


# Effects of Bioherbal Compounds on Performance and Intestinal Characteristics of Laying Chickens

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Received: 23 April 2023

Accepted: 03 June 2023

## ABSTRACT

Since the European Union banned the use of antibiotic growth promoters in poultry feed in 2006 (EC Regulation No 1831/2003), alternative feed additives have been investigated. The purpose of this study was to evaluate the effect of a bioherbal combination of probiotics and phytobiotics as a feed additive in drinking water on the performance and intestinal characteristics of male laying chickens. The study was performed on 200 male laying chickens for 60 days. This research method was a field experiment with a completely randomized design, consisting of four treatments and five replications. The treatments were T0 (drinking water without bioherbal, control), T1 (control + bioherbal code 1 M), T2 (control + bioherbal code 2 H), and T3 (control + bioherbal combination of 1M and 2H). The investigated parameters included growth performance and intestinal profile of the male laying chickens. The addition of bioherbal increased the number of villi in the intestines of the male laying chickens; however, there was no significant difference among other parameters. It can be concluded that the addition of bioherbal code 2H as a feed additive with a composition of herbal leaves can improve the performance and intestinal characteristics of male laying chickens.

**Keywords:** Intestinal Characteristic, Male Layings, Performance Production, Phytobiotic, Probiotic

## INTRODUCTION

Farmers strive to achieve high production results from male laying animals to increase their income. One effective way to achieve this is by regulating feed management, as feed constitutes the majority of production costs, accounting for around 60-70% of the total production costs (Ardiansyah et al., 2022). However, providing feed for animals also contributes significantly to land and water use, as well as greenhouse gas emissions (Adli, 2021).

To increase feed efficiency and reduce production costs, many farmers turn to feed additives. Antibiotics are commonly used as feed additives to suppress pathogenic digestive bacteria, leading to improved intestinal's health. However, the use of antibiotics as feed additives has been associated with many negative effects on both livestock and humans who consume them. Consequently, the Indonesian government banned the use of antibiotics as feed additives on January 1, 2018, as stipulated in the Minister of Agriculture Regulation Number 14/2017

Article 16, which applies to both finished products and raw ingredients for veterinary drugs that are mixed into feed (Adli et al., 2023; Shoilikin et al., 2023).

With the prohibition of using Antibiotics Growth Promoters (AGP), many researchers have been finding substitutes for antibiotics as additives, such as natural additives derived from herbals. Derived from natural ingredients, alternative feed additives (such as probiotics, prebiotics, enzymes, organic acids, and phytobiotics) are becoming increasingly popular in the livestock industry, particularly in poultry (Sami and Fitriani, 2019; Mirzaei et al., 2022). Bioherbal is an additive combination of probiotics and phytobiotics, where the synergistic of both are expected to provide a more optimal effect on increasing the productivity and health of livestock (Adli et al., 2023).

Probiotics are additives derived from live microorganisms that can increase growth and feed efficiency without leaving residues since the process of absorbing probiotics into the body of livestock does not occur (Mirzaei et al., 2022). Probiotics can be applied to

feed, medicine, and feed supplements in powder or liquid form, while phytobiotics are additives in the Natural Growth Promoter (NGP) group derived from herbs, spices, or other plants because they contain antimicrobial substances and antioxidants (Kurniawati et al., 2021). Some herbal plants often used as additives include aromatic ginger, curcuma, cinnamon, ginger, turmeric, kalmegh, moringa, papaya, and betel leaves (Saeed et al., 2018; Ardiansyah et al., 2022; Li et al., 2023).

Based on the above, this research was conducted to evaluate the effects of adding bioherbal, a feed additive combination of probiotics and phytobiotics, both rhizomes and leaves biopharmaceuticals, on performance and intestinal characteristics in male layings to achieve maximum productivity.

## MATERIALS AND METHODS

### Ethical approval

Ethical approval for the study was given by the Animal Care and Use Committee, University of Brawijaya, No.118-KEP-UB-2022 on August 21, 2022.

### Research materials

In this study, 200 male ISA brown laying chickens were growing for 60 days. Male ISA brown laying chickens were chosen due to regulations the Indonesian government was not allowed to sacrifice the female laying chickens. Open house cages, commercial starters, finisher feeds, bioherbal codes 1M and 2H, vaccines, medicines, and cage equipment were used as essential components of a well-managed poultry operation. The bioherbal compositions of 1M and 2H are presented in Table 1.

**Table 1.** Bioherbal compositions of 1M and 2H

1M	Consisted	2H	Consisted
Aromatic ginger	1ml	Kalmegh leaves	2ml
Curcuma	1ml	Betel leaves	2ml
Ginger	1ml	Moringa leaves	2ml
Turmeric	1ml	Papaya leaves	2ml
Actinocetes	1.2 x 10 <sup>6</sup> cfu/ml	Actinocetes	2.9 x 10 <sup>6</sup> cfu/ml
Lactic Acid Bacteria	1.2 x 10 <sup>6</sup> cfu/ml	Lactic Acid Bacteria	2.9 x 10 <sup>6</sup> cfu/ml
Photosynthetic Bacteria	1.2 x 10 <sup>6</sup> cfu/ml	Photosynthetic bacteria	2.9 x 10 <sup>6</sup> cfu/ml
Yeast	1.2 x 10 <sup>6</sup> cfu/ml	Yeast	2.9 x 10 <sup>6</sup> cfu/ml
Fermented Fungi	1.2 x 10 <sup>6</sup> cfu/ml	Fermented fungi	2.9 x 10 <sup>6</sup> cfu/ml

## Methods

The *in vivo* field experiment using a completely randomized design was employed to evaluate the effectiveness of bioherbal combinations of probiotics and phytobiotics as additives in drinking water. The experiment included two codes, namely codes 1M and 2H and was composed of four treatments, each with five replications. Thus, there were a total of 20 experimental units, with each replication consisting of 10 chickens.

In this study, four different treatments were given to evaluate the effectiveness of bioherbal combinations as additives in poultry production. The first treatment, T0, served as a control and involved providing drinking water without any bioherbal additives. The second treatment, T1, involved adding Bioherbal Code 1M to the control group. The third treatment, T2, involved adding Bioherbal Code 2H to the control group. Finally, the fourth treatment, T3, involved adding both Bioherbal Code 1M and 2H to the control group, alternating between the two every week. By comparing the results from each of these treatments, the study aimed to determine the impact of bioherbal additives on the health and productivity of poultry.

### Research variables

The variables measured in this study included performance and intestinal characteristics.

#### Performance

##### Feed consumption

Feed consumption can be determined by weighing the feed difference between the feed given and the remaining feed (Putri and Bintari, 2021).

Feed consumption =  $\Sigma$  feed given (g) -  $\Sigma$  remained feed (g)

##### Body weight gain.

Body weight gain for male layings can be determined by weighing the chickens by calculating the difference between the final body weight and the initial body weight.

Body weight gain = Final body weight (g) - Initial body weight (g)

##### Feed conversion ratio

Feed conversion ratio (FCR) expressed between feed offered and total body weight produced (Ardiansyah, et al., 2022).

FER =  $\Sigma$  feed consumption (g) / body weight gain (g)

##### Income over feed cost

Income over feed cost (IOFC) was expressed from total income by differences between amount of laying chicken harvested and total cost of feed at whole periods

(Fitro et al., 2015).

IOFC = (Body weight x chicken price (alive)) - (Σ feed consumption x feed cost)

**Intestinal characteristics**

*Number of villi, length of villi, surface area of villi, and depth of crypts*

The prepared villi were analyzed under a DIC Olympus BX51TF light microscope (Japan) connected to the Optilab application. Measurements of the number of villi, villi length, villi surface area, and depth of the crypt were carried out using the Image Raster application, which was adjusted for magnification at the time of observation.

**Statistical analysis**

For the statistical analysis, analysis of variance (ANOVA) using a general linear model (GLM) was carried out in SAS OnDemand for Academics (ODA, Cary, NC, USA). The results were presented as standard error mean (SEM). Moreover, probability values were

calculated using the least significant different testing. The following model was used by following Adli et al. (2022) and Ardiansyah et al. (2022).

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where,  $Y_{ij}$  signifies the parameters observed,  $\mu$  is the overall mean,  $T_i$  denotes the effect level of date seed flour, and  $e_{ij}$  determines the amount of error number. Furthermore, the probability values were calculated using the least significant difference of  $p < 0.05$ .

**RESULTS AND DISCUSSION**

Tables 2 and 3 show the results of observations and statistical analysis on performance and intestinal characteristics of male layings, including feed consumption, body weight gain, feed egg, income over feed cost, number of villi, length of villi, the surface area of villi and depth of crypt with four treatments and five replications.

**Table 2.** Effect of adding bioherbals on feed consumption, body weight gain, FER, IOFC, of 60 days male laying chicken during research

Parameters	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	SEM
FI (g/bird)	2362.51	2378.51	2344.00	2363.24	12.33
BWG (g/bird)	842.20	832.00	873.19	852.12	11.30
FER	2.69	2.74	2.58	2.66	0.12
IOFC (IDR/head/day)	4391.13	3592.06	5302.04	4493.82	89.77

FI: Feed intake, FER: Feed egg ratio, FBW: Final body weight, IOFC: Income over feed cost. <sup>a,b,c,d</sup> Means with different superscripts in the row differ significantly ( $p < 0.05$ ). T<sub>0</sub>= Drinking water without bioherbal (control); T<sub>1</sub>= Control + bioherbal code 1M, T<sub>2</sub>= Control + bioherbal code 2H, T<sub>3</sub>= Control + bioherbal code 1M and 2H, with alternate every week

**Feed consumption**

Table 2 presents feed consumption data from a field experiment by giving control drinking water and adding bioherbal. The results of data analysis showed that the addition of bioherbal to drinking water had no significant effect on the whole parameters of growth performance, including feed intake, body weight gain, feed egg ratio, and income over feed cost ( $p > 0.05$ ). This suggests that the bioherbal was not optimally absorbed by the laying chickens, and its content did not significantly impact feed intake ( $p > 0.05$ ). Similar findings were reported by Tini et al. (2020), who indicated that giving herbal supplements (ginger, turmeric, and curcuma) to quails did not have a significant effect on feed intake, possibly due to suboptimal absorption of the herbal ingredients.

The highest value of feed intake was found in T1 with a total of 2378.75 gram/bird when 1M bioherbal

was added. In contrast, the lowest value was 2344.00 gram/bird in T2 with the addition of 2H bioherbal. The content of essential oils and curcumin contained in 1M bioherbal can increase the appetite of chickens because these ingredients play a role in the rapid emptying of stomach contents, leading to higher feed consumption (Tini et al., 2020). palatability, environment, and livestock behavior are also important factors that influence feed consumption (Avianti et al., 2020). Palatability can be affected by the ingredients contained in bioherbal, which give a different taste, one of which is the bitter taste caused by turmeric, while the behavior of livestock is related to the feeding system (Ardiansyah et al., 2022). *Ad libitum* system can make it easier for livestock to consume feed every time. Ultimately, these related factors influenced the treatment of the laying chickens.

### Body weight gain

Table 2 presents the results of a field experiment on body weight gain by providing controlled drinking water and adding bioherbal. The data analysis showed that the addition of bioherbal to the drinking water did not have a significant effect ( $p > 0.05$ ) on the body weight gain of male layings. The average values from lowest to highest were T1 (832.00), T0 (842.20), T3 (852.15), and T1 (873.19) gram/bird. The highest body weight value of 873.19 g/bird was observed in T2 chickens with the addition of 2H bioherbal, while the lowest value of 831.00 g/bird was observed in T1 with the addition of 1M bioherbal. These findings are consistent with the results of a study conducted by [Sukmaningsih and Rahardjo \(2019\)](#), which reported that the use of probiotics and herbs in drinking water did not successfully increase the feed intake and body weight gain of broiler chickens.

Body weight gain is influenced by the amount of feed consumed. The more feed consumed, the more rapidly the body weight will increase. The feed consumed must meet the nutritional needs of livestock to be converted into the desired product. The increase in feed consumption was consistent with the increase in body weight in each treatment. The addition of 2H bioherbal to T2 showed that a small amount of feed consumption was able to produce maximum body weight gain, presumably because the bioherbal content was able to improve feed quality, allowing the body to utilize it better for increasing body weight. [Daud et al. \(2017\)](#) reported that the physiological function of livestock affects body weight gain because the feed consumed will preferably be used for the formation of body tissues. Several factors that affect livestock body weight gain are breed, feed, rearing management, and environmental conditions ([Sholikin et al., 2023](#)).

### Feed conversion ratio

Table 2 displays the results of FCR based on a field experiment involving the provision of control drinking water and the addition of bioherbal. The data analysis indicated that the addition of bioherbal to the drinking water did not have a significant effect on the FCR ( $p > 0.05$ ), with an average result from lowest to highest of T2 (2.58), T3 (2.66), T0 (2.69), and T1 (2.74). This lack of significant effect may be due to the relationship between feed intake and body weight gain, which did not show a significant effect and is used to calculate the feed egg ratio. Similar to the research conducted by [Sukmaningsih and Rahardjo \(2019\)](#), the addition of a combination of probiotics and herbs did not have a significant effect on

the feed egg ratio of broiler chickens. However, the addition of bioherbal did affect the feed egg ratio according to the treatment given. The optimal digestive tract ecosystem of livestock can be achieved through the use of certain leaves contained in the 2H bioherbal, which play a role in increasing body weight. For instance, the active compounds found in Moringa leaves, such as essential oils, flavonoids, and antioxidants, can increase productivity by promoting the optimal functioning of organs. Additionally, fragrant papaya leaves can increase consumption and body weight gain, which can affect the feed egg ratio ([Sami and Fitriani, 2019](#)). The feed egg ratio can be influenced by genetics, feed and water quality, type of livestock, and rearing management ([Daud et al., 2017](#)).

### Income over feed cost

Table 2 presents the income over feed cost (IOFC) of male layings based on a field experiment with control drinking water and the addition of bioherbals. The data analysis showed that the addition of bioherbals to drinking water had no significant effect ( $p > 0.05$ ) on IOFC, with an average from lowest to highest of T1 (3592.06), T0 (4391.13), T3 (4493.82), and T2 (5302.04) IDR/bird. This is allegedly due to the lack of optimal absorption of bioherbals, as evidenced by the non-significant effect of feed consumption in the statistical analysis. IOFC increased in each treatment, with the addition of 2H bioherbals to T2 resulting in the highest IOFC of 5302 IDR/bird compared to other treatments. The probiotics in the bioherbals provide benefits in terms of increased digestibility, nutrients, and feed efficiency, resulting in the high body weight of chickens produced. The IOFC is influenced by the body weight of chickens, feed intake, and the cost of feed during the rearing period, as well as the selling price of chickens at harvest. The cost of feed and the conditional selling price of chickens can result in unstable income. The IOFC value is proportional to the efficiency of feed use, where a more efficient IOFC value will be higher.

### Number of villi

Table 3 displays the results of the number of villi observed in the laboratory based on a field experiment that gave control drinking water and the addition of bioherbals. The data analysis revealed that the addition of bioherbals to drinking water had a significant effect ( $p < 0.05$ ) on the number of villi, with an average value ranging from lowest to highest: T1 (51.00), T0 (53.33), T3 (57.40), and T1 (61.60). The increase in villi number is attributed to the

probiotics and herbs containing essential oils in the bioherbals, which reduce the growth of pathogenic bacteria and stimulate optimal villi growth. Moreover, the production of lactic acid bacteria in the bioherbals affects villi density (Sjofjan et al., 2020). This finding is consistent with the research conducted by Sjofjan et al. (2020), which states that lactic acid bacteria produced by probiotics and turmeric can increase the number of villi in broiler chickens. Probiotics undergo a fermentation process, which produces short-chain fatty acids that help expand the area of nutrient absorption

and multiply intestinal epithelial cells. Furthermore, they protect the villi from damage caused by pathogenic bacteria, leading to optimal growth and functioning of the digestive tract walls (Elisa et al., 2017). The highest number of villi was observed in T2, which received the addition of 2H bioherbals compared to other treatments. This result may be attributed to the chlorophyll content in the bioherbals' leafy herbs. Chlorophyll has high antioxidant levels, which help counteract free radicals (Jumadin et al., 2016).

**Table 3.** Effects of adding bioherbals on number of villi, length of villi, surface area of villi, and depth of crypt in 60 days male laying chicken

Parameters	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	SEM
Number of villi (transversal cut)	53.33 <sup>a</sup>	51.00 <sup>a</sup>	61.60 <sup>ab</sup>	57.40 <sup>a</sup>	4.15
Length of villi (µm)	526.32	544.93	578.93	542.51	23.44
Surface Area of Villi (mm <sup>2</sup> )	0.60	0.61	0.622	0.64	0.03
Depth of Crypt (µm)	137.16	143.39	155.42	153.79	12.33

<sup>a,b,c,d</sup> Means with different superscripts in the row differ significant ( $p < 0.05$ ). T<sub>0</sub>= Drinking water without bioherbal (control); T<sub>1</sub>= Control + bioherbal code 1M; T<sub>2</sub>= Control + bioherbal code 2H; T<sub>3</sub>= Control + bioherbal code 1M and 2H, with alternate every week

### Length of villi

Table 3 presents the results of villi length based on a field experiment involving male layings given control drinking water and the addition of bioherbals, which were observed in the laboratory. Data analysis revealed that the addition of bioherbals to drinking water had no significant effect ( $p > 0.05$ ) on villi length, with an average range from lowest to highest of T<sub>0</sub> (526.32), T<sub>3</sub> (542.51), T<sub>1</sub> (544.93), and T<sub>2</sub> (578.93) µm. This could be due to suboptimal production of lactic acid bacteria in the intestine. This finding is consistent with Rahmah's (2013) research, which reported that adding herbals up to a level of 1.5% to feed did not improve broiler production performance due to the suboptimal effect of bioactive substances in the herbals. Lactic acid bacteria (LAB) in the digestive tract compete with pathogenic bacteria for feed nutrition. When the number of harmful pathogenic bacteria is high, a small amount of LAB will disrupt nutrient absorption, affecting the condition of the digestive tract.

### Surface area of villi

Table 3 presents the results of the surface area of villi based on a field experiment that involved providing control drinking water and the addition of bioherbals, which were observed in the laboratory. The data analysis showed that the addition of bioherbals to drinking water

did not have a significant effect ( $p > 0.05$ ) on the surface area of the villi. The average value of surface area from lowest to highest was T<sub>0</sub> (0.60), T<sub>1</sub> (0.61), T<sub>2</sub> (0.62), and T<sub>3</sub> (0.64) mm<sup>2</sup>. This is likely to be correlated with the villi length value, which also showed no significant effect. The surface area of villi can be determined by measuring the apical width, basal width, and length of villi. Villi with a broad surface have the potential to absorb optimal feed nutrients. The condition of the digestive tract has a positive impact on livestock productivity. A larger intestine size enables more nutrient absorption, resulting in more efficient feed consumption (Lisnahan et al., 2019).

### Depth of crypt

Table 3 presents the results of the depth of crypts in male layings based on a field experiment involving control drinking water and the addition of bioherbals, which were observed in the laboratory. The data analysis results indicated that the addition of bioherbals to drinking water had no significant effect ( $p > 0.05$ ) on the depth of crypts, with an average value ranging from lowest to highest as follows: T<sub>0</sub> (137.16), T<sub>1</sub> (143.39), T<sub>3</sub> (153.79), and T<sub>2</sub> (155.42) µm. This condition is presumed to be caused by insufficient dosage of bioherbal administration. The administration of additives in accordance with the livestock's needs is expected to have a positive effect on productivity. In line with the research conducted by Ramadhan et al. (2022),



the addition of herbs, such as turmeric and probiotics, did not have a significant effect on the depth of crypts.

The depth of crypts is closely related to the growth of villi and the absorption of nutrients from the ration. Additionally, Lisnahan et al. (2019) reported that a higher depth of crypt leads to increased nutrient digestion and absorption, which has a positive impact on the growth of organs in native chickens. Deep crypts in the intestine help absorb more nutrients, leading to optimal absorption in livestock organs and achieving maximum livestock growth.

## CONCLUSION

It can be concluded that the addition of 2H bioherbal gave the best result in improving the growth performance and intestinal profile of male laying chicken. Further studies are suggested to investigate using bioherbal mixed into the feed of laying chicken.

## DECLARATIONS

### Acknowledgments

The authors wish thanks to BioHerbal and Mr. Yayok and Team who have provided funding and facilitate on this project.

### Authors' contributions

Chafifah Mienati Permata Utami contributed to collecting data, analysis of proximate, data analysis and preparing original draft manuscript. Osfar Sjojfan contributed to the research design, supervision, and revised the draft of the manuscript, Muhammad Halim Natsir contributed to the research design and supervision. Danung Nur Adli contributed to revising the original draft and analyses of the data.

### Competing interests

No potential conflict of interest relevant to this article was reported.

### Ethical consideration

All authors have checked the ethical issues, plagiarism, fabrication and/or falsification, double publication, and redundancy.

### Availability of data and materials

This article includes all data generated or analyzed during this research.

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