

# NUTRITION



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# Research Article Effect of Fiber Feed on Rabbits During the Postweaning Period

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## Abstract

**Background and Objective:** This study was conducted to analyze the effect of the neutral detergent fiber (NDF) and fiber particle size on mortality, hematology, stress status, immunity and number of *E. coli* in New Zealand white (NZW) crossbred rabbits during the postweaning period. **Methodology:** A total of 144 NZW crossbred rabbits were used in the study. The trial design was a completely randomized  $3 \times 3$  factorial pattern, namely, 3 levels of the concentration of neutral detergent fiber (NDF) (F1, F2 and F3) and 3 sizes of fiber particles (P1, P2 and P3). Each treatment was repeated 4 times and each replication consisted of 4 trials. **Results:** The average mortality up to 12 weeks was 19.44%. The treatment combinations of F3P2 and F2P2 had the lowest mortality rate (0%) and low rates of *E. coli* infection at 2.55 and 2.71 × 10<sup>9</sup> CFU mL<sup>-1</sup>, respectively. The feed treatment affected the hematological values in rabbits during the postweaning period. The results indicated that there was a relationship between mortality and *E. coli* infection. Moreover, the F3P2 treatment was the best feed combination observed in this study. **Conclusion:** The feed treatment affected the mortality, immunity, hematology and stress status of NZW crossbred rabbits during the postweaning period.

Key words: Fiber feed, mortality, immunity, hematology, rabbit

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Data Availability: All relevant data are within the paper and its supporting information files.

#### **INTRODUCTION**

Rabbit cultivation in Indonesia has experienced many problems that prevent livestock performance from reaching its genetic potential. This condition has caused many farmers to suffer losses. In general, there are 3 problems that rabbit breeders face, including feed, heat stress and livestock health. The interaction of these factors causes a rather high rabbit mortality rate during the postweaning period. According to Lebas and Fortun-Lamothe<sup>1</sup>, the mortality rate in the postweaning and growth periods reached 11-12%, while the rate of rabbit mortality under general raising conditions in the subtropics was 8.5%. Moreover, the disease that often arises in the tropics is diarrhea, which causes a high mortality rate (20%). Farmers have found that the mortality rate of rabbits is increasing due to the presence of other diseases and other factors. This problem has not been addressed optimally and it has caused damages to rabbit breeders in Indonesia<sup>2</sup>.

Dietary fiber is crucial for rabbits, especially during the postweaning period. This fiber helps the digestion process, the microbial composition of the flora and the development of the digestive tract. Increasing the ratio of starch and fiber to less than 300 g of neutral detergent fiber (NDF) can lead to an interruption in the intestinal feed flow and bacterial biomass production in rabbits during the postweaning period<sup>3,4</sup>. Moreover, comparative levels of fiber and dietary deficiencies affect microbial communities in the cecum<sup>5</sup>. Reductions in NDF, e.g., from 300-250 g kg<sup>-1</sup> of feed, increased the microflora in the ileum and decreased the microflora in the secum<sup>4</sup>. Conversely, in terms of gastrointestinal health, excessive high fiber consumption leads to infection and an increase in the incidence of *colibacillosis*<sup>6</sup>.

If the composition of fiber feed is not homogenous in terms of particle size (i.e., the feed particles are different sizes), the proportion of the lignin content will be increased. The need for fiber particles in feed increased and was positively correlated with the presence of lignin<sup>7</sup>. In general, the size of the fiber particles for rabbits ranges from 0.5-1.5 mm before pellets are made. The particle size of the feed affects digestion and is essential for motility in the rabbit gut<sup>8</sup>. Particle size can cause a decrease in the quality of nutrients and promote stress-inducing digestive system disorders.

Some of the issues that have been discussed above include the main causes of high rabbit mortality, especially during the postweaning period. The postweaning period and the growth period are critical periods in the fattening process. Notably, during the postweaning period, antibodies are often completely depleted<sup>9</sup>. This observation is related to the immaturity of the immune system, which has not yet fully developed in this period. This study reviews the NDF-to-fiber particle size ratio and its effect on the mortality, hematology, stress status, immunity and number of *E. coli* in NZW crossbred rabbits in the postweaning period. Moreover, the results are expected to identify standard nutrient requirements for rabbit feed for the postweaning period, which can be used to improve rabbit health status and prevent high mortality, a prevalent problem in Indonesia, during this period. Based on the above descriptions, a study was conducted to address the challenges of improving the performance of postweaning period rabbits based on the interaction of the ratio of NDF content and the fiber particle size.

#### **MATERIALS AND METHODS**

This study used 144 28-day-old New Zealand white (NZW) crossbred postweaning period rabbits. These rabbits were produced through a process of artificial insemination (IB) by 5 NZW males with 50 local does that were mated simultaneously using gnRH stimulation. The does and postweaning period rabbits used in this study were kept in closed enclosures and a maximum temperature of 29°C was used as the optimal limit of rabbit comfort<sup>10</sup>. The level of ammonia (NH<sub>3</sub>) in the cage was controlled to minimize any effects other than the treatment.

This experiment was conducted to study the interaction between the level of NDF and the fiber particle size. The experimental design was a completely randomized  $3 \times 3$ factorial pattern, i.e., 3 NDF levels (F1, F2 and F3) and 3 fiber particle sizes (P1, P2 and P3) (Table 1). Each treatment was repeated 4 times. Each colony consisted of 4 rabbits in the postweaning period. The NDF value, the fiber particle size and the composition of feed ingredients were not shown as a requirement of patent processing by the Directorate General of Intellectual Property, Ministry of Law and Human Rights of the Republic of Indonesia.

Rice straw and kale straw were the sources of fiber in the forage feed and were milled to obtain fiber particles of 3 different sizes, namely, P1, P2 and P3. The concentrated feed ingredients consisted of corn, pollard, soybean meal, palm oil, molasses and premix. The raw ingredient feed that was used as a treatment ration and was once used as a complete feed was analyzed to determine the nutritional content. Proximate analysis was performed using the AOAC method<sup>13</sup> and NDF analysis was performed using the Van Soest *et al.*<sup>7</sup> method. These methods were performed on both the feed ingredients and the complete feed. This analysis was conducted at the Laboratory of Animal Science and Technology, Faculty of Animal Science Bogor Agricultural University.

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Nutrient contents*	F1	F2	F3	Standard**
Dry material (%)	88.75	88.11	87.48	-
Crude protein (%)	15.00	15.02	15.03	14.20-16.00
Ash (%)	6.19	6.49	6.79	-
Crude fat (%)	6.19	6.14	6.09	Free
Crude fiber (%)	CF 1	CF2	CF3	15.00-16.00
NDF (%) (F)	F1	F2	F3	33.00-35.00
DE (kcal kg <sup>-1</sup> )	2428	2428	2428	2428
Fibrous particle size (P) (mm)				0.5-1.5
The ratio of NDF (F)/fibrous particle size (P)				
P1	F1/P1	F2/P1	F3/P1	
P2	F1/P2	F2/P2	F3/P2	
Р3	F1/P3	F2/P3	F3/P3	

\*Feed treatment consists of kale straw, rice straw, corn, soybean meal, pollard, palm oil, Molasses and premix based on the analysis at the Laboratory of Animal Science and Technology, Faculty of Animal Husbandry, Bogor Agricultural University (IPB\*\*)<sup>11,12</sup>.

The variables observed in this study were mortality, hematology (e.g., leukocytes, eosinophils, neutrophils and lymphocytes), stress status, immunity (immunoglobulin A/IgA) and number of *E. coli* in NZW crossbred rabbits that were in the postweaning period. The concentration of IgA was measured in the intestinal fluid and serum. The results of hematology, stress status, immunity and number of *E. coli* were used to evaluate health status and these variables were correlated with the causes of high mortality rates in NZW crossbred rabbits in the postweaning period. A comparison between the neutrophils and lymphocytes (N/L) was used as an indicator to determine the stress status of the experimental animals<sup>14</sup>.

The primary repertoire phase was the period of rabbit raising during the postweaning period (i.e., 28-42 days), while the secondary repertoire phase was the period of rabbit raising during the growth period (i.e., 43-70 days)<sup>15</sup>. Blood, serum, ileal fluid and cecum content samples were collected once on day 42 of the experiment. The methods of Yun *et al.*<sup>16</sup> and Nurliyani *et al.*<sup>17</sup> with minor modifications were used for the intestinal collection. The IgA analysis was performed using a Rabbit IgA ELISA kit (Cat No: E0249Rb ELISA sandwich kit, Bioassay Technology Laboratory) and was conducted at the Clinical Pathology Laboratory, Faculty of Medicine, Gajah Mada University. The leukocyte differential analysis was performed at the Animal Health Laboratory of Central Java Provincial Government using a hematology analyzer.

All actions undertaken in this study were approved by the Animal Ethics Commission, Bogor Agricultural Institute with ethical approval number 86-2017 IPB, dated December 19, 2017. The data for each observed variable were analyzed verbally and the average differences were tested using Duncan's multiple range test<sup>18</sup>.

#### RESULTS

The average temperature in the enclosure during the study was 26.01°C (closed house) and the outside temperature was between 23.9 and 32.2°C. Furthermore, the humidity in the enclosure was between 70.66 and 97.66%, with an average of 86.51%.

The interaction between the NDF concentration and fiber particle size had a significant effect (p<0.05) on the cumulative mortality of NZW crossbred rabbits from 5-12 weeks of age. The F2P2 and F3P2 treatment combinations showed the lowest mortality rates (0%) (Table 2). The average mortality in all treatment groups up to 12 weeks of age was 19.44%. The results in this study showed that the highest mortality occurred at 8 and 9 weeks of age. The mortality in the F1P2 and F2P3 treatment groups at 9 weeks of age was 18.75% and the total mortality rate in all treatment groups was 6.25% (Table 2). The treatment group with the lowest NDF level (F1) showed the highest mortality rate (14.58%) at 8 weeks of age and the average mortality rate in all NDF feed grade groups was 6.94% (Table 2).

Fiber particle size had a significant effect (p<0.05) on the number of leukocytes, eosinophils and neutrophils in NZW crossbred rabbits that were in the postweaning period. Increases in fiber particle size could increase leukocyte levels from  $3.83-6.57 \times 10^9$  dL<sup>-1</sup>, eosinophil levels from  $0.04-0.07 \times 10^9$  L<sup>-1</sup> and neutrophil levels from  $2.18-3.96 \times 10^9$  L<sup>-1</sup> in rabbits in the postweaning period. These values were above the normal standards (Table 2).

The interaction between the NDF level and fiber particle size had a significant effect (p<0.05) on the number of lymphocytes in postweaning period rabbits, with the mean of  $1.66 \times 10^9 \, \text{L}^{-1}$ , although this value was still within the normal

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		F1	F2	F3	Average***
Table 2: The effect of NDF-to-particle size ratio on mortality rate, hematology and stress status of NZW crossbred rabbits in the postweaning period					

		F1	F2	F3	Average***
Mortality at week VIII	P1	12.50	6.25	6.25	8.33
	P2	18.75	0.00	0.00	6.25
	P3	12.50	6.25	0.00	6.25
Average**		14.58ª	4.17 <sup>b</sup>	2.08 <sup>b</sup>	6.94
Mortality at week IX*	P1	0.00 <sup>Q</sup>	6.25 <sup>Q</sup>	0.00 <sup>Q</sup>	2.08
	P2	18.75 <sup>P</sup>	0.00 <sup>Q</sup>	0.00 <sup>Q</sup>	6.25
	P3	0.00 <sup>Q</sup>	18.75 <sup>p</sup>	12.50 <sup>PQ</sup>	10.42
Average		6.25	8.33	4.17	6.25
Mortality from week V-XII*	P1	31.25 <sup>PQ</sup>	12.50 <sup>QR</sup>	12.50 <sup>QR</sup>	18.75
	P2	43.75 <sup>P</sup>	0.00 <sup>R</sup>	0.00 <sup>R</sup>	14.58
	P3	25.00 <sup>PQ</sup>	31.25 <sup>PQ</sup>	18.75 <sup>QR</sup>	25.00
		33.33	14.58	10.42	19.44
Leukocytes (10 <sup>9</sup> L <sup>-1</sup> )	P1	4.10±1.88	4.27±1.96	3.13±1.44	3.83±1.76 <sup>b</sup>
	P2	4.70±2.16	$5.53 \pm 2.54$	5.63±2.59	5.29±2.43ª
	P3	8.53±3.92	6.47±2.97	4.70±2.16	6.57±3.01ª
Average		5.78±2.65	5.42±2.49	4.49±2.06	5.23±2.40
Eosinophils (10 <sup>9</sup> L <sup>-1</sup> )	P1	0.04±0.02	0.04±0.02	0.03±0.02	$0.04 \pm 0.02^{b}$
	P2	0.04±0.02	$0.05 \pm 0.03$	0.06±0.03	0.05±0.03ª
	P3	0.09±0.05	$0.07 \pm 0.04$	0.04±0.02	$0.07 \pm 0.04^{a}$
Average		0.06±0.03	$0.05 \pm 0.03$	0.04±0.02	$0.05 \pm 0.03$
Neutrophils (N) (10 <sup>9</sup> L <sup>-1</sup> )	P1	2.13±1.14	2.33±1.25	2.07±1.10	2.18±1.16 <sup>b</sup>
	P2	3.10±1.65	3.27±1.74	2.80±1.49	3.06±1.63ª
	P3	5.17±2.76	4.13±2.21	2.57±1.37	3.96±2.11ª
Average		3.47	3.24	2.48	3.06±1.24
Lymphocytes (L) (10 <sup>9</sup> L <sup>-1</sup> )*	P1	1.57±0.63 <sup>Q</sup>	1.57±0.63 <sup>Q</sup>	0.77±0.31 <sup>Q</sup>	1.30±0.53
	P2	1.17±0.47 <sup>Q</sup>	1.73±0.70 <sup>Q</sup>	2.27±0.92 <sup>p</sup>	1.72±0.70
	P3	2.50±1.01 <sup>P</sup>	$1.60 \pm 0.65^{\circ}$	1.73±0.70 <sup>Q</sup>	1.94±0.79
Average		1.74	1.63	1.59	1.66±0.67
Stress status N L <sup>-1*</sup>	P1	1.43±0.55 <sup>Q</sup>	$1.57 \pm 0.60^{\circ}$	2.94±1.13 <sup>P</sup>	1.98±0.76
	P2	2.77±1.06 <sup>P</sup>	1.90±0.73 <sup>Q</sup>	1.14±0.44 <sup>Q</sup>	1.94±0.74
	P3	1.98±0.76 <sup>Q</sup>	$2.39 \pm 0.92^{\circ}$	1.69±0.65°	2.02±0.77
		2.06	1.96	1.93	1.98±0.80

\*Same capital letter in the same combination of row and column shows a nonsignificant difference (p>0.05). \*\*Same letter case in the same row shows no significant difference (p>0.05). \*\*Same letter case in the same column shows no significant difference (p>0.05) by Duncan's test<sup>18</sup>. Standard Normal Period Postweaning period: Leukocytes: 2.6-12.7 × 10<sup>9</sup> L<sup>-1</sup>, Eosinophils: 0.00-0.03 × 10<sup>9</sup> L<sup>-1</sup>, Neutrophils: 0.5-5.4 × 10<sup>9</sup> L<sup>-1</sup> and Lymphocytes: 0.8-6.4 × 10<sup>9</sup> L<sup>-1</sup><sup>10,19</sup>

range (0.8-6.5×10<sup>9</sup> L<sup>-1</sup>) (Table 2). Fiber particle size had a significant (p<0.05) effect on neutrophils, with an average count of  $3.06 \times 10^9$  L<sup>-1</sup> in the postweaning period rabbits but this value was still within the normal limits (Table 2). Interestingly, the interaction between the NDF level and fiber particle size had a significant effect (p<0.05) on the stress status (neutrophils/lymphocytes) of rabbits in the postweaning period (Table 2).

The NDF level and fiber particle size each had a significant main effect (p>0.05) on the number of *E. coli* bacteria in the contents of the caecum, with an average of  $3.01 \times 10^9$  CFU mL<sup>-1</sup> during the NZW rabbit postweaning period but there was no interaction. Level F1 showed the highest average value of *E. coli* bacterial contamination at  $3.37 \times 10^9$  CFU mL<sup>-1</sup>. Level P2 showed the lowest average *E. coli* bacterial contamination value at  $2.70 \times 10^9$  CFU mL<sup>-1</sup> (Table 3).

The interaction between the NDF and fiber particle size ratio had a significant effect (p<0.05) on serum

immunoglobulin A (IgA) levels (Table 3). Different-sized fiber feed particles had a significant effect (p<0.05) on the level of immunoglobulin A (IgA) in the ileal liquid in the postweaning period of NZW crossbred rabbits. Furthermore, the mean blood serum IgA concentration in the F2P2 treatment group in the postweaning period (83.77 ng mL<sup>-1</sup>) was lower than the average value of all treatment combinations (127.75 ng mL<sup>-1</sup>) (Table 3).

The blood IgA concentrations in the postweaning period were the lowest in the F2P2 group (83.77 ng mL<sup>-1</sup>) and the F3P1 group (70.73 ng mL<sup>-1</sup>). The interaction between different NDF concentrations and particle sizes of fiber feed had a significant effect (p<0.05) on the ileal fluid IgA levels (Table 3). The results of this study showed that in the primary repertoire period, or postweaning period (i.e., age: 28-42 days), the ileal fluid IgA concentration (32.48 ng mL<sup>-1</sup>) was lower than the serum IgA concentration (127.75 ng mL<sup>-1</sup>) (Table 3).

The treatment combinations of F3P2 and F2P2 had the lowest mortality rates (Table 2). Furthermore, the F3P2 and

Table 3: The effect of NDF-to-particle size ratio on serum immunoglobulin A (IgA) and *Escherichia coli* in the cecum contents of NZW crossbred rabbits during the postweaning period

		F1	F2	F3	Average***
lgA serum (ng mL⁻¹)*	P1	138.67±78.85 <sup>PQ</sup>	156.23±88.83 <sup>PQ</sup>	70.37±40.01 <sup>R</sup>	121.76±69.23
	P2	147.23±83.72 <sup>PQ</sup>	83.77±47.63 <sup>R</sup>	163.76±93.12 <sup>p</sup>	131.59±74.82
	P3	131.26±74.64 <sup>PQ</sup>	131.79±74.94 <sup>PQ</sup>	126.63±72.00 <sup>Q</sup>	129.90±73.86
Average		139.05	123.93	120.26	127.75±72.64
IgA lleal liquid (ng mL <sup>-1</sup> )	P1	29.30±4.72	39.13±6.30	51.71±8.32	40.05±6.45ª
	P2	1.41±0.23	46.38±7.47	71.10±11.45	39.63±6.38ª
	P3	6.80±1.10	6.30±1.01	40.17±6.47	17.76±2.86 <sup>b</sup>
Average**		12.50	30.60	54.32	32.48±5.23
<i>Escherichia coli</i> ×10 <sup>9</sup> (CFU mL <sup>-1</sup> )*	P1	3.47±0.46	2.74±0.36	2.98±0.39	3.06±0.41ª
	P2	2.84±0.38	2.71±0.36	2.55±0.34	2.70±0.36 <sup>b</sup>
	P3	3.80±0.50	3.41±0.45	2.64±0.35	3.28±0.43ª
Average		3.37±0.45ª	2.95±0.39 <sup>b</sup>	2.72±0.36 <sup>b</sup>	3.01±0.40

\*Same capital letter in the same combination of row and column shows a nonsignificant difference (p>0.05). \*\*Same letter case in the same row shows no significant difference (p>0.05). by Duncan's test<sup>18</sup>

F2P2 treatments had the lowest values of *E. coli* infection at 2.55 and 2.71×10<sup>9</sup> CFU mL<sup>-1</sup>, respectively (Table 3). The high blood serum immunoglobulin A (IgA) content of the F3P2 treatment group in the postweaning period was 163.76 ng mL<sup>-1</sup> and this treatment group also had a low level of *E. coli* infection, which was  $2.55 \times 10^9$  CFU mL<sup>-1</sup> (Table 3).

The F3P2 treatment combination increased the IgA concentration in serum and ileal liquid by 163.76 and 71.10 ng mL<sup>-1</sup>, respectively (Table 3), showing the highest value of IgA among the different treatment combination groups. The ability of this treatment combination to produce higher levels of IgA could suppress the growth rate of *E. coli* and promote the lowest stress level at  $2.55 \times 10^9$  CFU mL<sup>-1</sup> (Table 3) and 1.14, respectively (Table 2).

The different NDF concentrations and fiber particle sizes had significant main effects on ileal fluid IgA levels (p<0.05). However, there was no significant interaction effect (p>0.05) of the two treatments on ileum fluid IgA concentration in postweaning period rabbits (Table 3).

#### DISCUSSION

Effect of the feed treatment on mortality rate, hematology and stress status of NZW crossbred rabbits in the postweaning period: The ratio of NDF and fiber particle size had a highly significant interaction between 5 and 12 weeks of age. The mortality rate during the first 5 weeks even reached its minimum value. The average mortality rate in 12 weeks was almost 20%. The average mortality rate of rabbits raised in the tropics was approximately the same as that found in this study<sup>2</sup>. According to Raharjo *et al.*<sup>2</sup>, maintenance of rabbits in the tropics has a mortality rate above 20%. In contrast, the results from 8-9 weeks of age showed that the total number of deaths was lower than that at 12 weeks but the overall group increased from 0-6.25%. The results obtained in this study were in line with Gutierrez *et al.*<sup>20</sup> who stated that the administration of 2 different concentrations of NDF (360 and 300 g NDF kg<sup>-1</sup>) in feed had no effect on weaning rabbits (age of 25 days) but dietary differences had an effect on mortality if feed was given to older rabbits (older than 39 days).

The mortality rate at 8 weeks was lower than that from 9-12 weeks and the overall NDF value slightly decreased. The main cause of death during this period was diarrhea. In rabbits, diarrhea often occurs in 4-8 weeks of age<sup>21</sup>. This diarrhea is caused by a digestive system disorder due to a feed fiber composition that is not in accordance with the needs of the weaning or growth periods of the rabbits<sup>1</sup>.

There were significant effects of the NDF level and fiber feed on the leukocyte numbers but they did not interact significantly with the feed particle size during the postweaning period, which could increase the number of leukocytes, causing the observed levels of stress<sup>22</sup>. Moreover, low feed quality or NDF levels that did not meet the needs of rabbits could cause stress<sup>8</sup>. This result showed that the number of leukocytes was within the normal range of  $3.3-12.2 \times 10^9$  dL<sup>-1</sup> (Table 2).

Parasitic infections due to low feed quality would cause an increase in eosinophil production<sup>23</sup>. Increasing fiber particle size had an impact on eosinophil levels during the postweaning period and values were above the normal range. Eosinophils are weak phagocytic cells in comparison to neutrophils. A parasitic infection occurred during the weaning period due to the large size of the feed particles and eosinophils were produced in large quantities. These eosinophils migrated to the tissues and eosinophils were also found in areas of allergic reactions<sup>23</sup>. The NDF-to-fiber particle size ratio and its effect on lymphocytes were still within the normal range. Differences in the number of lymphocytes occurred because of the treatment response that resulted in the formation of antigens in the body due to different feed qualities. According to Tizard<sup>24</sup>, the main function of lymphocytes is to respond to antigens by forming antibodies.

Similar to the eosinophils, the effect of feed particle size on the average number of neutrophils also improved but it was still within the normal range of values. The increase in the number of neutrophils in circulation was called a left shift and was found when an acute infection occurred. The number of abnormal neutrophils with hypersegmentation was called a right shift and was found in cases of chronic infection or stress<sup>12</sup>. The ratio of neutrophils/lymphocytes (N/L) was used as the stress status index<sup>8</sup>. Additionally, the interaction between the NDF and fiber particle size had a significant effect on stress status during the postweaning period.

There was no interaction effect of the treatment combinations of the NDF level and the size of the fiber particles on the number of *E. coli* but each treatment had a significant main effect. The best average value of E. coli occurred in level F1, while P2 had the worst value. Giving or consuming high fiber to improve the health conditions of the digestive tract caused infections or increased the incidence of colibacillosis<sup>6</sup>. When livestock were infected with microorganisms in the blood, the number of leukocytes generally increased and the ratio of neutrophils and monocytes increased in relation to the number of leukocytes, indicating that cattle tended to be infected with bacteria (E. coll) and viruses. If the eosinophil ratio increased with the number of leukocytes, animals were infected with parasites. Additionally, if the ratio of basophils increased relative to the number of leukocytes, the livestock experienced allergic disturbances<sup>25</sup>.

Effect of NDF and particle size ratio on IgA and *E. coli* in the cecum contents of NZW crossbred rabbits during the postweaning period: The essential interaction of the NDF and particle size ratio had a significant effect on the serum IgA level during the postweaning and growth periods. The same conditions affected the concentration of IgA in the ileal liquid during the postweaning period. This result was because the lymphoid organs develop in the gastrointestinal tract of rabbits from the primary repertoire period up to the secondary repertoire period. The process of lymphoid organ development is strongly influenced by the quality of dietary fiber<sup>3,4</sup>. Lymphoid organs had begun to form during the growth period around the age of 43-70 days. After entering

the secondary repertoire period, the lymphoid organs in the digestive tract began to produce their own IgA. At this time, rabbits were beyond the period with the highest mortality rate, i.e., the primary repertoire period. The results showed that the serum had the smallest mean IgA concentration relative to all treatment combinations, while similar conditions were observed in the growth period. This result indicated that the lowest infection rate was in the combination treatment in each of these periods.

The lowest blood IgA concentrations were in the F2P2 and F3P1 treatments. The low blood IgA concentration in the F2P2 treatment group showed that the combination of feed in this group was able to suppress infections, resulting in a low mortality rate of 0% (Table 1 and 2)<sup>6</sup>.

There was a significant interaction that affected the ileal fluid IgA levels in the different treatment combinations. This result was because the IgA concentrations in the postweaning period and early periods of growth are more commonly produced in the gut-associated lymphoid tissue (GALT) than in the gastrointestinal lymphoid organs<sup>15</sup>. Based on the results, the ileal IgA concentration was lower than the serum IgA concentration at 28-42 days of age.

The digestive system in rabbits has 2 functions, to digest nutrients and to protect against pathogenic microorganisms. Both functions are active from birth but they do not reach optimum performance until rabbits reach the ages of 8-10 weeks<sup>15</sup>. The results showed that the F3P2 and F2P2 combinations had the lowest values in terms of both mortality rate and the amount of *E. coli* contamination, which showed that there was a relationship between these variables. Treatment group F3P2 also had a large serum IgA value and a low *E. coli* level. The high serum IgA concentration in the F2P2 treatment group showed that the combination of feed in this group was able to suppress infection, especially in periods of weaning, which resulted in a mortality of 0% (Table 1 and 2)<sup>6</sup>.

The highest values of IgA in the serum and ileal liquid were in treatment group F3P2 and these values reduced stress status to its lowest level. This result was because more IgA in the postweaning period was produced in the GALT than in the gastrointestinal tract<sup>15</sup>. The results of this study indicated that in the primary repertoire period or postweaning period (age 28-42 days), the concentration of ileal liquid IgA (32.48 ng mL<sup>-1</sup>) was lower than the concentration of serum IgA (127.75 ng mL<sup>-1</sup>) (Table 3).

For ileal fluid IgA levels, the treatment combinations had a significant effect but there was no interaction during the postweaning period. This result suggested that both the quality of dietary fiber in the NDF and the feed particle size (Table 1) affected the number of infections in NZW crossbred rabbits<sup>3,4</sup>. In the postweaning period, F1 had the lowest feeding NDF level and P3 had the highest particle feed size. The low IgA concentration of the ileal fluid in these 2 periods showed the low rate of lymphoid organ development of the small intestine that occurred in both periods. This phenomenon indicated that the minimal development of the gastrointestinal lymphoid organs during the period of weaning (primary repertoire) and the low feed quality (feed stress) affected the ileal fluid IgA concentration<sup>8</sup>. Furthermore, this finding indicated that feed quality may affect the development of the lymphoid glands in the gastrointestinal tract<sup>3,4</sup>.

#### CONCLUSION

It is concluded that the mortality rate up to the 12th week of development in NZW rabbits was 19.44%. The treatment combinations F3P2 and F2P2 showed the lowest mortality rate (0%), as well as low *E. coli* infections of 2.55and  $2.70 \times 10^9$  CFU mL<sup>-1</sup>, respectively. This result showed that there was a relationship between mortality and *E. coli* infection. The feed treatment affected the hematological values of rabbits in the postweaning period. Differences in infection rates and stress in postweaning period rabbits were also observed as a response to differences in feed. This study showed that feed affected mortality, immunity, hematology and stress status of rabbits in the postweaning period. The F3P2 treatment combination was the best feed tested in this study.

#### SIGNIFICANCE STATEMENT

This study found a possible effect of the combination of NDF and fiber particle size. Furthermore, this effect can be used to reduce mortality and improve the health status of NZW crossbred rabbits that are raised in tropical areas in general and specifically, in Indonesia. These results will help researchers identify the critical portion of the weaning period that is recorded to have the highest mortality rate of all rabbit raising periods. Thus, a new theory about the combination of NDF and the size of fiber particles in rabbit feed can be developed for the weaning period.

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