicken carcasses of 72.94%. Broiler strain, age, rearing management, gender, type of feed, or natural conditions such as humidity, environmental temperature, and air pressure cause carcass weight and percentage variations.

An increase in body weight is the cause of an increase in carcass percentage because the higher the body weight, the higher the carcass presentation. This shows that bitter grape, red ginger, and bee glue extracts act as growth promoters. This is confirmed by Lillehoj *et al.* (2018) stated that phytochemical compounds, namely phenolics, flavonoids, saponins, and tannins, can be used as Antibiotic Growth Promoters (AGP) for poultry because these compounds can have antibacterial properties, which in turn can reduce the growth of pathogenic bacteria in the poultry digestive tract.

The abdominal fat percentage result shown that in the treatment groups (P1, P2, P3, and P4) compared to the control (P0). The highest fat percentage was observed in P0 (1.46%), while P3 and P4 (0.77%) had the lowest fat accumulation. Based on a statistical analysis, the Abdominal fat in the group of chickens given bitter grape, red ginger, and bee glue extracts given alone, a combination of two ingredients, and a combination of three ingredients showed significant results ($P<0.05$) in the group of chickens that were not given additional extracts drinking water (P0). In this study, the percentage of abdominal fat in broilers aged 28 days ranged from 0.77% to 1.46%. This is slightly different from (Annisa *et al.* 2020) those who reported that the abdominal fat of broilers aged 42 days ranged from 3.55% to 5.52%; this is because the age of the broilers is different. The extended maintenance resulted in more fattening and fat accumulation in chickens. The high metabolism in broiler chickens due to herbal administration causes low abdominal fat, so a relatively higher body weight compensates for it. This is in line with research (Tahalele *et al.*, 2018) stating that adding herbal concoctions to drinking water significantly affects the percentage of abdominal fat ($P<0.01$).

Many factors, such as polysavones, can suppress the development of abdominal fat. Polysavone, compounds containing flavonoids and saponins, show actual activity in enhancing immunity, perhaps due to a simple combination of the functions of the individual components or synergism between the active ingredients (Dong *et al.*, 2007). In lipid metabolism through cytokines the immune system plays a vital role, this is the same as the excretory system in regulating lipolysis or lipogenesis through hormones; for example, interleukin-1 has an essential role in lipid metabolism by regulating insulin levels under physiological conditions (Matsuki *et al.* 2003). Therefore, reducing abdominal fat deposits caused by the active substance flavonoids and saponins should be associated with increased immune response.

The cholesterol levels varied across treatments, with the highest level recorded in the control group (P0: 287.75 mg/dL). The lowest cholesterol level was observed in P4 (230.00 mg/dL), followed by P2 (234.50 mg/dL) and P1 (247.75 mg/dL). Based on the results of statistical analysis of cholesterol levels, it was shown that in chickens treated with bitter grape extract, red ginger, and bee glue, which were given alone, a combination of two ingredients and a combination of three ingredients showed results that had a significant effect on the group of chickens that were not given additional extracts in their drinking water. The herbal ingredients contain bioactive substances such as antioxidants, which can help reduce chicken blood cholesterol levels. Antioxidants that can neutralize free radicals, neutralize cholesterol, and have anticancer properties include flavonoids and eugenol. The antibiotic properties contained in these compounds can prevent the growth of bacteria, viruses, or fungi that harm the body.

Flavonoids can reduce cholesterol levels to reduce LDL levels in the blood (Bjune *et al*., 2024; You *et al*., 2008). Bee glue, which plays a significant role as an antioxidant to increase the activity of the glutathione enzyme or contains components such as essential fatty acids that suppress the activity of 3-hydroxy-3-methylglutaryl coenzyme, is the cause of decreased triglyceride and cholesterol values. The liver has a reductase, the main regulatory enzyme in cholesterol synthesis (Babińska *et al*., 2013).

Cooking loss, which measures the amount of weight loss after cooking, was significantly reduced in the treatment groups. The highest cooking loss was recorded in P0 (49.40%), while the lowest was in P4 (22.30%). Statistical analysis shows that the administration of bitter grape extract, red ginger, and bee glue given singly or in combination has a significant effect ($P<0.05$) on cooking loss of broiler chicken meat. The research results showed that the treatment with 1% bee glue extract had a lower cooking loss percentage range, namely around 22.30%. Meat that has good meat quality is meat that has low cooking loss because fewer nutrients are lost. According to Rafli *et al*. (2022), adding mixed herbal in broiler feed had a better cooking loss than using commercial feed treatment.

Bitter grape extract, red ginger, and bee glue, which contain tannins, cause a decrease in carcass percentage. Das *et al*. (2020) and Ribeiro *et al*. (2022) stated that tannin is a compound that can bind and precipitate proteins and is soluble in water (especially hot water). The hot water temperature causes the weight of the meat to decrease, causing the protein content in the meat to decrease. The high intramuscular fat content in meat can cause cooking loss, loss of liquefaction, oxidative stability of beef, and percentage of cooking loss (Adetunji *et al*., 2019). The loss of meat during cooking, which is relatively high in the range of 35-50%, is related to the substantial lipid content because some of the lipids will be lost during cooking (Xia *et al*., 2009). When cooking, the amount of water lost greatly influences cooking losses. The more air that is trapped, the less meat protein. This is because meat protein can bind air. Air is released, which results in lower cooking losses (Kartikasari *et al*., 2018).

CONCLUSION

The results of this research indicate that the addition of bitter grape extract, red ginger extract, and bee glue whether administered individually, in pairs, or as a combination of all three has a significant positive effect on broiler immunity and carcass quality, including carcass percentage, abdominal fat, blood cholesterol, and cooking loss.

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NOVELTY STATEMENT

Bitter grape, red ginger extract, and bee glue, either single or combine application, may increase the immunity and carcass quality of the broiler.

AUTHOR'S CONTRIBUTION

DDP, N, BFP, and AO conceived and planned the experiments. DDP, BFP, and AO performed the experiments in the laboratory and in the field. DDP, N, BFP, and AO contributed to interpreting the results. DDP, VRP and RN led the writing of the manuscript. All authors provided critical feedback and analyzed and revised the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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