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Performance of Broiler Chickens Fed Diet Added with Buffaloes Rumen Fluid Enzymes from Slaughterhouses

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ABSTRACT

This study was conducted to evaluate the effect of addition of rumen fluid enzymes of buffaloes from slaughterhouse in diet on feed intake, slaughter weight, daily weight gain, feed conversion ratio, absolute carcass weight, relative carcass weight, and digestive organs of broiler. Materials used was two hundred unsexed 1 day old chicks of broilers. The study used a completely randomized design with five treatments and four replications consisted of 10 birds each replication. Treatment groups were as follows P0 = diet without addition of rumen fluid enzymes (control); P1 = diet with addition of rumen fluid enzymes of 0.75%; P2 = diet with addition of rumen fluid enzymes of 1.5%; P3= diet with addition of rumen fluid enzymes of 2.25%; P4= diet with addition of rumen fluid enzymes of 3%. The variables observed were feed intake, slaughter weight, daily weight gain, feed conversion ratio, absolute carcass weight, relative carcass weight, and digestive organs. Results of the study showed that the treatments gave a non-significant different (P>0.05) on feed intake, slaughter weight, daily weight gain, feed conversion ratio, absolute carcass weight, relative carcass weight, and digestive organs of broiler chicken. It could be concluded that the use of rumen fluid enzymes of buffaloes in the diets up to 3% level did not increase performance and digestive organs of broiler chicken.

Keywords: Broilers, Buffalo, Digestive organs, Performance, Rumen fluid enzymes

Introduction

The use of commercial enzymes in poultry feeds has been carried out with the aim of increasing digestibility of food substances, increasing growth and improving feed consumption of broiler chicken (Zhao *et al*., 2011), but the use of commercial enzymes available in the market with various purpose must be limited because it is related to its relatively expensive price, so that it can also result in an increasing price of feeds. Another alternative that can be done is by utilizing buffalo rumen fluids from slaughterhouses (RPH) as a source of inexpensive enzymes, because it is a waste that is often wasted and not utilized.

The results of previous studies have reported that cellulase, mannanase, amylase, phytase and protease enzymes contained in cattle rumen fluid could increase the digestibility of some local feed (Budiansyah, 2010a). Budiansyah (2010b) reported that the waste rumen fluid from RPH has high carbohydrase enzyme activity and enzyme characteristics which are potential to be used as feed additive sources of enzymes to improve the quality of poultry feed. The cow's rumen fluid contains cellulase enzymes, xylanase, mannanase, amylase, proteases and phytase which are able to hydrolyze local feed ingredients Budiansyah, (2010). These enzymes generally not only digest fiber, but it also can improve the process of nutrient absorption in broiler body transform into meat and can increase the availability of phosphorus, energy and protein (Ketaren *et al*., 2002; Herawati, 2010). The rumen fluid enzyme can reduce phytic acid and increase dissolved glucose levels derived from NSP (Non-Starch Polysaccharide) on some feed ingredients (Mehri *et al*., 2010). Resmi *et al*. (2013) reported that some local feed ingredients of poultry feeds contains phytic acid such as polishes of 6.73 ± 0.34%, soybean meal as much as 6.37 ± 0.29%, palm kernel cake 5.83 ± 1.28% and rice bran as much as 6.75 ± 0.86%. The use of cow rumen fluid enzymes on some feed ingredients can increase dissolved glucose levels derived from the breakdown of Non-Starch Polysaccharide/NSP (Zhao *et al*., 2011). The highest glucose increase in some feed ingredients such as palm kernel meal, rice bran, and coconut cake was obtained after the ingredients were incubated as much as

2% of cattle rumen fluid enzymes, in fish meal 2.5% and soybean meal 3% (Resmi *et al*., 2013).

Research on the ability of buffalo rumen fluid to hydrolyze food substances in feed ingredients is currently not widely carried out, but it is thought that the enzymes contained in buffalo rumen fluid have the same ability as cow rumen fluid enzymes. Wahyudi and Masduqie (2004) reported that buffalo rumen fluid contains more cellulolytic microbes compared to other ruminants. In buffalo rumen fluid, seven colonies of cellulolytic microbia are found (group of *Ruminococcus sp*.), while in cattle there are only four colonies. The percentage of cellulolytic bacteria in cattle is 19.5% and in buffalo 42.3% of total bacteria. The main groups of cellulolytic bacteria in the rumen include *Ruminucoccus albus*, *Ruminococcus flavefaciens*, and *Bacteroides succinogenes* (Chesson and Forsberg, 1988; Suryahadi *et al*., 1996). Suryahadi *et al*. (1996) stated that the activity of cellulolytic bacteria from buffalo cattle is higher than that of cattle. Based on the various reports, the ability of buffalo fluid enzymes is thought to be better than the rumen fluid of cattle in matter of its ability to digest high fibrous feed ingredients. Some studies also reported that the use of high fibrous feed ingredients and anti-nutrients such as phytic acid in feeds can affect the weigh of carcass and digestive organs (Skomorucha *et al*., 2008; Herawati, 2010). Li *et al*. (2010) reported that the higher feed containing crude fiber will affect the enlargement and thinning of the proventricular organ causing an increase in the proventricular weight. Lesson and Summer (1997) suggested that feeding which contains high phytate will cause an increase in liver weight, because the liver must work harder to neutralize phytic acid so that it will affect carcass weight. The use of enzymes in feeds will increase digestibility, especially high-fiber feed ingredients and high phytic acid so that the quality of the feed is expected to be improved. Thus, it is expected that daily body weight gain, final body weight and carcass weight will also increase and non carcass weights such as digestive organs tend to decrease.

Based on the description above, a study was conducted on "The Performance of Broiler Chickens Fed Diet by Addition of Enzymes from the Buffalo Rumen Fluid from Animal slaughterhouses".

Materials and Methods

Enzyme extraction from buffalo rumen fluid

The contents of rumen were taken when cutting buffalo in the slaughterhouse (RPH) of the Jambi city. The rumen liquid of buffalo was taken from the rumen of buffalo by filtration (filtering with cotton fabric) under cold conditions using ice cubes. Filtered rumen liquid was then cleaned from impurities and microbial cells by modifying the use of centrifuges with a filtering method using three layers of cotton fabric as a filter which was rotated using a washing machine for 10 minutes at 4ºC.

The rumen fluid that comes out of the filter results in the form of a supernatant was then taken as a source of coarse enzymes. The supernatant consisting of these enzymes was then reacted with ammonium sulfate at a saturation level of 60% (w/v) and stirred using a stirrer for approximately 30 minutes and left overnight at 4ºC. The supernatant was then centrifuged at 4,000 rpm for 15 minutes at 4ºC. The precipitate (crude enzyme) obtained was taken then dissolved in distilled water with a ratio of 10: 1 (every 100 ml of the rumen supernatant was dissolved in 10 ml of distilled water), then the enzyme solution was used in the feed. The process of extracting rumen fluid could be seen in Figure 1.

Feed and broiler

Treatment feeds were formulated according to the needs of broilers in the NRC table (1994). The formulation of feed ingredients and nutrient content of the research feed for the starter period and finisher period were presented in Table 1.

The broiler used were one day old (DOC) as many as 200 birds, Lohman strains without sex separation (unsexed). The addition of enzymes into experimental diet was done by mixing and stirring manually before the diet was given to chicken. The enzyme used was enzyme obtained from the extraction of buffalo rumen fluid which was taken from RPH (slaughterhouses) of Animal Husbandry Service of Jambi City. The extracting method of buffalo rumen fluid could be seen in Figure 1. Enzyme activity of buffalo rumen fluid consisted of cellulase enzyme 13.6070±7.9986, amylase 4.1751±0.0927, mannanase 1.8864±0.5226 and xylanase 0.6595±0.0525 (Budiansyah *et al*., 2013).

The diet was arranged based on the broiler needs in the starter period (0-3 weeks) and the finisher period (4-6 weeks) according to the NRC Table (1994). The feed ingredients used consisted of local feed ingredients which were yellow corn, soybean meal, rice bran, fish meal, coconut meal, and other ingredients such as calcium carbonate (CaCO3), DL-methionine, L-lysine, and premix B. Treatments that were given to chicken consisted of 5 kinds of treatment feeds, that were as follows: P0 = feed without addition of rumen fluid enzyme (control), P1 = feed with addition of 0.75% rumen fluid enzyme, P2 = feed with addition of 1.5% buffalo rumen fluid enzyme, P3 = feed with the addition of 2.25% buffalo rumen fluid enzyme and P4 = feed with the addition of 3% buffalo rumen fluid enzyme.

Each treatment consisted of 4 replications, so that there were 20 units of experimental cages and each replication consisted of 10 chickens. The chickens were kept for 35 days in a cage made of wire which was equipped with a place to take feed and drinking water and a heater that came from a 40 watt electric lamp in every unit of

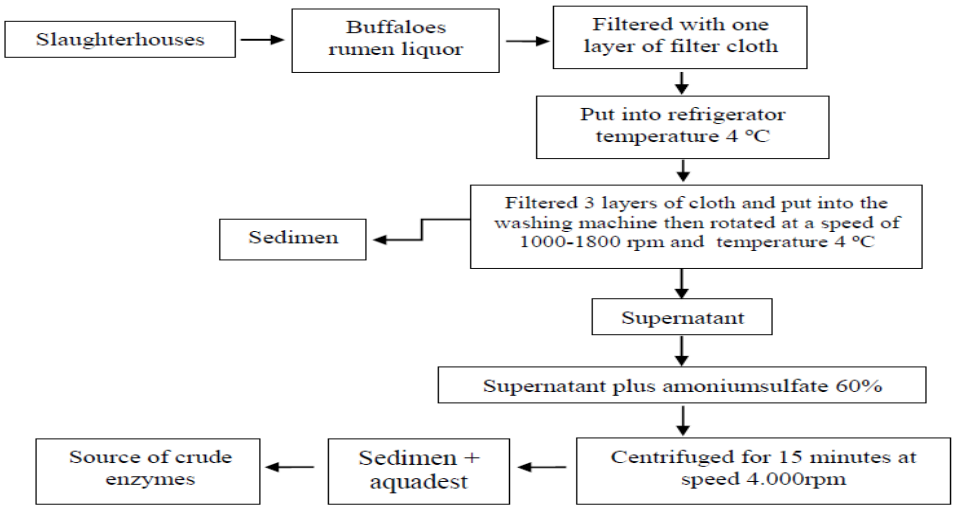

Figure 1. The cow rumen fluid extraction workflow from slaughterhouses, a modified use a washing machine (Budiansyah *et al.,* 2010).

Table 1. The composition of feed ingredients and nutrient contents of experiment diet in the starter and finisher period

Feed Ingredient %	Starter period%	Finisher period %
Yellow corn	34	34
Soybean meal	30	25
Fish meal	10	10
Coconut meal	5	10
Rice bran	16	16
Corn oil	3.5	3.5
Premix	0.2	0.5
$CaCO_3$	0.8	0.5
DL- methionine	0.25	0.25
L- lysine	0.25	0.25
Nutrient contents of the experiment diet	Starter period %	Finisher period %
(Dry matter* %	89.04	88.99
Ash* %	8.22	8.06
Crude protein* %	24.59	22.38
Crude fat* %	4.6	4.65
Crude fiber* %	5.15	3.45
Calcium* %	0.75	0.17
Phosphorus* %	0.60	0.36
Metabolic energy** kcal/kg	3,105.45	3,069.70

Explanation: * Analysis results at Laboratory of Animal Feed Nutrition, Faculty of Animal Science, Jambi University, 2017)
** The calculation results.

the cage. The cage was also equipped by thermometer with celsius degree to determine the temperature conditions in the cage. Feeding was started at DOC, according to treatment and drinking water was available *ad libitum*. At the end of the study, two chickens were taken from each cage to be slaughtered and analyzed its carcasses and digestive organs.

The variables observed were feed consumption, daily body weight gain, feed conversion, slaughter weight, carcass weight (absolute and relative), weight of digestive organs (liver, proventriculus, ventriculus and pancreas), weight and length of broiler's small intestine.

The design used in this study was a completely randomized design. To analyze the treatment effect on the observed variables, the data obtained were analyzed by Variant analysis according to the design used with the linear model as follows:

$$Y_{ij} = \mu + \rho 1 + €_{ij}$$

Where :
Y_{ij}= response to observations result that received i treatment i and j replication
μ= average general population
ρ1= the effect of the i treatment
$€_{ij}$= the effect of the error on the j replication unit and i treatment

Result and Discussion

The measurement results of feed consumption could be seen in Table 2. The results of the variant analysis showed that the use of buffalo rumen fluid enzymes in the broiler chicken feed in the starter period and finisher period had no significant effect (P>0.05) on feed consumption. This result was similar to the results of a study reported by Budiansyah (2010a)

regarding the use of cow rumen fluid enzymes in broiler chickens that the addition of cow rumen fluid enzyme did not cause an increase or decrease in consumption of broiler chicken feeds. This was caused by the addition of buffalo rumen fluid enzymes did not change the composition of the nutrient in the diet, especially metabolic energy content. Although buffalo rumen fluid enzymes had high enzyme activity of carbohydrase enzymes and good enzyme characteristics, the addition of these enzymes did not have much effect on the energy content of the feed and the energy content in all treatments was relatively the same. Wahju (1988) suggested that feed consumption was strongly influenced by the energy content in the feed, high metabolic energy content in the feed led to decreased feed consumption and conversely the low metabolic energy content in the feed would lead to increased consumption of feeds. In addition, the addition of buffalo rumen fluid enzymes did not change the shape and colour of the feeds and feeds given to all treatments in this study, relatively similar to mash (flour). As reported by Hussain *et al*. (2012) that the physical form of the feed affected the amount of feeds consumed, and poultry tended to consume feeds in the form of crumble and pellets. The average consumption of starter period feeds in this study ranged from 240.26 to 637.07 g/bird/week. The finisher period ranged from 605.55 to 618.52 g/bird/week. The results of this study were lower than the research of Sumiati *et al*. (2011) who stated that the average consumption of starter feed ranged from 154.40-451.10 g/bird/week and the finisher period ranged from 380.60-2,188.80 g/bird/week.

Based on the results of variance analysis showed that effect of the use of buffalo rumen fluid enzymes in feeds on daily body weight gain, feed conversion, slaughter weight, absolute carcass weight and relative carcass weight of 35 days broiler chickens had no significant effect (P> 0.05) (Table 3).

This indicated that the increase in the level of enzyme use to 3% in feed resulted in daily body weight gain, feed conversion, slaughter weight and carcass weight relatively the same as the control. This condition was thought to be caused by several things, including the consumption of the same feed between treatments. Fanatico *et al*. (2005) stated that daily body weight gain and slaughter weight were closely related to feed consumption, increasing feed consumption would increase the daily body weight gain and final body weight or slaughter weight and vice versa. Mehri *et al*. (2010) and Vahjen *et al*. (2005) state that slaughter weight was influenced by the amount of feed consumed and the quality of feeds. The average slaughter weight of this study results ranged from 1219.75 - 1285.37 (g/bird). This result was higher than the study results reported by Durrani *et al.* (2006) that the average slaughter weight of 5-week-old broiler chicken ranged from 1012 g/ bird. Compared to the results of the study by Durrani *et al* (2006) that the results of this study were better. Gunal *et al*. (2006) reported that the average slaughter weight of 5-week-old broiler chicken ranged from 2,333.25±19.38 g/bird. The results of this study were lower than those reported by Gunal *et al*. (2006) This was caused by the amount of feed consumption in this study was lower.

There were no significant effect on daily body weight gain, feed conversion, slaughter weight and carcass weight in this study, it was assumed that the feeds given had good quality and easy to digest so that the addition of enzymes from buffalo rumen was not needed in order to increase digestibility of feed substances. It could be seen that the feed given contained less than 6% crude fiber, while the crude protein content was 24.59% in the starter period and 22.38% in the finisher period which was relatively high. NRC (1994) recommended that the protein content of the starter period was 21% and the finisher period

Table 2. The effect of using buffalo rumen fluid on feed intake

Treatments (%)	Starter period (g/bird)	Finisher period (g/bird)
P0 (0.0%)	253.68 ± 14.69	615.38 ± 6.39
P1 (0.75%)	263.07 ± 0.83	606.73 ± 8.11
P2 (1.5%)	259.10 ± 7.14	605.55 ± 4.55
P3 (2.25%)	240.26 ± 20.07	608.34 ± 28.93
P4 (3.0%)	252.32 ± 12.16	618.57 ± 17.55

P0 = feed without addition of rumen fluid enzymes 0% (control); P1= feed with addition of rumen fluid enzymes 0.75% ; P2= feed with addition of rumen fluid enzymes 1.5%; P3 = feed with addition of rumen fluid enzymes 2.25%); P4= feed with addition of rumen fluid enzymes 3%.

Table 3. Effect of the use of rumen fluid enzymes in diets on slaughter weight, daily weight gain, feed conversion ratio, absolute carcass weight, relative carcass weight of broiler chicken

Variable	Treatments				
	P0	P1	P2	P3	P4
Slaughter weight (g)	1,219 ± 55.65	1,281 ± 84.51	1,257 ± 72.78	1,285 ± 79.14	1,272 ± 58.72
Average daily weight gain (g)	33.77±1.59	35.52±2.41	34.84±2.07	35.64±2.26	35.25±1.67
Feed conversion ratio	1.69±0.06	1.62±0.11	1.64±0.10	1.56±0.13	1.62±0.07
Absolute carcass weight) (g)	839.12 ± 55.90	871.62 ± 53.49	866.62 ± 56.01	846.50 ± 72.23	868.87 ± 37.38
Relative carcass weight (%)	68.75± 2.19	68.06 ± 0.84	68.91 ± 0.80	65.82 ± 2.93	68.33 ± 1.91

P0 = feed without addition of rumen fluid enzymes 0% (control); P1= feed with addition of rumen fluid enzymes 0.75% ; P2= feed with addition of rumen fluid enzymes 1.5%; P3 = feed with addition of rumen fluid enzymes 2.25%); P4= feed with addition of rumen fluid enzymes 3%.

was 23%. The feed ingredients used were materials which were commonly used in the preparation of poultry feeds and generally contained low crude fiber. Crude fiber was a substance that was often became a limiter in the preparation of poultry feeds. Zhao *et al*. (2011) reported that daily body weight gain and final body weight or poultry slaughter weight were influenced by the quality and quantity of food provided. The higher quality of feed ingredients given, the higher the performance of broiler chickens produced.

Based on the variant analysis results, the use of buffalo rumen fluid enzymes in the feed had no significant effect (P> 0.05) on absolute carcass weight. This was thought to be due to carcass weight was depended on slaughter weight, and all treatments that use enzyme produced carcass weights that were relatively the same and in line with slaughter weight. This was in accordance with the opinion of Haroen (2003) who stated that carcass production was closely related to the slaughter weight because the more slaughter weight, the carcass weight would increase. The average absolute carcass weight in this study was 839.12-871.62 g/bird/week lower than the results of Sofyan *et al*. (2012), that were carcass weight of five week old broilers 1,912.90±55.50 g/bird /week. The carcass weight that was not different between treatments was related to slaughter weight and feed consumption which did not differ between the treatment of enzyme use 0.75%, 1.5%, 2.25% and 3%. Nahashon *et al*. (2005) reported that carcass weight was strongly influenced by the weight of life produced. The higher the live weight, the higher the carcass weight and vice versa.

The results of the variant analysis in Table 3 showed that the use of buffalo rumen fluid enzymes had no significant effect (P>0.05) on relative carcass weight. The study results showed a non-significant effect (P> 0.05) on relative carcass weight. This meant that the use of enzymes from buffalo rumen fluid to the level of 3.0% did not interfere or did not improve the growth of broilers in forming body tissue, especially for the formation of meat and bone which could be seen from the slaughter weight and absolute carcass weight obtained.

The average relative carcass weight in this study ranged from 65.82 to 68.91%, this number was in accordance with the opinion of Skomorucha *et al*. (2008) who stated that the average carcass weight of chickens ranged from 65.45 to 67.13% of life weight. The results of relative carcass weight percentage in this study were quite good, this was supported by Boskovic *et al*. (2012) who stated that the normal carcass weight for roosters was 75.56% -76.56% while for female chicken 65.45%-67.13% of body weight.

The same relative carcass weight generated from treatment feeds P0, P1, P2, P3, and P4 was probably due to the speed of tissue growth, carcass and non carcass (fur, viscera) were relatively balanced. Broilers needed feed substances that were suitable with their needs, especially for the growth their body tissues.

The results of the variant analysis in Table 4 showed that the use of buffalo rumen fluid enzymes had no significant effect (P> 0.05) on digestive organs (proventricular weight, ventricular weight, intestinal weight and length, liver weight, pancreatic weight). it meant the use of buffalo rumen fluid to the level of 3% as a source of enzymes did not cause interference with the work of all digestive organs.

The Proventrikulus works to secrete enzymes and HCL (hydrochloric acid) and wet food with enzymes and HCL so that when food enters ventriculus, the food gets wet and soft and makes it easier for the ventriculus to grind food. Proventriculus weight that did not differ between treatments, this was similar to that reported by Wu and Ravindran (2004) that the treatment of the addition of xylanase and phytase enzymes both individually and in combination did not affect the proventricular or gizzard weights. It was suspected that the amount of rumen fluid enzyme used was at a low level. The same physical form of feed in the form of mash, was thought to be the cause of the proventricular and ventricular (gizzard) weights was not different in all treatments. This result was confirmed by the opinion of Li *et al*. (2010) who stated that the factors that affect the proventriculus weight were crude fiber. Li *et al*. (2010) reported that the higher the feed containing crude fiber would affect the enlargement and thinning of the proventricular organ, causing an increase in the proventricular weight. Garriga *et al*. (2006) reported that the factors that influence the weight of the proventriculus were feed, age, strain and genetic herds. The average percentage of the

Table 4. Effect of the use of rumen fluid enzymes in diets on digestive organs (proventriculus weight, ventriculus weight, weight and length of the small intestine, liver weight and weight of the pancreas) of broiler chick*en*

Variable	Treatments				
	P0	P1	P2	P3	P4
Proventriculus weight (%)	0.53±0.178	0.53±0.079	0.44±0.636	0.44±0.059	0.54±0.077
Ventriculus weight (%)	1.9±0.396	1.9±0.964	2.1±0.221	2.1±0.107	2.0±0.214
Small intestine weight (%)	3.9±0.3095	3.9±0.4203	3.9±0.4760	3.6±0.5058	3.7±0.7788
length of the small intestine (cm)	141.9±0.85	142.4±1.43	142.4±1.43	141.1±1.60d	140.4±0.25
Liver weight (%)	2.4±0.477	2.2±0.199	2.4±0.452	2.3±0.161b	2.2±0.168b
Pancreas weight (%)	0.3±0.039	0.3±0.040	0.3±0.059	0.3±0.020	0.4±0.025

P0 = feed without addition of rumen fluid enzymes 0% (control); P1= feed with addition of rumen fluid enzymes 0.75% ; P2= feed with addition of rumen fluid enzymes 1.5%; P3 = feed with addition of rumen fluid enzymes 2.25%); P4= feed with addition of rumen fluid enzymes 3%.

proventriculus weight produced in this study was 0.44%-0.54% of body weight, the results of this study were slightly lower than Basir and Toghyani's study (2017) 0.71±0.02% of body weight that used waste lemon (*Citrus aurantifulia*) to the performance of broiler chickens.

The results of the variant analysis showed that ventricular weight was relatively the same (not significantly different). The average weight of ventriculus in this study ranged from 1.9-2.1% of body weight, this number was in accordance with the opinion of Rahman *et al*. (2005); Ghorbani *et al*. (2009); Gracia *et al*. (2003) and Shirzadi *et al*. (2009) who stated that ventricular weight of broiler chickens ranged from 1.6 to 2.5% of body weight. This was because the physical form of the feed for each treatment was relatively the same so that the ventriculus was not different between treatments in carrying out activities to grind and destroy food particles into smaller particles. Amerah *et al*. (2007) stated that ventriculus work in grinding and refining food was influenced by the physical form and texture of the feed. The crude fiber content of treatment feed was still within the normal range so that it did not affect ventricular work, the limit of crude fiber content in broiler chicken feed was maximum 6% (Wu and Ravindran, 2004). The role of enzymes was relatively small because the muscles of the stomach work more dominantly to grind food rather than hydrolyze, so the weight ventriculus that was obtained was relatively the same.

The results showed that the treatment of buffalo rumen fluid enzyme addition in the feed had no significant effect (P> 0.005) on the intestine weight and length. These results indicated that the use of buffalo rumen fluid enzymes in the feed produced the same small intestine weight and length between treatments. This meant that the addition of enzymes did not affect the function and work of broiler small intestine. The results were contrary to the reports of Wu and Ravindran (2004) and Akyurek *et al*. (2009). Wu and Ravindran (2004) reported that the addition of xylanase and phytase enzymes to wheat-based feeds reduced the length of the small intestine by 15%, while the duodenal parts decreased by 17.8%, jejeunum was reduced by 15.8% and the ileum was reduced by 14.6%. This result was supported by the report of Akyurek *et al*. (2009) that the addition of enzymes (multi cellulase enzymes, β-glucanase and xylanase) which was combined with phytase not only significantly reduced the relative length of the duodenal digestive tract, jejunum, ileum and cecum, but also decreased the weight of these parts. Decreasing the size and weight of the digestive tract was caused by an increase in the digestion process of carbohydrates by exogenous enzymes. No significant difference in the weight and length of the small intestine in this study, it was thought that this was due to the addition of buffalo rumen fluid enzymes in feed was still at a low level and the secretion of endogenous enzymes was not affected by the addition of exogenous enzymes. Experimental feed which was given was relatively similar and crude fiber content in the treatment feed was still within the normal range so it did not affect the work of the small intestine in digesting and absorbing food substances. Though the addition of rumen fluid enzymes was thought to be able to help digestion and absorption of protein, if the absorption was a much it would affect the weight of the intestine and the length of the intestine that would increase. Al-Kassie (2009) stated that the small intestine functioned as a place of digestion and absorption of food substances, where the small intestine wall secreted liquid containing pepsin which functioned to digest proteins which were then absorbed, some of the food consumed would be absorbed in the small intestine.

Hernandez *et al*. (2004) stated that the increase in intestinal weight was due to increase in intestinal work in digesting a number of feeds, so that the intestines work harder in digesting. In fact, the addition of buffalo rumen fluid enzymes did not affect digestion and absorption, which was seen in the slaughter weight produced that was relatively the same. Thought digestion and absorption of food substances due to the addition of buffalo rumen fluid enzymes that was carried out occured less, because the role of enzymes in digestion and absorption was relatively small, so the weight and length of the small intestine produced was relatively the same. This result was supported by the opinion of Amerah *et al*. (2007) who stated that the length and weight of the small intestine varied depending on the physical form of the feed consumed, it would affect the digestion and absorption of food substances.

The results showed that intestinal weights obtained were 3.6-3.9% and intestinal length 140.4-142.4 cm, lower than the study of Ibrahim *et al*. (2000) where intestinal length ranged from 151.87 to 165.62 cm. Abidin (2010) intestinal weight ranged from 2.43-3.05%, almost the same as the statement Hernandez *et al*. (2004) that the relative weight of the small intestine in broilers was 2.45g/100g slaughter weight. Supported by the opinion of Ortatali *et al*. (2005) several factors that affect the length and weight of the small intestine, one of which was the quality of the feed.

The results of the variant analysis showed that the addition of buffalo rumen fluid enzymes to the level of 3% showed no significant effect (P>0.05) on the liver and pancreas weight of the broiler chicken. This showed that the addition of buffalo rumen fluid enzyme did not affect the weight of the liver and pancreas of broiler chickens. This was contrary to the reports of several researchers. Nadeem *et al*. (2005) reported that the addition of enzymes in broiler chicken feed reduced liver weight, whereas Ghorbani *et al*. (2009), Rahman *et al*. (2005) and Gracia *et al*. (2003) reported that the addition of enzymes in the feed did not affect liver weight. The similarity in liver weight between treatments in this study was thought to be due to the amount of phytic acid and other anti-nutrients in all

treatments was relatively the same, so that the liver weight was relatively the same. Ortatali *et al*. (2005) stated that feeding which contained high phytate would cause an increase in liver weight, as a result of the liver having to work harder in neutralizing phytic acid. Resmi *et al*. (2013) reported that feed ingredients that made up feeds containing phytic acid consisted of polish (6.37±0.341%), soybean meal (6.37±0.29%), palm kernel cake (5,83±1.28%) and fine bran of (6.75 ± 0.86%), the addition of rumen fluid enzyme was only able to reduce phytic acid levels in fine bran 14.73%, palm kernel cake 46.69%, polish 29.94%, and soybean meal 19.67%, so that only a small portion of phytic acid could be digested. Resmi *et al*. (2013) reported that the role of cattle rumen fluid enzymes did not appear to be significant in palm kernel meal, polish, fine bran, and soybean meal. The this possibility also occured in the use of buffalo rumen fluid enzymes in this study, consequently the ability of enzymes to digest phytic acid was low, so this caused the weight of the liver was not significantly different. In this study liver weight ranged from 2.2 to 2.4% of body weight. The weight produced was higher compared to the opinion of Kumar and Balachandran (2009) that liver weight in poultry reaches 25-35 g or 1.7-2.3% of live weight.

The results of the variant analysis showed that the use of buffalo rumen fluid enzymes in the treatment feed had no significant effect (P>0.05) on the weight of the pancreas. This result was different from that reported by Gracia *et al*. (2003) that the addition of amylase enzyme to corn-based feeds and soybean meal reduced the weight of the pancreas. Decreased pancreatic weight was thought to be due to decreased endogenous amylase secretion by the pancreas due to exogenous amylase. It is known that the pancreas works to secrete amylase and protease enzymes (trypsin and chymotrypsin). The results of this study did not showed a tendency to decrease or increase the weight of the pancreas. This showed that the addition of buffalo rumen fluid enzymes to broiler feeds did not affect the pancreas in secreting enzymes, this could be seen from the similarity weight of the pancreas that was obtained. It was suspected that pancreatic secretions in the form of enzymes were relatively stable not affected by the addition of rumen fluid enzymes, thus the worked of the pancreas was relatively stable and produced relatively similar pancreatic weights. One of the functions of the pancreas was to secrete amylase, protease and lipase enzymes that help the digestion process of carbohydrates, proteins and fats. The pancreas also produced lipolytic, amylolytic and proteolytic enzymes (Basir and Toghyani, 2017). In this study the pancreatic weight obtained 0.3-0.4% did not have a significant effect (P>0.05) on the weight of the pancreas. This was not different when compared to that obtained by Basir and Toghyani (2017) who obtained the results of pancreatic weight percentage in the range of 0.39 ± 0.04%.

Conclusions

Based on the results of this study, it can be concluded that the use of buffalo rumen fluid enzymes from slaughterhouses in the diet to the level of 3% did not increase feed consumption, slaughter weight, body weight gain, feed conversion, absolute carcass weight, relative carcass weight, and digestive organs of broiler.

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