# The Visceral Organ, Gastrointestinal Tract and Blood Characteristics in Pegagan Ducks Fed Ration Fermented by Tape Yeast with Different Moisture Content

*by* Miksusanti Salbi

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# The Visceral Organ, Gastrointestinal Tract and Blood Characteristics in Pegagan Ducks Fed Ration Fermented by Tape Yeast with Different Moisture Content

**[1]Fitra Yosi, [1]Sofia Sandi and [2]Miksusanti**

*[1]Department of Animal Science, Faculty of Agriculture, University of Sriwijaya, South Sumatera, Indonesia*
*[2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Sriwijaya, South Sumatera, Indonesia*

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Corresponding Author:
Fitra Yosi
Department of Animal Science, Faculty of Agriculture, University of Sriwijaya, South Sumatera, Indonesia
E-mail: fitrayosi@unsri.ac.id

**Abstract:** The purpose of this study was to assess the effect of administration diet containing locally sourced materials fermented by tape yeast with different moisture content on the weight of the visceral organs, gastrointestinal tract and blood chemistry and profile of Pegagan ducks. As many as 200 Pegagan ducks aged 3 days were kept for 6 weeks in colony cages. This study used a completely randomized design that consisted of 5 treatments and 4 replicates. The treatments tested were symbolized as P1, P2, P3, P4 and P5 based on the level of moisture content used in the fermentation process of ration, namely control, 40, 50, 60 and 70%. Data were analyzed by analysis of variance continued by Duncan's multiple range test at 5%. The weight of gizzard, pancreas, crop-oesophagus, proventriculus, the total of small intestine and ceca were significantly ($p<0.05$) affected by treatment, but did not significantly affect ($p>0.05$) on the weight of bile, liver, spleen, heart, duodenum, jejunum, ileum and rectum. Furthermore, blood cholesterol, triglyceride and LDL of Pegagan ducks were significantly ($p>0.05$) different. It can be concluded that the fermentation process of locally sourced ration using a moisture content of 50% gives the optimal result on the weight of visceral organ, gastrointestinal tract and also blood chemistry and profile of Pegagan ducks.

**Keywords:** Blood, Gastrointestinal Tract, Moisture Content, Tape Yeast, Visceral Organ

## Introduction

The use of local ingredients in reducing the cost of feed is widely utilized. However, their use for poultry is limited because of a high crude fiber content. This is due to poultry has limitation to digest crude fiber, such as cellulose and hemicelluloses (Yosi *et al*., 2016). Therefore, it needs a feed processing technology, such as fermentation, to reduce the fiber content and increase the nutrient digestibility. Yeast is a natural ingredient that is widely used as an inoculum in fermentation processes of poultry feedstuff (Khempaka *et al*., 2014; Sandi *et al.,* 2016; Yosi *et al*., 2016). In addition, the yeast is also used as a feed supplement in poultry rations that can stimulate the growth process (Yalçın *et al*., 2013). The use of yeast in the feed has been widely studied and have a significant effect on performance and physiological responses to poultry, such as increasing the immune response (Asli *et al*., 2007; Yalçın *et al*., 2010; Haldar *et al*., 2011), lowering the blood cholesterol levels (Yalçın *et al*., 2010) and increasing the digestibility of nutrients (Gao *et al*., 2008).

One type of yeast that could potentially be used is the tape yeast (Yosi *et al*., 2016). The tape yeast contains *Saccharomyces cerevisiae* (Bidura *et al*., 2012), which is able to produce enzymes to digest complex compounds, such as cellulose and hemicellulose and produce simple monosaccharide compounds. It is reported that one affects the growth of *S. cerevisiae* during the fermentation process is the moisture content (Rohmawati *et al*., 2015). In the process of fermentation solids, moisture content used is in the range 30-80%. If the moisture content is too low, microbial metabolism is inhibited and growth will be disturbed. Conversely, if the moisture content is too high, the porosity of the substrate becomes lower.

Consequently, aeration and mass transfer processes in the metabolism of microbes are inhibited.

By knowing the optimal moisture content in the fermentation process of locally sourced rations, it is expected that the growth of *S. cerevisiae* to be better so that the nutritional digestibility of the ration is increasing. It is known that the high nutrient digestibility will affect the activity of visceral organs and digestive tract, which can be measured by weight changes (Hetland *et al*., 2005). In addition, this will also affect the increase of erythrocyte numbers, hemoglobin and hematocrit (Huff *et al*., 2010), as well as stimulate the immune system to generate more antibodies (Shanmugasundaram *et al*., 2013). Based on this, further studies to find out the effect of feeding the local ration fermented by tape yeast with different moisture content on bird's performances need to be implemented.

## Materials and Methods

### *Birds, Feed and Housing*

A total of two hundred of Pegagan ducks aged 3 days was allocated into 5 treatments of experimental fermented rations and kept for 6 weeks. All the ducks were placed in 20 plots (l × w × h; 1.5 × 1.2 × 0.8 m) made of bamboo, with containing 10 ducks per plots. Each plot is equipped with 40-watt bulb lamp and switched on for 24 h as a heater until the age of 2 weeks. After 2 weeks, the position of the lights is raised and used only for illumination at night. Rations used is feed by using local raw materials, including refined corn meal, palm kernel cake, coconut pulp, cassava leaf meal, water hyacinth meal, lamtoro leaf meal, kale leaf meal, snail meal, egg shell meal and premix. The composition and nutrient content of the ration can be seen in Table 1.

### *Method of Fermentation*

The treatments ration was symbolized as P1, P2, P3, P4 and P5 based on the level of moisture content used in the fermentation process of ration, namely control (without adding water), 40, 50, 60 and 70%. Determination of water content in the ration according to (AOAC, 1995). The fermentation process of ration refers to Bidura *et al*. (2009) modified. The first stage was the ration was inserted into the container, then added to warm water (50-60°C) to each ration treatment in accordance with a certain amount. The mixture of feed and water was stirred until uniform, then aerated for 5 min. After that, the tape was inserted into the yeast ration as much as 0.3% of the weight ration and stirred until blended. The ration was then covered with plastic and stored for 7 d for aerobic fermentation. After 7 d, the ration is put into the oven at 45°C for 6 h. Rations and drinking water are given ad libitum. In this study, tape yeast used is brand "Ragi Tape NKL", which is produced locally and is commonly used in the manufacture of Indonesian food called "Tape".

Table 1. Composition and nutrient content of the experimental ration

| Feed ingredients | % material (w/w) |
|---|---|
| Refined corn meal | 57.00 |
| Coconut pulp | 5.00 |
| Palm kernel cake | 4.00 |
| Snail meal | 17.00 |
| Lamtoro leaf meal | 5.00 |
| Cassava leaf meal | 4.00 |
| Water hyacinth meal | 3.00 |
| Kale leaf meal | 4.00 |
| Eggshell meal | 0.50 |
| Premix | 0.50 |
| Total | 100.00 |
| Nutrient content of ration | |
| Crude Protein (%) | 17.27 |
| Crude Fat (%) | 7.04 |
| Crude Fiber (%) | 9.59 |
| ME (kcal/kg) | 2921.39 |
| Ca (%) | 0.73 |
| P (%) | 0.30 |
| Methionine (g/100g) | 0.21 |
| Lysine (g/100g) | 0.53 |

### *Measurement of the Weights of Visceral Organ and Gastrointestinal Tract*

A total of 10 ducks on each treatment, at the end of the experiment, was randomly selected as many as 2 birds. Ducks were then individually weighed and slaughtered, feathered and eviscerated. The weights of the gizzard, spleen, liver, pancreas, bile and heart were calculated and expressed as a percentage of BW (Rahbar *et al*., 2011). The empty weight of crop-esophagus, proventriculus, intestinal, duodenum, jejunum, ileum, cecum and rectum parts were recorded. Duodenum was measured from gizzard outlet to the end of the pancreatic loop, jejunum was from the pancreatic loop to Meckel's diverticulum and ileum was from Meckel's diverticulum to the cecal junction. The weight of gastrointestinal tracts parts to slaughtering weight was calculated and expressed as a percentage (Yalçın *et al*., 2013)

### *Analysis of Blood Profile and Chemistry*

As many 3 ml of venous blood samples from 2 birds per pen, at the end of experiment, were collected by puncture of the brachial vein using sterilized syringes containing an anticoagulant (Ahmad *et al*., 2008). Then, the syringes were capped and carried to the laboratory for counting the number of erythrocytes, leukocyte, hemoglobin, hematocrit, Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC). Blood samples were put into the tubes containing no anticoagulant and then centrifuged at 3.220 × g for 8 min at 4°C. Serum

was taken and stored at -20°C (Yalçın *et al*., 2013) for testing of triglyceride, cholesterol, Low-Density Lipoprotein (LDL) and High-Density Lipoprotein (HDL).

### *Statistical Analysis*

The data were Analyzed with Analysis of Variance (ANOVA) using the SPSS software with version 17. The significance of mean differences among treatments was tested by Duncan's multiple range test at 5% of a significance level.

## Results

### *The Weight of Gastrointestinal Tract and Visceral Organ*

Based on Table 2, the treatment significantly ($p<0.05$) affected the weight of the crop-oesophagus, proventriculus, total of small intestine and ceca, but not significantly ($p>0.05$) affected the weight of duodenum, jejunum, ileum and rectum. The weight of crop-oesophagus, total of the small intestine and ceca of ducks fed rations fermented with a moisture content of 50% (P3) was significantly ($p<0.05$) the highest among the other treatment ration, namely 1.30, 3.09% and 0.48%, respectively. Meanwhile, the ducks consumed rations fermented with moisture content above 50% produced the weight of the proventriculus and small intestine that was significantly ($p<0.05$) higher than that under 50%. However, the weight of the proventriculus of ducks fed rations fermented with 50-70% of moisture content was not significantly different ($p>0.05$), which was 0.78 to 0.81%.

The different water content fermentation process also significantly affected ($p<0.05$) the weight of some organs, such as the gizzard and pancreas (Table 3). Moreover, the gizzard and pancreatic weights on ducks fed ration fermented with moisture content above 50% were significantly ($p<0.05$) higher than that under 50%. However, the weight of the bile, liver, spleen and heart in this study was not significantly ($p>0.05$) affected by treatment.

### *Blood Profile and Chemistry*

Based on Table 4, it can be observed that the treatment significantly ($p<0.05$) effected on the number of leukocytes, but the number of erythrocytes, hematocrit, hemoglobin, MCV, MCH and MCHC was not significantly ($p>0.05$) affected by the treatment. The number of leukocytes in ducks consumed rations fermented with a moisture content of 70% was significantly ($p<0.05$) the highest among the other treatment ration, i.e., 35,700 per $mm^3$. Furthermore, the ducks fed rations of fermented with a moisture content of 40 to 60% had a number of leukocytes, which was not significant ($p>0.05$).

The administration of a ration fermented by tape yeast with a different water content had a significant difference ($p<0.05$) on the level of cholesterol, triglycerides and LDL in the blood of Pegagan ducks, but had not a significant difference ($p>0.05$) on the HDL levels in the blood (Table 5). Moreover, levels of blood cholesterol and LDL of ducks fed rations fermented with moisture content above 50% were significantly ($p<0.05$) lower than that under 50%. If the water level used in fermentation exceeds 50%, the levels of blood cholesterol produced did not differ significantly. The triglyceride level in the blood of ducks fed rations fermented with a water content of 70% was significantly ($p<0.05$) the lowest among other treatments, which is 133.75 mg $dL^{-1}$. The HDL in the duck's blood that was consumed rations fermented with 40-70% moisture content was relatively equal, i.e. 35.50 to 38 mg/dl.

Table 2. The mean of the weight of the gastrointestinal tract of Pegagan ducks aged 6 weeks

| | Treatments | | | | |
|---|---|---|---|---|---|
| Variable | P1 | P2 | P3 | P4 | P5 |
| Gastrointestinal tract (%): | | | | | |
| Crop-oesophagus | $0.93^{a}\pm0.08$ | $0.97^{a}\pm0.04$ | $1.30^{c}\pm0.09$ | $1.10^{b}\pm0.08$ | $0.90^{a}\pm0.07$ |
| Proventriculus | $0.64^{a}\pm0.11$ | $0.61^{a}\pm0.05$ | $0.81^{b}\pm0.08$ | $0.78^{b}\pm0.10$ | $0.81^{b}\pm0.08$ |
| Duodenum$^{ns}$ | 0.43±0.180 | 0.40±0.050 | 0.60±0.190 | 0.48±0.050 | 0.50±0.030 |
| Jejunum$^{ns}$ | 0.96±0.140 | 1.06±0.230 | 1.22±0.070 | 1.00±0.150 | 1.02±0.280 |
| Ileum$^{ns}$ | 1.00±0.110 | 1.03±0.070 | 1.28±0.180 | 1.14±0.120 | 1.15±0.160 |
| Total of small intestine | $2.39^{a}\pm0.33$ | $2.49^{a}\pm0.23$ | $3.09^{b}\pm0.28$ | $2.63^{ab}\pm0.31$ | $2.68^{ab}\pm0.34$ |
| Ceca | $0.35^{a}\pm0.03$ | $0.37^{a}\pm0.08$ | $0.48^{b}\pm0.05$ | $0.32^{a}\pm0.060$ | $0.40^{ab}\pm0.05$ |
| Rectum$^{ns}$ | 0.48±0.07 | 0.55±0.060 | 0.68±0.260 | 0.64±0.1400 | 0.53±0.080 |

Description: The same superscripts in the same column indicate significantly different ($p<0.05$). ns = non significant. P1 = Locally sourced ration fermented without adding water (control). P2, P3, P4 and P5 = locally sourced ration with a moisture content of 40, 50, 60 and 70%, respectively

Table 3. The mean of the weight of visceral organs of Pegagan ducks aged 6 weeks

| | Treatments | | | | |
|---|---|---|---|---|---|
| Variable | P1 | P2 | P3 | P4 | P5 |
| Visceral organs (%): | | | | | |
| Gizzard | 4.69$^{a}$±0.220 | 5.13$^{a}$±0.330 | 5.78$^{b}$±0.35 | 5.77$^{b}$±0.63 | 5.74$^{b}$±0.19 |
| Bile$^{ns}$ | 0.34±0.120 | 0.35±0.040 | 0.34±0.130 | 0.33±0.060 | 0.351±0.09 |
| Liver$^{ns}$ | 3.66±0.750 | 3.26±0.670 | 3.78±0.350 | 3.63±0.810 | 3.61±0.550 |
| Pancreas | 0.42$^{a}$±0.04 | 0.43$^{a}$±0.04 | 0.57$^{b}$±0.07 | 0.50$^{ab}$±0.09 | 0.56$^{b}$±0.04 |
| Spleen$^{ns}$ | 0.07±0.04 | 0.09±0.02 | 0.13±0.07 | 0.14±0.070 | 0.16±0.040 |
| Heart$^{ns}$ | 0.75±0.06 | 0.70±0.09 | 0.82±0.12 | 0.80±0.110 | 0.84±0.050 |

Description: The same superscripts in the same column indicate significantly different ($p<0.05$). ns = non significant. P1 = Locally sourced ration fermented without adding water (control). P2, P3, P4 and P5 = locally sourced ration with a moisture content of 40, 50, 60 and 70%, respectively

Table 4. Mean values of blood profiles of Pegagan ducks aged 6 weeks

| | Treatments | | | | |
|---|---|---|---|---|---|
| Variable | P1 | P2 | P3 | P4 | P5 |
| ∑ erythrocytes ($10^{6}$/mm$^{3}$)$^{ns}$ | 2.75±0.0600 | 2.60±0.180 | 2.50±0.200 | 2.53±0.100 | 2.68±0.100 |
| ∑ Leukocytes ($10^{3}$/mm$^{3}$) | 29.90$^{a}$±2.50 | 29.40$^{a}$±2.72 | 29.80$^{a}$±3.02 | 31.00$^{a}$±0.88 | 35.70$^{b}$±0.74 |
| Hematocrit (%)$^{ns}$ | 36.00±1.410 | 34.00±1.830 | 33.50±2.520 | 32.38±1.970 | 35.75±1.000 |
| Hemoglobin (g/dl)$^{ns}$ | 11.35±0.240 | 10.93±0.640 | 10.70±0.470 | 10.64±0.290 | 11.40±0.440 |
| MCV (fl)$^{ns}$ | 130.90±3.26 | 130.88±2.17 | 134.09±3.93 | 128.20±5.47 | 133.71±3.76 |
| MCH (pg)$^{ns}$ | 41.28±0.870 | 42.05±0.500 | 42.90±1.710 | 42.14±0.520 | 42.70±3.100 |
| MCHC (%)$^{ns}$ | 32.23±0.220 | 32.13±0.180 | 32.01±0.280 | 32.37±0.590 | 32.11$^{ab}$±2.16 |

Description: The same superscripts in the same column indicate significantly different ($p<0.05$). ns = non significant. P1 = Locally sourced ration fermented without adding water (control). P2, P3, P4 and P5 = Locally sourced ration with a moisture content of 40, 50, 60 and 70%, respectively

Table 5. Mean values of blood chemistry of Pegagan ducks aged 6 weeks

| | Treatments | | | | |
|---|---|---|---|---|---|
| Variable | P1 | P2 | P3 | P4 | P5 |
| Cholesterol (mg/dl) | 146.25$^{a}$±2.87 | 145.25$^{a}$±2.87 | 143.00$^{ab}$±2.16 | 140.50$^{b}$±3.42 | 139.75$^{b}$±0.74 |
| Triglyceride (mg/dl) | 153.25$^{a}$±1.89 | 152.00$^{a}$±6.48 | 152.25$^{a}$±5.12 | 152.75$^{a}$±6.95 | 133.75$^{b}$±4.11 |
| LDL (mg/dl) | 84.75$^{a}$±2.870 | 83.25$^{a}$±5.910 | 76.75$^{b}$±2.990 | 77.25$^{b}$±4.350 | 77.00$^{b}$±2.160 |
| HDL (mg/dl)$^{ns}$ | 38.00±3.160 | 36.50±1.290 | 35.50±4.2000 | 36.75±2.9900 | 36.00±2.1500 |

Description: The same superscripts in the same column indicate significantly different ($p<0.05$). ns = non significant. P1 = Locally sourced ration fermented without adding water (control). P2, P3, P4 and P5 = locally sourced ration with a moisture content of 40, 50, 60 and 70%, respectively

## Discussion

In principle, *S. cerevisiae* can affect the development of mucosa in the small intestine. *S. cerevisiae* can also promote the development of the small intestine by stimulating the growth of villi (de los Santos *et al*., 2007), so that ultimately can improve the function of the small intestine (Kidd *et al*., 2013) and improve the digestibility of nutrients (Akhavan-Salamat *et al*., 2011). Bradley *et al*. (1994) reported that supplementation of *S. cerevisiae* in ration caused a change in the morphology and the number of goblet cells in the chicken intestine. In line with this, Santin *et al*. (2001) also reported that administration of 0.2% *S. cerevisiae* in the ration could increase the villus height, located in the small intestine mucosa, which is thought to affect the weight of the small intestine. Furthermore, Rahbar *et al*. (2011) reported that the use of *S. cerevisiae* into the basal diet containing Peganum harmala seed powder significantly influenced the villus height in the jejunum, but no significant effected on the duodenum and ileum.

The gizzard and pancreatic weights of ducks were significantly affected by treatment. The gizzard is a muscular organ that plays a role to reduce the particle size of ingested rations and blends them with several digestive enzymes (Nishii *et al*., 2015). During the fermentation process, *S. cerevisiae* most likely hydrolyzes the fiber component in the ration into short chains and thus lowers the grinding activity of the gizzard. Hetland *et al*. (2005) reported that it was difficult to grind coarse insoluble fiber and consequently increased the size of gizzard. It is reasonable to assume that the more gizzard has grinding activity, the more gizzard developed. The same results also occurred in

pancreatic weight. During the digestion process, *S. cerevisiae* extract might have stimulated the secretion of the pancreatic digestive enzyme, such as α-amylase, which is effective in the digestion of dietary starch. This is as reported by Matur *et al*. (2010) that influence of *S. cerevisiae* extract on pancreatic amylase could be useful in the digestion of dietary starch. Moreover, β-glucan, known as a component of *S. cerevisiae*, could stimulate cholecystokinin from enteroendocrine cells, where cholecytokinin was effective to stimulate the pancreatic secretion (Chandra and Liddle, 2009; Matur *et al*., 2010). However, the weight of the bile, liver, spleen and heart in this study was not affected by treatments. The non-significant results were also reported by Rahbar *et al*. (2011) that the weight of the spleen and liver of broilers added to *S. cerevisiae* in the rations were not significantly different.

The high number of leukocytes can be used as an indicator to determine the immune status of poultry. The higher number of immune cells in the blood means that the better the immune system of poultry. Related to the use of *S. cerevisiae* in feed, Savage and Zakrzewska (1996) reported that *S. cerevisiae* had a significant role to stimulate the immune system in the body, which would increase the levels of IgG and IgA. This has an impact on the improvement of the immune system and can increase poultry antigenic response in the intestinal mucosa (Santin *et al*., 2001). In line with this, Gao *et al*. (2008; 2009) also stated that chickens consume product fermented by *S. cerevisiae* could improve the lymphocyte cell so that the body immunity was increasing. The same results also reported by Al-Homidan and Fahmy (2007) and El-Husseiny *et al*., 2008) that a product fermented by *S. cerevisiae* might modulate the immune system so that the birds consuming the product could handle the stress better.

The level of cholesterol, triglycerides and LDL in the blood of Pegagan ducks were affected by treatment. The mechanism associated with the cholesterol-lowering effect of yeast could be linked with an inhibitor of 3-hydroxyl-3-methylglutaryl- coenzyme reductase (Saleh *et al*., 2013). In line with this, Paryad and Mahmoudi (2008) reported that dietary supplementation of 1% *S. cerevisiae* significantly lowered plasma cholesterol and triglyceride concentrations in broiler chickens, while HDL was increased. The results obtained slightly different to that reported by Yalçın *et al*. (2008) that administration of *S. cerevisiae* in rations added oilseed meal had no effect on cholesterol and blood triglycerides.

## Conclusion

It was concluded that the feeding of locally sourced ration fermented by yeast tape with a moisture content of 50% provides optimal results to the weight of visceral organs and gastrointestinal tract and also on blood profile and chemistry of Pegagan ducks.

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## Author's Contributions

**Fitra Yosi:** Conducted to the research, analyzed the data and wrote the manuscript.

**Sofia Sandi and Miksusanti:** Conducted to the research.

## Ethics

All of authors confirm that this article is original and contains unpublished materials and has no ethical issues involved.

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