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Growth Performance, Mortality, Relative Organ Weight, Blood Biochemistry, and Intestinal Microbial of Arbor Acres Broiler Fed Diets Containing Mannan-Riched Fraction (Mrf) and Probiotic-Enhanced Liquid Acidifier

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ABSTRACT

The research purpose was to carry out the effect of mannan-riched fraction (MRF) and probiotic enhanced water as natural growth promoters (NGPs) on Growth Performance, mortality, relative organ weight, blood biochemistry, and intestinal microbial flora. A total of 3000 day old chicks (DOC) Arbor Acres broiler were randomly allocated to 4 dietary treatments and 4 replications of 187 broilers per cage. Four treatments used in research were as follows: i) CON, basal diet, ii) basal diet, CON+ MRF (Actigen™) 80g/100kg/feed , iii) basal diet, CON+ 0.2% drinking water + 2 ml/L Combination feed additive (Acid-Pak 4-way®), and iv)) basal diet, CON+ MRF (Actigen™) 80g/100kg/feed+ drinking water 2 ml/L Combination feed additive (Acid-Pak 4-way®). The results showed that using mannan riched fraction (MRF) (feed) and combination with probiotic-enhanced liquid acidifier (drinking water) presented significant difference (P>0.05) on body weight gain at 1-28 days and intestinal microbial. On the blood biochemistry, the effect of combination began to reduce the amount of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) at 21 days periods. To sum up, the addition of mannan-riched fraction and combination with probiotic enhanced liquid acidifier doesn't impacted on growth Growth Performance, blood biochemistry, relative organ weight but give significant effect on intestinal microbial and reduces mortality of broiler.

Keywords: Blood biochemistry, Broiler, Growth Performance, Mannan-rich fractions, Micro flora

Introduction

Poultry industry serves an essential role in supporting the availability of cheap animal protein in Indonesia. This condition is reflected based on the demand for poultry products nationally. In 2017, broiler production increased approximately 6.82% compared to 6.34% of the population in 2016 (equivalent to 1.6 billion broilers) (Agriculture Ministry of Indonesia, 2017). However, the poultry industry mostly involves the use of antibiotics as a growth promoter. These antibiotics growth promoters (AGPs) have been in poultry diets as feed additives for more than 40 years in Europe. Lately, the use of antibiotic compounds has decreased, and several European countries has suppressed the use of antibiotics growth promoters (AGPs) as non-nutritive feed additives. Numerous efforts have been undertaken to develop suitable alternatives to counteract the anticipated drawbacks associated with the ban of AGPs. According to the newest regulation, Indonesia has banned the use of antibiotics in poultry production (Sjofjan *et al.,* 2020). First the antibiotics can be toxic to consumers. Secondly, antibiotics can create resistant microorganisms in the body of humans or livestock (especially pathogenic bacteria) (Adli *et al.,* 2018).

The use of antibiotics are still prevalent in the poultry business. As a result the quality of meat is depends on the feed and antibiotics use by farmers as feed additives. Different substances often referred to as natural growth promoters (NGPs) are supposed to achieve high consumer acceptance since they do not usually pose any risk that will lead to bacterial (Adli *et al.,* 2017).

Although the amount of antibiotic used as growth promoters is relatively small, it improves the feed efficiency to help farmers obtain more enormous profits. Using feed additives is one method to improve the quality of feed (Jet *et al.*, 2014) The antibiotics are provided as a growth promoter; however, they cause bacterial resistance and residue in the carcass. Alternative feed additive such as mannan-riched fraction (MRF) and probiotic enhanced liquid acidifier has

been the center of attention for many studies during the past five years due to its beneficial effect on feed efficiency. Mannan-riched fraction belongs to the family of prebiotics using new techniques such as nutrigenomics. Both prebiotics and probiotics replaces use of the antibiotics because they are safer and act as a natural growth promoter (NGPs) in the broiler (Adli *et al.,* 2018).

Research in several countries used prebiotics and probiotics combined for poultry to enhance their overall Growth Performance and health. The use of prebiotics combined with probiotics, acidifier's even electrolytes in Indonesia has so far reported to their ability to maintain health, prevent digestive tract disorder by utilizing the microbes for balancing and increasing the population of non-pathogenic bacteria. The addition of probiotics, prebiotics, and acidifier is expected to detoxify toxins and their metabolites to improve absorption of nutrients and reduce cholesterol level in blood (Sjofjan *et al.,* 2020)

Probiotics are the hot prospects for feed additives that can be provided to animal in both solid and liquid forms. The used of prebiotics are to balance pH, lactic acid bacteria colony, and decreasing the nutrient of pathogen bacteria that can survive in the intestines (Jet *et al.*, 2014). The role of prebiotics has a synergistic effect in which lactic acid bacteria (LAB) can inhibit the growth of pathogenic microbes especially *Escherichia coli* and *Enterococcus sp*. The activator of the prebiotics increases the number of feed intake for the growth of the internal organ of broiler (Natsir *et al.,* 2010). Therefore, the purpose of this research was to investigate the effect of mannan-riched fraction (MRF) and probiotic enhanced liquid acidifier on growth Growth Performance, relative organ weight, blood biochemistry, and intestinal microbial of broilers.

Materials and Methods

Animals, housing, and experimental design

A total of 3000 (Arbor Acres) broiler with an initial body weight (BW) of 39.43±1.23 g were used in a 5-wk trial. Treatments were randomly assigned to pens within gender using the random number generator in excel. Pens were assigned in a randomized complete block design to compensate for known position effects in the experimental facility. After randomization of treatments within gender was completed, average initial BW for each treatment was checked to make sure it was equal (4 replicates with 187 per replication pen). Treatments were as follows: i) CON, basal diet, ii) basal diet, CON+ MRF (Actigen™) 80g/100kg/feed, iii) basal diet, CON+ 0.2% Drinking water + 2 ml/L Combination feed additive (Acid-Pak 4-way®), and iv)) basal diet, CON+ MRF (Actigen™) 80g/100kg/feed+ Drinking water 2 ml/L Combination feed additive (Acid-Pak 4-way®). The Actigen™ was obtained from a commercial company (Alltech Inc., Nicholasville, Kentucky: USA). A prebiotic was used throughout this experiment derived from the surface of cell wall membrane e of a specific strain of yeast - *Saccharomyces cerevisiae* 1062. MRF contains a greater concentration of mannan reactive units (alpha 1,3 mannan). The feed additive used in this research was probiotics-enhanced liquid acidifier consists citric acid, sodium chloride, potassium chloride, acetic acid, sodium citrate, ethyl vanillin, zinc sulphate, iron sulphate, magnesium sulphate, dried *Aspergillus niger* fermentation extract, and dried *Bacillus subtilis* fermentation extract. All broiler was allowed ad libitum access to feed and water through a self-feeder and nipple drinker throughout the experimental period. All broiler was housed in an environmentally controlled room. The target room temperature and humidity were 29°C and 64%, respectively. The rice hull-littered floor pens with height of 3.3 (1.8 x 1.8) m^2 per pen. The lighting program was set at 23 hours light and one hour darkness.

Growth performance

The arbor acres broilers were individually weighed at the beginning of the experiment, and every week thereafter till the end of the experiment. The gain in body weight (BWG) of broilers per week was calculated as the difference between the initial and end weight at a given week (7 days, 14 days, 21 days, 28 days, and 35 days). The feed intake was calculated by the difference between the offered and remained amounts weekly. The feed intake was calculated after correction for that used by dead broilers. The feed gain ratio was calculated by dividing the amount of feed consumed in a certain period by the gain in weight at the same period (with consideration of dead broilers), expressed in the same weight units. During the experimental period, daily mortalities were recorded for each group and mortality rate was calculated (Sjofjan *et al.,* 2019).

Relative organ weight

The 42 broilers from each group, close to the average live BW, were selected at the days 21, 28, and 35 days broilers will be sacrificed of the experiment. Broilers were slaughtered by electrical shock method to obtained organ weight. In addition, the relative weight of organs, such as the liver, spleen, pancreas, thymus, bursa, and spleen of broiler was assessed and its relation to the live BW of the broiler, in percentage, was calculated.

Blood biochemistry

Blood samples were collected from the heart in each group at 21, 28, and 35 days of age. The Blood non-EDTA tubes obtained from the samples of broiler and allowed to clot for one hour, at room temperature, Blood samples was immediately centrifuged using the cryogenic centrifuge (Hettich Universal 320R, Germany) for 15min at 3000 rpm. The blood biochemistry and samples was kept in tubes at−20 °C until chemically analyzed. At the time of analysis, the samples were thawed and analyzed for GOT:

glutamic oxaloacetic trasaminase; GPT : glutamic pyruvic transaminase; TP: total protein, ALB : Albumin; GLB : globulin; A/G : albumin/ globulin ratio; TGL: triglyceride; TCHOL: total cholesterol; BUN: blood urea nitrogen; GLC: glucose using Clinical biochemistry Analyzer (CA) (Nicholasville: Kentucky: USA) (Adli *et al.,* 2019)

Intestinal morphometric

The sample about 6 cm from the middle of duodenum, jejunum, and ileum were excised and flushed with ice-cold saline and immediately placed in combination of liquid of NA2PO4 2%; $NA_2H_2PO_4$ 2%, 24% Formaldehyde; and 900 ml reverse osmosis water for morphometric analysis. The method used were Hematoxylin eosin staining coloring. The indices of villus height, crypt depth (µm) and villus height and crypt depth ratio were measured using computer-aided light microscope image software m-shot digital image system with 200x zooming according to method from (Adli *et al.,* 2019).

Intestinal microbial

Chyme from jejunum and ileum, including lactic acid bacteria and coliform bacteria were determined at the end of the experiment. One gram of the chyme sample from each broiler was diluted with 9 mL of 10 g/kg peptone broth (Becton, Dickinson and Co., USA) and homogenized. Then, 10-fold dilutions of chime samples were performed (ranging from 10−4 to 10−6) and then cultivated onto MacConkey agar plates (Difco Laboratories, Detroit, MI, USA) for the enumeration of coliform bacteria and lactobacilli agar plates (Medium 222; DSMZ, Braunschweig, Germany) for the enumeration of lactic acid bacteria. The MacConkey agar plates were incubated for 24 hours at 37°C. The lactobacilli agar plates were then incubated for 48 hours at 37°C under anaerobic conditions. The lactic acid bacteria and coliform bacteria colonies were counted immediately after removal from the incubator. Concentration of microflora was finally expressed as log10 colony-forming units per gram of chime (Adli *et al.,* 2019).

Data analyses

The statistical analyses were performed Analysis of variance (anova) tests were used for analyses of variance accompanied by Duncan's multiple range test to detect the differences between the treatments with help software using SAS (version 9.4, SAS University Ver. Inc.,). The results are presented as standard error mean (SEM) among group treatment. Probability values less than 0.05 ($p<0.05$) was considered significant (Widiyawati *et al.,* 2020).

Results and Discussion

Growth performance

According to the Table 2 shows that the growth performance, diets containing MRF and probiotics-enhanced liquid acidifier level was presented no significant difference ($P<0.05$) on body weight, feed intake, and feed/gain but on body weight gain at 1-28 days for all treatment (T1, T2, and T3) increased compared with control (1138.73, 1128.63, 1192.09 vs. 1001.12 g/broiler; $P>0.05$). The treatment (T3) presented greater than all treatment since it combination mix both give in drinking water and experimental diet may stimulation the increasing of body weight gain.

Table 1. Experimental diet

Feed nutrient (%)	Starter (1-21 days)	Finisher (22-35days)
Yellow corn	57.11	69.66
Dehulled soybean meal	36.53	26.65
L-Lysine	0.10	0.10
DL-methionine	0.55	0.55
Dicalcium phosphate	1.67	1.55
Limestone	1.13	1.02
Salt	0.30	0.30
Soy oil	2.81	0.06
Vitamin premix*	0.05	0.05
Mineral premix**	0.05	0.05
Choline	0.10	0.10
	100	100
Dry matter (%)	87.00	87.00
ME (Kcal/kg)	3050	3150
Ash (%)	9.00	9.00
Crude protein (%)	22.00	18.00
Fat (%)	6.00	6.00
Crude fibre (%)	3.00	2.50
Ca (%)	1.00	0.95
P (%)	0.70	0.75
Copper (ppm)	30	50
Zinc (ppm)	120	120

*vitamin premix (per kg of diet); vitamin A 12,500 IU; Vitamin D3, 2,500; Vitamin E 20 IU; Vitamin K3 2.5 Mg; Vitamin B_1 2Mg; Vitamin B_2 5 Mg; Vitamin B_6 3Mg; Vitamin B_{12} 0.012 Mg; Niacin 35 Mg; Pathonenic acid 12Mg; Folic Acid 1Mg;

**Mineral premix (Per kg of diet); Fe 70 mg, Zn, 90 mg; CU, 10 mg; Mn, 80 mg

The treatment use four treatments, and four replicates. The treatments were:

T0: Basal diet + without feed additive (control); T1: Basal diet + MRF (Actigen™) 80g; T2 : Drinking water + 2 ml/L Combination feed additive (Acid-Pak 4-way®); and T3: Basal diet + MRF (Actigen™) 80g + Drinking water 2 ml/L Combination feed additive (Acid-Pak 4-way®).

Table 2. Effect of average group of MRF and probiotics-enhanced liquid acidifier on the body weight and body weight gain of broiler

	Treatments[1]				
Day	T0	T1	T2	T3	SEM
			—Body weight, g/broiler—		
1	43.72	43.65	43.65	43.78	0.30
21	718.40	713.70	704.39	715.92	37.20
28	1212.20	1245.20	1220.20	1235.70	37.60
35	1842.55	1889.30	1842.00	1775.25	70.60
			—Body weight gain, g/broiler—		
1-21	674.64	670.05	671.98	668.98	42.55
1-28	1001.12[b]	1138.73[a]	1128.63[a]	1192.09[a]	47.70
21-35	1525.10	1537.10	1574.70	1486.90	65.20
1-35	1856.90	1842.70	1878.40	1786.60	167.20

[a,b] Mean values not sharing the same superscripts in a row differ significantly (P < 0.05);
[1]T0= control; T1= MRF 80g/100kg; T2=Drinking water + 2 ml/L (Acid-Pak 4-way®); T3=MRF 80g/100kg+ Drinking water 2 ml/L (Acid-Pak 4-way®).

The combination of MRF and probiotic helps the treatment (T3) in blood and increase body weight gain of broilers. Compared with Brennan *et al.* (2014) stated the used of MRF give significant difference (P<0.05) on body weight gain at 21 d and 35 d compared than control (877 g (MRF 400 g/ton (21 d); 50 g/tonne (35d)) vs 819 g control) due it combination both of mannan and probiotic. The result according to the table 2 got additional statement from Brennan *et al.* (2014) stated that the result of performance broiler were according due to factor rearing condition, the broiler will increase the body weight gain when the environment (bedding are clean) (Brennan *et al.,* 2014).

The result continued to the Table 3 showing the feed intake was no significant difference (P>0.05) at whole periods. The feed intake result shown on the table 3 were no significant difference at 1-28 days treatment (T1) better than control (2018.40 vs 1874.60 g/broiler), 21-35 days (T1, and T2) give best result compared than control (2414.40, and 2273.40 vs 2264.70 g/broiler) and followed by result at 1-35 days (T1, and T2) better than control (T0) (2810.80, and 2690.10 vs 2668.60 g/broiler). The feed intake increased may due correlating with body weight and body weight gain, when both of thus variable growth increase the feed intake will also increase. The increase may due to rearing condition, the way of feed give ad-libitum made broiler eat more. According to Brennan *et al.,* (2018) stated feed intake at 21 d and 35 d compared than control (1.33 (MRF 400 g/ton (21 d); 1.49/ton (35d)) vs 1.23 control) due its rearing condition factor example the way and quality of feed given. The mortality result on table 3 showed the used of MRF combination with probiotic liquid acidifier on treatment (T2 and T3) give no significant differences (P>0.05) reduces to 1.31% compared to control 3.94%. The mortality were also parameters observed on the research to combine the relevance it with treatment reduces percentage or not. The result may include the composition in the feed additive can prevent factors dead factors such as coccidian during the research. In the composition the *Saccharomyces cerevisiae* 1062, citric acid, sodium chloride, potassium chloride, acetic acid, sodium citrate, ethyl vanillin, zinc sulphate, iron sulphate, magnesium sulphate, dried *Aspergillus niger* fermentation extract, and dried *Bacillus subtilis* helps to stimulate immune modulation of broilers, Contrary to these findings, it was reported from study Biswas *et al.* (2018) cannot help to reduce mortality in male broilers at 0-21 d and 0-42 d (3.38 vs 0.28 (control)) and (4.51 vs 1.69 (control)) because the experimental the broiler reared under stocking density stress (43 kg live weight per m^2 floor space) (Biswas *et al.,* 2018).

Relative organ weight (%)

The result was not significantly difference (P>0.05) for liver, spleen, bursa of fabricius, thymus, and pancreas of organ weight at 21, 28, and 35 days. However, the immune organ such as thymus are better on the (T1, T2, and T3) compared to control (T0) (table 4) at 21 days for all treatment (0.38, 0.31, and 0.33 vs. 0.30) although, it not significant difference (P>0.05) on statistics.

Table 3. Effect of MRF and probiotics-enhanced liquid acidifier on the feed intake, feed/gain, and mortality of broiler

	Treatments[1]				
Day	T0	T1	T2	T3	SEM
			—Feed intake, g/broiler—		
1-21	909.80	979.10	855.40	807.30	21.06
1-28	1874.60	2018.40	1822.90	1727.80	53.70
21-35	2264.70	2414.40	2273.40	2102.00	70.00
1-35	2668.60	2810.80	2690.10	2499.00	76.80
			—Feed/gain, g/broiler—		
1-21	1.33	1.49	1.22	1.22	0.09
1-28	1.73	1.72	1.54	1.44	0.23
21-35	1.52	1.58	1.54	1.49	0.37
1-35	1.48	1.54	1.50	1.44	0.14
			—Mortality, (%)—		
1-35	3.94	5.26	1.31	1.31	4.36

[1]T0= control; T1= MRF 80g/100kg; T2=Drinking water + 2 ml/L (Acid-Pak 4-way®); T3=MRF 80g/100kg+ Drinking water 2 ml/L (Acid-Pak 4-way®)

The bursa of fabricius in 35 days presented not significant difference (P>0.05) (Table 5) (0.12, 0.12, and 0.15 vs. 0.12). In the experiment, the stabile weight of bursa of fabricius could have an initial positive signal for the development of broiler immune systems. However, as broiler age more, the bursa of fabricius will disappear according to the maturation of the immune system of the broiler.

The weight of bursa fabricius (percentage of live weight) using MOS 0.1% and 0.2% are not significance difference (P>0.05) (0.28 and 0.32 vs 0.29 (control)) at finisher periods of broiler (Biswas *et al.,* 2018). The weight of liver, spleen and pancreas also are not significant difference (P>0.05) (Biswas *et al.,* 2018). The exact explanation may due to prebiotic helps protect proliferating immature bursal B cells and thymic T lymphocytes from oxidative stress (Biswas *et al.,* 2018). The studies from the used MOS in relative organ weight of male broiler at level 1 g/kg were not significant influenced (P>0.05) (0.19 (MOS) vs. 0.20) it because the activities from MOS sometimes not occur (Bozkurd *et al.*, 2009). In addition, the bursa of fabricius is an organ of the immune system and is responsible for maturation of B-lymphocytes (Biswas *et al.,* 2018).

Intestinal microbial

Based on table 5 the used MRF and probiotic-enhanced liquid acidifier as a feed additive on intestinal microbial were not significant different (P>0.05) on the *Lactobacillus* and *Coliform* at 21, 28, and 35 days both jejunum and ileum. At 28 days the *Coliform* in the ileum parts the trends were decreasing even though, were not significant different (P>0.05) (2.74 (T1), 3.03 (T2), 3.11 (T3) vs. 3.23 (T0) Log cfu/g, DM). However, the *Lactobacillus* decreasing at small amount at ileum parts (2.33 (T1), 3.81(T2), and 3.40(T3) vs 4.36 (T0) Log cfu/g, DM). At 35 days (table 7) the *Lactobacillus* were not significant difference (P>0.05) for T1,T2,T3 in line but still better than control (T0) (4.78, 4.56, 4.72 vs 4.32 Log cfu/g, DM). At 28 days, the positive microbial population may starts to stabilize indicated the treatment may help the microbial stable on bacterial fermentation, but at 35 d the condition began unstable at several cases.

Table 4. Effect of average group MRF and probiotics-enhanced liquid acidifier on the relative organ weight of broiler

		Treatments[1]				
Day	Item(g)	T0	T1	T2	T3	SEM
		— — (Organ weight/body weight) x 100 — —				
21	Liver	3.31	3.16	2.62	3.24	0.41
	Spleen	0.10	0.10	0.12	0.12	0.02
	Bursa	0.22	0.21	0.32	0.20	0.09
	Thymus	0.30	0.38	0.31	0.33	0.11
	Pancreas	0.39	0.46	0.43	0.45	0.07
28	Liver	2.85	2.83	2.83	2.18	0.42
	Spleen	0.17	0.11	0.17	0.09	0.04
	Bursa	0.20	0.17	0.17	0.14	0.05
	Thymus	0.28	0.28	0.31	0.26	0.10
	Pancreas	0.32	0.30	0.31	0.29	0.05
35	Liver	1.86	1.89	2.02	1.83	0.15
	Spleen	0.08	0.11	0.12	0.12	0.02
	Bursa	0.12	0.12	0.12	0.15	0.03
	Thymus	0.32	0.29	0.33	0.26	0.10
	Pancreas	0.20	0.21	0.20	0.22	0.02

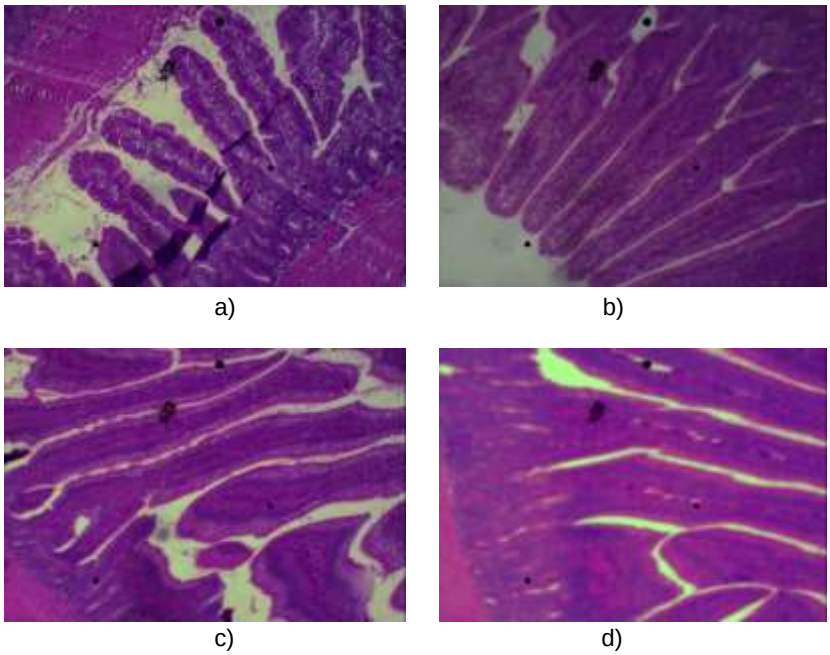
[1]T0= control; T1= MRF 80g/100kg; T2=Drinking water + 2 ml/L (Acid-Pak 4-way®); T3=MRF 80g/100kg+ Drinking water 2 ml/L (Acid-Pak 4-way®)

Table 5. Effect of average group MRF and probiotics-enhanced liquid acidifier on the intestinal microbial of broiler

		Treatments[1]				
Bacterial	Day	T0	T1	T2	T3	SEM
Jejunum	21	— — — — Log cfu/g, DM — — — —				
Lactobacillus		2.47	2.72	1.83	2.66	0.87
Coliforms		2.44	1.51	1.55	3.50	1.73
Ileum						
Lactobacillus		2.34	2.83	1.73	2.08	0.79
Coliforms		2.62	3.33	3.52	2.43	0.09
Jejunum	28	— — — — Log cfu/g, DM — — — —				
Lactobacillus		3.17	3.35	2.98	3.44	0.63
Coliforms		2.85	3.04	3.21	3.00	1.73
Ileum						
Lactobacillus		4.36	2.33	3.81	3.40	1.09
Coliforms		3.23	2.74	3.03	3.11	1.59
		— — — — Log cfu/g, DM — — — —				
Jejunum	35					
Lactobacillus		4.56	4.44	3.96	3.57	1.25
Coliforms		2.34	2.12	2.77	3.32	1.56
Ileum						
Lactobacillus		4.32	4.78	4.56	4.72	0.35
Coliforms		2.56	2.78	2.54	2.31	1.13

[1]T0= control; T1= MRF 80g/100kg; T2=Drinking water + 2 ml/L (Acid-Pak 4-way®); T3=MRF 80g/100kg+ Drinking water 2 ml/L (Acid-Pak 4-way®)

The result showed on Table 6 the used MRF and probiotic-enhanced liquid acidifier as a feed additive on intestinal properties were significant different (P<0.05) on the jejunum part. At 21 days, (Table 8) Villus height were significant difference (P<0.05) the treatment (T1: MRF 80g/100kg;T2: *Drinking water + 2 ml/L (Acid-Pak 4-way®)*,T3: *MRF 80g/100kg + Drinking water 2 ml/L (Acid-Pak 4-way®)*) help to increase surface area of intestinal compared to control (593.00 (T1), 597.50 (T2), and 569.50 (T3) vs 442.75 (T0) µm). However, based on table 8 VH/CD whole are not significant difference (P>0.05). The result may be correlated with the treatment of probiotics, and prebiotics that had helped to increase the surface area of the morphology of small intestine (jejunum). Compared Brennan *et al.,* (2014) The used of MRF 7 d to 21 (400 g/tonne); 21 to 42 d (200 g/tonne) better than control (1267.3 vs 796.6) increase villus height of broiler (P<0.05), villus height in jejunum tissue increase is a positive indicator of intestinal health and increased of absorptive area F. The level addition of a probiotic to broiler increased the villus height leading to increased intestinal surface area and therefore to increased digestion and absorption of nutrients in the basal diet (Brennan *et al.,* 2014). The result (table 8) at 35 days the used of MRF and probiotic liquid acidifier give significant difference (P<0.05) on villus height and crypth depth for the treatment (T1: MRF 80g/100kg;T2: Drinking water + 2 ml/L (Acid-Pak 4-way®),T3: MRF 80g/100kg+ Drinking water 2 ml/L (Acid-Pak 4-way®)).



a) b) c) d)

Figure 1. Representative condition of a) 21 days intestinal characteristic condition; b) 28 days intestinal characteristic condition; c) 35 days intestinal characteristic condition; d) VH/CD at 35 days intestinal characteristic condition (T_3).
Note: (a shallow crypt is positive factors for the development of an immune status and efficient for the small intestine).

Table 6. Effect of average group MRF and probiotics-enhanced liquid acidifier on the intestinal microbial at 21, 28, and 35 days of age

	Treatments[1]				
Jejunum	T0	T1	T2	T3	SEM
21 days	— — — — µm— — — —				
VillusHeight	442.75[b]	593.00[a]	597.50[a]	569.50[a]	57.57
Crypt depth	83.50	134.75	143.75	121.75	46.50
VH/CD	5.30	4.40	4.15	4.67	6.74
28 days					
VillusHeight	378.00[c]	617.00[ab]	666.00[a]	508.50[bc]	63.72
Crypt depth	105.00[b]	140.00[ab]	170.00[ab]	136.25[ab]	25.01
VH/CD	3.60	4.40	3.91	3.73	1.34
35 days					
VillusHeight	598.50[b]	713.75[a]	695.50[a]	718.50[a]	49.34
Crypt depth	112.50[b]	148.75[ab]	149.00[ab]	156.50[a]	25.17
VH/CD	5.32	4.79	3.99	4.59	3.45

[a,b] Mean values not sharing the same superscripts in a row differ significantly (P<0.05);
[1]T0= control; T1= MRF 80g/100kg; T2=Drinking water + 2 ml/L (Acid-Pak 4-way®); T3=MRF 80g/100kg+ Drinking water 2 ml/L (Acid-Pak 4-way®)

The treatment (T3) give the greater compared to all treatment and control (718.50 vs 713.75 (T1), 695.50 (T2), and 598.50 (control) µm; villus height). It may the combination both of MRF and probiotic liquid enhanced acidifier helps to increase surface are at final days. While, the villus height increase it correlation with crypt depth also increase (148.75 (T1), 149.00 (T2), and 156.50 (T3) vs 112.50 (control) µm). The MRF given at uniform dose (200, 400 mg/kg) are not significant different in crypt depth ratio (42d) (Di Giola and Biavati, 2018). A larger area of cryth depth may positive and faster growing in tissue for helps maintenance energy requirements (Spring et al., 2015). Based on the figure 1, a shallow crypt is positive factors for the development of an immune status and efficient for the small intestine. With a lower renewal rate, the cells in the intestinal become mature and allowing more efficient digestive enzyme production and absorption (Van Nevel *et al.,* 2005).

Blood biochemistry

Based on Table 7 and 8 the used MRF and probiotic-enhanced liquid acidifier as a feed additive on serum blood biochemistry were not significantly different (P>0.05) but the results on Glutamic oxaloacetic transaminase (GOT) at 21 days began to reduce. The treatment better compared to control (206.25 (T1), 208.25 (T2), and 228.00 (T3) vs. 238.50 U/L). The criteria for GOT were < 40 U/L for broiler. Continued to GPT the treatment were began trends were reduced at 28 days compared to control (table 6) (1.75 (T1), 2.00 (T2), 2.00 (T3) vs 2.25 U/L) even though, were not significantly different (P>0.05). The indicator normal for broiler were at < 41 U/L. The amount of GOT and GPT were unstable at 35 days (Table 7). The GOT treatment better compared to control (300.80 (T1), 265.80 (T2), and 343.30 (T3) vs. 329.50 U/L) and GPT were (3.00 (T1), 1.75 (T2), and 2.00 (T3) vs. 2.75 U/L). The result may due to the treatment cannot help to reduce the amount of GPT and GOT. The GPT and GOT were the indicator in the liver that the treatment cause negative effect or not. The dietary treatments did not have significant effects on the activities of GOT and GPT because the ability from probiotics does not always occur it depends on the optimum dose, frequency, and duration of treatments (An *et al.,* 2008).

Based on Table 7 and 8 the used MRF and probiotic-enhanced liquid acidifier as a feed additive on serum blood biochemistry were not significantly different (P>0.05) but the results on total protein (TP) at 21 days still stabile. The treatment better compared to control (2.57 (T1), 2.52 (T2), and 2.55 (T3) vs. 2.57 g/dL (21 days)) and (2.97 (T1), 2.97 (T2), 2.92 (T3) vs 2.95 g/dL (35 days)). The criteria for TP were < 2.55 g/dL for broiler. Continued to albumin (ALB) the treatment were cannot help to reduce trends at 21-35 days

Table 7. Effect of average group MRF and probiotics-enhanced liquid acidifier on the serum blood biochemistry of broiler at 21 days of age

	Treatments[1]				
Item[3]	T0	T1	T2	T3	SEM
GOT (U/L)	238.50	206.25	208.25	228.00	21.97
GPT(U/L)	2.00	1.50	2.25	2.75	0.61
TP (g/dL)	2.57	2.57	2.52	2.55	0.17
ALB (g/dL)	1.05	1.02	1.05	1.12	0.15
GLB (g/dL)	1.55	1.57	1.37	1.62	0.12
(A/G)	0.70	0.67	0.70	0.65	0.09
TGL(mg/dL)	140.25	141.75	142.00	144.25	97.65
TCHOL(mg/dL)	130.25	131.75	118.00	122.25	17.70
BUN(mg/dL)	1.27	1.05	1.06	1.27	0.25
GLC(mg/dL)	255.50	278.75	238.50	234.75	36.83

[1]T0= control; T1= MRF 80g/100kg; T2=Drinking water + 2 ml/L (Acid-Pak 4-way®); T3=MRF 80g/100kg+ Drinking water 2 ml/L (Acid-Pak 4-way®)

Table 8. Effect of average group MRF and probiotics-enhanced liquid acidifier on the blood biochemistry broiler at 35 days of age

	Treatments[1]				
Item[3]	T0	T1	T2	T3	SEM
GOT (U/L)	329.50	300.80	265.80	343.30	115.20
GPT(U/L)	3.00	1.75	2.00	2.75	0.97
TP (g/dL)	2.97	2.97	2.92	2.95	0.29
ALB (g/dL)	1.27	1.27	1.17	1.22	0.16
GLB (g/dL)	1.70	1.70	1.77	1.70	0.18
(A/G)	0.77	0.77	0.67	0.72	0.11
TGL(mg/dL)	32.25	30.25	30.50	29.00	5.66
TCHOL(mg/dL)	128.50	128.75	114.50	123.50	12.38
BUN(mg/dL)	0.97	0.47	0.62	0.47	0.32
GLC(mg/dL)	251.00	205.00	213.75	227.00	29.10

[1]T0= control; T1= MRF 80g/100kg; T2=Drinking water + 2 ml/L (Acid-Pak 4-way®); T3=MRF 80g/100kg+ Drinking water 2 ml/L (Acid-Pak 4-way®)

compared to control (table 6) (1.05 (T1), 1.02 (T2), 1.05 (T3) vs 1.12 g/dL (21 days)) and (1.27 (T1), 1.27 (T2), 1.17 (T3) vs 1.22 g/dL (35 days)) even though, were not significantly different (P>0.05).

The indicator normal for broiler were at < 1.00 g/dL. The amount of GLB and TGL were unstable at 35 days (table 7). The GLB treatment better compared to control (1.70 (T1), 1.70 (T2), and 1.77 (T3) vs. 1.70 (T0) g/dL) and TGL were (30.25 (T1), 30.50 (T2), and 29.00 (T3) vs. 32.25 mg/dL). High triglycerides may contribute to hardening of the arteries or thickening of the artery walls (arteriosclerosis) — which increases the risk of stroke, heart attack and heart disease. The result may due to the treatment cannot help to reduce the amount of TP, ALB and GLB. The ALB and GLB were the indicator in the liver that the treatment cause negative effect or not. The dietary treatments did not have significant effects on the activities of ALB and TGL because the levels in treatment doesn't helps, which contains antioxidants (An et al., 2008).

Based on Table 7 and 8 the used MRF and probiotic-enhanced liquid acidifier as a feed additive on serum blood biochemistry were not significantly different (P>0.05) but the results on total cholesterol (TCHOL), blood urea nitrogen (BUN), and glucose (GLC) at 21 days began to reduce. The treatment (T2 and T3) better compared to control (206.25 (118.00 (T2), and 122.25 (T3) vs. 130.25 mg/dL). The criteria for GOT were < 115 mg/L for broiler. Continued to BUN the treatment were began trends were reduced at 35 days compared to control (table 6) (0.47 (T1), 0.62 (T2), 0.47 (T3) vs 0.97 U/L) even though, were not significantly different (P>0.05).

The indicator normal for broiler were at < 0.47 U/L. The amount of GLC were unstable at 21 days (table 7). The GLC treatment (T2 and T3) better compared to control (238.50 (T2), and 234.75 (T3) vs. 255.50 mg/dL). When our glucose levels are optimal, it often goes unnoticed. But when they stray from recommended boundaries, while glucose notice the unhealthy effect it has on normal functioning. The dietary treatments did not have significant effects on the activities of TCHOL, BUN, GLC because the ability from probiotics does not always occur it depends on the optimum dose, frequency, and duration of treatments (An et al., 2008).

Conclusion

To sum up, the addition of mannan-riched fraction and combination with probiotic enhanced liquid acidifier doesn't impacted on growth Growth Performance, blood biochemistry, relative organ weight but give significant effect on intestinal microbial and reduces mortality of broiler.

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Refferences

Adli, D. N., O. Sjofjan, and M. Mashudi. 2017. Dried of Poultry Waste Urea-Molasses Block (dpw-umb) as Potential for Feed Supplementation. Jurnal Agripet 17: 144-149. DOI: https://doi.org/10.17969/agripet.v17i2.8391.

Adli, D. N. and O. Sjofjan. 2018. Nutrient content evaluation of dried poultry waste urea molasses block (DPW-UMB) on In-vitro analysis. Sains Peternakan: Jurnal Penelitian Ilmu Peternakan 16: 50-53. DOI: 10.20961/sainspet.16.2.50-53.

Adli, D. N., O. Sjofjan, and M. Mashudi. 2018. Nutrient content evaluation of dried poultry waste urea-molasses block (dpw-umb) on proximate analysis. Jurnal Ilmu-ilmu Peternakan 28: 84-89. DOI: 10.21776/ub.jiip.2018.028.01.09.

Adli, D. N., O. Sjofjan, and M. Mashudi. 2019. Nutrient content evaluation of dried of poultry waste urea-molasses block (dpw-umb) on proximate analysis. JITP. 6(2), 72-76.

Adli, D. N., Y. Chi, J. W. Lee, and O. Sjofjan. 2019. Supplementation Mannan-Rich Fraction (MRF) and/or Combination with Probiotic-Enhanced Water Acidifier on Dietary Female Broiler at 28 days as Natural Growth Promoters (NGPs). Research Journal of Advanced Engineering and Science. 4(3): 427-429. DOI: 10.5281/zenodo.3459284.

Agriculture Ministry of Indonesia. 2017. Livestock animal health statistics [Internet]. http://ditjenpkh.pertanian.go.id/userfiles/File/Buku_Statistik_2017_(ebook).pdf?time=150512744%093012 [cited 2020 Mar 6].

An, B. K., B. L. Cho, S. J. You, H. D. Paik, H. I. Chang, S. W. Kim, C. W. Yun, and C. W. Kang. 2008. Growth Growth Performance and antibody response of broiler chicks fed yeast derived β-glucan and single-strain probiotics. Asian-Australas. J. Anim. Sci. 21 1027-1032. DOI: 10.5713/ajas.2008.70571.

Biswas, A., N. Mohan, M. Raza, N. A. Mir, and Mandal. 2018. A production Growth Performance, immune response and blood biochemical parameters in broiler broiler fed diet incorporated with prebiotics J. Anim. Physiol. Anim. Nut. 103, 1-9. doi: 10.1111/jpn.13042.

Bozkurd, M., K. Küçükyılmaz, A. U. Çatlı, and M. Çınar. 2009. Effect of dietary mannan oligosaccharide with or without oregano essential oil and hop extract

supplementation on the Growth Performance and slaughter characteristics of male broilers Afr. J. Anim. Sci. 39, 223-232. http://dx.doi.org/10.4314/sajas.v39i3.49157.

Brennan, K. M., M. A. Paul, R. S. Samuel, B. Lumpkins, J. L. Pierce, S. R. Collett, and G. F. Mathis. 2014. Comparison of Growth Performance and intestinal morphology of broilers using step-down supplementation with a mannan-rich fraction versus bacitracin methylene disalicylate. J. Appl. Anim. Nut. 2: 1-7. DOI: https://doi.org/10.1017/jan.2014.6.

Charalampopoulos, D. and R. A. Rastall. 2009. Prebiotics and Probiotics, Springer, UK.

Di Giola, D. and B. Biavati 2018. Probiotics and prebiotics in animal health and food safety, Springer Press, Switzerland.

Jet, S. M., H. Soetanto, O. Sjofjan, B. Tulung. 2014. The effect of native gedi leaves (Abelmoschus manihot l. medik) of northern Sulawesi-Indonesia as a source of feedstuff on the Growth Performance of broilers. Int J. Bio. Sci. 3, 82-91. doi: http://dx.doi.org/10.12692/ijb/3.10.82-91.

Natsir, M. H., O. Sjofjan, K. Umam, A. Manab, and E. Widodo. 2010. Effects of liquid and encapsulated lactic acid in broiler diets on Growth Performance, intestinal characteristics and intestinal microflora. J. Poult. Sci. 47: 240-243. https://doi.org/10.2141/jpsa.009099

Spring P., C. Wenk, A. Conolly, and A. Kiers. 2015. A review of 733 published trials on bio-mos®, a mannan oligosaccharide, and actigen®, a second generation mannose rich fraction, on farm and companion animals. J. of Appl. Anim. Nutr. 3, 1-11. doi: 10.1017/jan.2015.6

Sjofjan, O., M. H. Natsir, D. N. Adli, D. D Adelina, and L. M. Triana. 2020. Effect of symbiotic flour (lactobacillus sp. and fos) to the egg quality and performance of laying hens. IOP Conf. Series: Earth and Environmental Science. 465: 012-033. doi:10.1088/1755-1315/465/1/012033

Sjofjan, O., D. N. Adli, P. K. Hanani, and D. Sulistiyaningrum. 2019. The utilization of bay leaf (*Syzygiumpolyanthum*Walp) Flour in feed on carcass quality, microflora instestine of broiler. International Journal of Engineering Technologies and Management Research. 6: 1-9. DOI: 10.5281/zenodo.3563042.

Sjofjan, O., D. N. Adli, M. H. Natsir, and A. Kusumaningtyaswati. 2020. Pengaruh kombinasi tepung kunyit (Curcuma domestica Val.) dan probiotik terhadap penampilan usus ayam pedaging. Jurnal Nutrisi Ternak Tropis dan Ilmu Pakan 2: 19-24. DOI: 10.24198/jnttip.v2i1.26587.

Van Nevel, C. J., J. A. Decuypere, N. A. Dieric, and K. Moll. 2005. Incorporation of galactomannans in the diet of newly weaned piglets: Effect on bacteriological and some morphological characteristics of the small intestine. J. Anim. Nutr. 59, 123-138. Doi: 10.1080/17450390512331387936.

Widiyawati, I., O. Sjofjan, and D. N. Adli. 2020. Peningkatan kualitas dan persentase karkas ayam pedaging dengan subtitusi bungkil kedelai menggunakan tepung biji asam (*Tamarindus indica L*) fermentasi. Jurnal Nutrisi Ternak Tropis 3: 35-40. DOI: 10.21776/ub.jnt.2020.003.01.7.