



Multi-strain Probiotics Effects on Laying Hens' Performance, Egg Quality, Digestive Organ Weight and Small Intestine Histomorphology

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ABSTRACT

Background: Antibiotic growth promoters (AGPs) in animal diets are no longer recommended due to antibiotic resistance and presence of residues in animal product. Probiotics are alternative feed additive that can be given to poultry in a single or multi-strains probiotic (MP) through the diet or drinking water.

Methods: Two hundred laying hens, 32-week old, were randomly distributed into five treatments and four replicates, 10 hens each replicate. Experimental diets included a basal ration only (MP0), a basal ration with 0.2% of MP (MP2), a basal ration with 0.4% of MP (MP4), a basal ration with 0.6% of MP (MP6) and a basal ration with 0.8% of MP (MP8). The variables included the initial and final weight, feed consumption, hen day production (HDP), mass of eggs, feed conversion ratio (FCR) and quality of egg, digestive organ weight and histomorphology of the small intestine. The data analysis employed variance analysis and the Duncan comparison test.

Result: Adding of commercial MP to the laying diet increases feed consumption, HDP, the thickness of the eggshell, weight of the small intestine and villus height at duodenum and jejunum ($p < 0.05$), other parameters were unaffected ($p > 0.05$). Therefore, commercial MP in laying hen increased feed consumption, HDP, eggshell thickness and villus height at the duodenum and jejunum. The optimum dose rate is 0.6% in the diet.

Key words: Conversion, Index, Intake, Probiotic, Production, Thickness.

INTRODUCTION

The use of synthetic antibiotic growth promoters (AGPs) in animal diets is no longer recommended due to concern over antibiotic resistance, the presence of residues in animal-derived products and environmental contamination (Sugiharto and Ranjitkar, 2019), resulting in a negative effect on human health. Many studies have been carried out to identify feed additives, that do not affect the microbial ecosystem of the digestive tract, ensure safe animal products and are environmentally friendly (Sugiharto and Ranjitkar, 2019). Therefore, probiotics, prebiotics, phytobiotics, enzymes and organic acids are alternatives additives that are added to the diets. Probiotics are however an appropriate additive following restrictions on synthetic antibiotics as a growth promoter in poultry nutrition. They can be used as single probiotic or multi-strain probiotics (MP) through the diet or drinking water.

Probiotics are living bacteria when it administered in sufficient amounts, stabilize the intestinal microflora, offering health advantages to the host (Hill *et al.*, 2014) by preventing the proliferation of harmful microbes within the gut (Sugandhi, 2018). Probiotics strains include representatives of the genera *Bacillus*, *Enterococcus*, *Lactobacillus*, *Pedococcus*, *Streptococcus*, *Propionibacterium*, *Bifidobacterium*, *Saccharomyces*, *Debaryomyces*, *Micrococcus* and *Photobacterium* (Babot *et al.*, 2018). The groups of *Bifidobacteria* and *Lactobacillus* have been proven to restrict the development of *Escherichia coli*, which causes diarrhoea (Song *et al.*, 2019). Study by Chapman *et al.*

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(2013) showed that MP may have superior inhibitory impacts on enteropathogens and provide greater advantages than single-strain probiotics. Furthermore, a combination of two species of *Bifidobacteria* and *Lactobacillus* may alter the intestinal environment, depressing pathogen bacteria, while boosting beneficial bacteria (Toscano *et al.*, 2017). The colony of *Lactobacillus* and *Bifidobacteria* was elevated in the ceca of laying hen given a multi-strain *Bacillus*-based probiotic (Oketch *et al.*, 2024).

Multi-strain probiotics in poultry improves the height of the villus (HV), the number of goblet cells in the jejunum and the ratio between the HV and crypt depth (CD) in the ileum (Kazemi *et al.*, 2019) and significantly increased villi height at the section of the duodenum, jejunum and ileum

(Kalita *et al.*, 2021; Khatun *et al.*, 2022). MP supplementation in laying hens' diet improves the FCR (Premavalli *et al.*, 2018) and decreases the rate of broken eggs (Balevi *et al.*, 2001). MP improves the quality of the eggs and has no effect on productivity (Chung *et al.*, 2015). These results show that by improving digestive function and controlling the intestinal microbiota, probiotics can enhance health and growth performance (Yang *et al.*, 2012).

Nowadays trends in poultry production are increasingly using MP in feed preparation, rather than relying on a single strain. There are no studies reported on the use of commercial MP of "Lacto-Bact" in laying hens. Therefore, this study aims to investigate the impacts of "Lacto-Bact" on laying hens' performance, quality of egg, digestive organ weight and small intestine histomorphology.

MATERIALS AND METHODS

Ethical approval

The approval of the experimental protocols was issued by the Committee of Animal Care and Use of the Universitas Tadulako, Palu and the approval number was 8089/UN28.9/PT/2024. The experiment was carried out at the teaching farm of the Faculty of Animal Husbandry and Fishery, Universitas Tadulako, Palu at Sibalaya-Sigi, Central Sulawesi, Indonesia.

Experimental birds

Two hundred 32-week-old Lohmann Brown laying hens were kept in wire cages for eight weeks (June-August, 2024), following a 10-day adaptation period. The cages were placed in a house with partial openings and equipped with nipple drinkers and trough feeders.

Experimental diets

Basal ration primarily consisted of yellow maize, rice bran and soybean cake, with an 18% protein level (Table 1) and composition is based on NRC (1994). MP (Lacto-Bact; PT. Sekawan Mitra Abadi) was purchased from the commercial market. The experimental rations were a basal ration only (MP0), a basal ration with 0.2% of MP (MP2), a basal ration with 0.4% of MP (MP4), a basal ration with 0.6% of MP (MP6) and a basal ration with 0.8% of MP (MP8). The diets and drinking water were available throughout the study.

Parameters and measurement

The parameters included the initial and final weight of the hens, feed consumption (FC), hen day production (HDP), the egg mass, ratio between FC and egg mass (FCR), weight of egg, yolk percentage, yolk index, albumin percentage, albumin index, eggshell percentage and thickness, digestive organ weight and small intestine histomorphology. The weight of the hens was measured at the beginning and end of the study using a digital scale. FC was recorded weekly, while the total quantity and weight of eggs were noted daily.

One average-weight egg was taken each week from each experimental unit to assess the quality. The proportions

of egg yolk, albumin and shell to the overall weight were calculated after they were separated. A digital height and depth gauge was used to record the height of the albumen and yolk and a digital caliper was applied for the width and length of albumin and yolk measurement. The eggshells, albumin and yolk were weighed using a digital scale. Eggshell thickness was measured using a digital micrometre at three distinct points.

Two laying hens from each experimental unit were taken for small intestine and digestive organ assessment. The hens were slaughtered following 8 hours of fasting and slaughter weights were recorded. The small intestine and other digestive organs were gently removed and weighed individually. Their weight were adjusted using their respective slaughter weights. Small intestine were separated into duodenum, jejunum and ileum sections. Three cm of length from each section was taken and put in the pot containing 10% formalin solution for histomorphology evaluation. The samples underwent dehydration, clearance and paraffin impregnation. A microtome was used to cut the tissues into 6µm pieces after they had been treated with paraffin wax. Before transferring the sliced tissues on slides coated with 10% poly-L-lysine, they were flattened in water at 55-60°C. The slides were stained with eosin and hematoxylin. The HV and CD were measured using a microscopic image analyzer (Motic Images, 2000 1,2 Scion Image, Japan).

Experimental design, statistical and chemical analysis

Experimental hens were randomly allocated to five treatments and four replicates, with 10 hens in each

Table 1: Ration composition.

Ingredients	%
Yellow maize	55.00
Deoiled soybean	16.50
Fish meal	6.00
Rice bran	15.00
Cooking oil	0.50
Salt	0.10
CaCO ₃	4.30
Mineral-mix ¹	2.30
Lysine	0.10
Methionine	0.20
Chemical composition (% DM)	
ME (kcal/kg)	2900.45
Moisture	11.91
Crude protein	18.04
Crude fiber	6.70
Extract ether	4.23
Ash	14.89

1) Each kg contains: Ca 0.325 kg; P 0.1 kg; Fe 6 g; Mn 4 g; I 0.075 g; Cu 0.3 g; Zn 3.75 g; Vit. B12 0.5 mg; Vit. D3 50,000 IU; Vit. A 1,200,000 IU; Vit. E 800 IU; Vit. K 0.2 g; Vit. B1 0.2 g; Vit. B2 0.5 g; Vit. B6 0.05 g; Vit. C 2.5 g; Ca-D-pantothenate 0.6 g; Niacin 4 g; Met. 3 g; Lys. 3 g; Santoquin 1 g and Zinc bacitracin 2.1 g.

replicate. The data analysis employed an analysis of variance (ANOVA) and the Duncan mean test at $p < 0.05$ (Steel and Torrie, 1991) and feed chemical analysis used AOAC (2005) method.

RESULTS AND DISCUSSION

As shown in Table 2, the initial body weight of laying hens was approximately 1.47 kg and their HDP was 82.99%. These values are lower than the Lohmann Brown standard, which reports an initial body weight of 1.90 kg and a HDP rate of 95.00% (Anonymous, 2021). Although there was an improvement in body weight, it was lower than the expected ideal weight. Despite these restrictions, laying hens still produce eggs, although at a lower level than the potential production of a similar strain under standard management condition as reported in the management guide (Anonymous, 2021).

The MP enhanced feed intake by 9.33% ($p < 0.05$), with the greatest value seen at a dosage rate of 0.8% in the diet. This improvement is in accordance with the findings by Tang *et al.* (2017). Their study showed an improvement in feed intake of laying hens given a mixture of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, *Streptococcus faecium* and *Aspergillus oryzae*. Sultan *et al.* (2024) reported that there was an increase in feed intake of broiler-given MP with a rate of 50 and 100 mg/kg compared to the control group. Similarly, hens received MP enhance feed intake (Getachew *et al.*, 2016; Oketch *et al.*, 2024). Increased feed consumption is most likely due to probiotics' capacity to stimulate laying hen appetites (Nahashon *et al.*, 1996). This could also be connected to the increase body weight of the supplemented laying hens, even though it was a non-significant increased ($p > 0.05$). In contrast, MP in laying hens' diet did not modify the feed intake compared to the control group (Ray *et al.*, 2022).

The results also proved that MP inclusion had significant ($p < 0.05$) effects on HDP and the highest value of HDP was achieved at the rate of 0.6% MP. The HDP was increased by 17.69% compared to the unsupplemented hens. This is in line with Ray *et al.* (2022) and Hamada *et al.* (2023) who found that MP enhances daily egg yield in both laying hens and laying Japanese quail, respectively.

Similarly, Getachew *et al.* (2016) reported that a single or mixed of three types of bacteria supplemented to laying hens enhances egg production. In contrast, Aalaei *et al.* (2018) found no substantial impact on egg yield by including MP in the diet.

Hen day production increased as feed intake was elevated with FCR decreased non significantly until dose rate of 0.6% MP. This indicates that supplementation with MP improved production at the optimal dose rate of 0.6%, with the lowest FCR value of 2.17. The change in FCR according to the MP applied, may related to the metabolic activity. Probiotics can enhance FCR by altering bacterial metabolism in the gut, sustaining a beneficial microbial community (Premavalli *et al.*, 2018) and boosting digestion and absorption of the feed (Sheoran *et al.*, 2018). Meanwhile, Yang *et al.* (2012) reported that probiotics inclusion can increase growth performance and health by speeding digestion and controlling the gut flora.

Table 3 shows that MP increased egg quality, however it only had a significant effect on the thickness of the eggshells ($p < 0.05$). The current findings are consistent with Wang *et al.* (2021) and Ray *et al.* (2022). The results discovered that administration of MP to laying hens improves the eggshells thickness. Similarly, Mikulski *et al.* (2020) showed a significant improvement in eggshell thickness in the probiotics-laying hens. In contrast, other studies have revealed no impact of probiotics on the eggshells thickness (Aalaei *et al.*, 2018).

The main concerns of egg producers is maintaining the quality of the eggshell because thicker eggshells will make eggs safer in collection, packing and transportation. This fact is relevant to our findings, which showed increased eggshell thickness from the addition of MP to the ration and it is also consistent with the results in laying birds by Mazanko *et al.* (2019). Similarly, eggshell thickness is increased by providing multi-strain *Bacillus*-based probiotics in laying hen diets (Oketch *et al.*, 2024). All of these data showed that probiotics can enhance the growth of beneficial bacteria, allowing in the buildup of short-chain fatty acids (SCFAs) (Forte *et al.*, 2016). This allowed a reduction in intestinal pH, increasing Ca-solubility and boosting Ca-absorption (Kishino *et al.*, 2002). Therefore,

Table 2: Initial weight, final weight, feed consumption, egg production, egg mass and feed conversion ratio of laying hens given a different dose rate of multi-strain probiotics.

Items	Treatments					SEM	p-value
	MP0	MP2	MP4	MP6	MP8		
IW, kg/hen	1.49	1.48	1.45	1.46	1.46	0.01	0.68
FW, kg/hen	1.51	1.51	1.47	1.48	1.48	0.01	0.76
FC, g/hen/day	103.29 ^a	110.92 ^{ab}	108.43 ^{ab}	106.93 ^{ab}	112.93 ^b	1.14	0.04
HDP, %	77.70 ^a	82.22 ^b	83.89 ^b	88.55 ^c	82.61 ^b	0.99	0.00
EM, g/hen/day	44.99	47.05	47.08	49.44	45.42	0.63	0.17
FCR	2.30	2.37	2.34	2.17	2.49	0.04	0.08

IW: Initial weight, FW: Final weight, FC: Feed consumption, HDP: Hen day production, EM: Egg mass, FCR: Feed conversion ratio, SEM: Standard error means. Different letters on the same line are significantly different ($p < 0.05$).

the mineral Ca and P content of eggshells was significantly increased following supplementation with probiotics (Wang *et al.*, 2021). As a result, the quality of eggshells may have improved due to probiotics' ability to increase laying hen serum calcium, absorption and retention levels, as well as stimulate calcium and other mineral deposition on shell glands (Upadhaya *et al.*, 2019).

The present results also showed that there were no significant impacts on the weight of the selected digestive organs of hens supplemented with MP ($p > 0.05$), except for small intestine weight (Table 4). Dose rate of 0.8% significantly increased the weight of the small intestine compared to the control group ($p < 0.05$) and other treatments showed no significant difference. This study proved that different dosages of MP has little or no effect on the digestive organs, such as the pancreas, liver, gizzard, or proventriculus. One explanation for this could be that the basal ration was similar for every treatment. Meanwhile, one of the goals of probiotic use is more closely linked to the improvement of the intestinal mucosa (Toscano *et al.*, 2017) and the activity of different kinds of non-pathogenic bacteria in the intestine. Probiotic organisms have been shown to lengthen the small and large intestines for enhance nutritional absorption by improving absorptive ability (Chen *et al.*, 2005), resulting in a heavier gut. Additionally, there is an improvement in the length of the villus and the quantity of goblet cells in the jejunum (Kazemi *et al.*, 2019). This is supported by the data in Table 5, in which MP improved the size of the villus of the small intestine. All the described facts, collectively resulted in the small intestine becoming heavier with the inclusion of MP in the diets.

Data presented in Table 5 indicated that MP generally has a major effect ($p < 0.05$) on the small intestine histomorphology, allowing elevation of nutrient absorption in the digestive tract. Increased villus height and decreased CD, or an increase in the villus height to CD ratio, shows an increase in the surface area of the digestive tract where nutritional absorption occurs. Taller villi increase both surface area and the activity of enzymes released by the villi. Furthermore, the formation of villi reflects the health of the livestock's digestive system (Ologhobo *et al.*, 2015). According to Viveros *et al.* (2011), tall villi and short CD, or a high HV/CD ratio, promote higher nutritional absorption, disease resistance and animal performance. The present results revealed a positive correlation between small intestine development (Table 4 and 5) and laying hen performance data (Table 2).

The inclusion of MP significantly improved HV, CD and HV/CD ratio at duodenum section ($p < 0.05$). In the jejunum, MP significantly influenced the HV and CD ($p < 0.05$). Meanwhile, in the ileum, MP had a significant effect on the CD and the ratio of HV to CD ($p < 0.05$), without significantly affecting the value of HV. These improvement patterns agree with Kazumi *et al.* (2019), who demonstrated that MP in poultry diets improves VH, the number of goblet cells in the jejunum and the ratio of HV to CD in the ileum. Similarly, there was a remarkably increased height of villus in the duodenum and jejunum and no significant effect in the ileum of chicken given two strains of probiotics in their diet (Mirsalami and Mirsalami, 2024) and MP increases villi height at the duodenum, jejunum and ileum (Khatun *et al.*, 2022).

Table 3: Egg quality of laying hens given a different dose of multi-strain probiotics.

Items	Treatments					SEM	p-value
	MP0	MP2	MP4	MP6	MP8		
Weight, g	55.59	56.92	56.98	56.62	56.60	0.45	0.89
Albumin, %	65.54	66.27	66.13	65.70	64.67	0.26	0.38
Albumin index	0.07	0.08	0.06	0.07	0.07	0.00	0.60
Yolk, %	24.46	23.58	23.79	23.97	24.64	0.21	0.50
Yolk index	0.48	0.47	0.48	0.48	0.48	0.00	0.91
Eggshell, %	10.00	10.14	10.07	10.33	10.60	0.10	0.37
Eggshell thickness, mm	0.54 ^a	0.57 ^{ab}	0.58 ^{ab}	0.61 ^b	0.61 ^b	0.01	0.01

SEM: Standard error means. Different letters on the same line are significantly different ($p < 0.05$).

Table 4: Digestive organ of laying hens given a different dose of multi-strain probiotics.

Items, g/kg	Treatments					SEM	p-value
	MP0	MP2	MP4	MP6	MP8		
Proventriculus	4.33	4.16	4.38	4.64	4.06	0.08	0.17
Gizzard	27.03	25.87	25.98	26.91	26.49	0.70	0.89
Liver	17.11	17.57	16.95	19.32	16.23	0.46	0.31
Pancreas	2.04	1.94	1.97	2.19	1.96	0.06	0.79
Small intestine	27.81 ^a	28.99 ^{ab}	30.00 ^{ab}	30.44 ^{ab}	33.79 ^b	0.66	0.03
Ceca	6.65	7.26	7.35	8.17	8.54	0.28	0.22

SEM: Standard error means. Different letters on the same line are significantly different ($p < 0.05$).

Table 5: Small intestine histomorphology of laying hens given a different dose of multi-strain probiotics.

Items	Treatments					SEM	p-value
	MP0	MP2	MP4	MP6	MP8		
Duodenum							
- HV, μm	751.99 ^a	875.60 ^b	784.80 ^{ab}	812.30 ^{ab}	842.16 ^{ab}	14.17	0.01
- CD, μm	59.05 ^a	42.95 ^b	65.34 ^{ac}	48.83 ^b	66.940 ^c	2.54	0.00
- Ratio HV/CD	12.74 ^c	20.36 ^a	12.12 ^c	16.63 ^b	12.58 ^c	0.87	0.00
Jejunum							
- HV, μm	517.00 ^{ab}	505.90 ^b	521.20 ^{ab}	599.49 ^a	607.96 ^a	13.94	0.01
- CD, μm	27.25 ^a	30.65 ^a	37.80 ^a	66.15 ^b	37.05 ^a	4.16	0.00
- Ratio HV/CD	19.16	16.95	17.03	9.06	16.48	1.45	0.22
Ileum							
- HV, μm	542.60	560.26	518.30	500.01	547.00	8.93	0.21
- CD, μm	59.40 ^a	41.27 ^{ab}	27.20 ^b	23.74 ^b	37.32 ^{ab}	4.24	0.03
- Ratio HV/CD	10.65 ^a	13.71 ^{ab}	20.22 ^b	21.07 ^b	14.76 ^{ab}	1.38	0.04

SEM: Standard error means. HV= Height of villus, CD= Crypt depth. Different letters on the same line are significantly different ($p < 0.05$).

CONCLUSION

In conclusion, MP in the laying hen diet enhanced feed intake, egg production (HDP), eggshell thickness and small intestine development. The optimum dose rate of MP is 0.6% in the diet.

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Disclaimers

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Informed consent

All animal procedures for experiments were approved by the Committee of Animal Care and Use of the Universitas Tadulako, Palu, Indonesia and the approval number was 8089/UN28.9/PT/2024.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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