

The Balancing of Safflower Oil and Inositol to Intestinal Morphometric of Sentul Chicken

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Abstract. This study was aimed to evaluate the use of safflower oil (*Carthamus tinctorius L*) and inositol on the digestive profile of male Sentul chickens. A total of 182 Sentul chickens aged 17 weeks were reared up to 23 weeks in 91 units of battery cage (6 chickens/unit). The research was conducted in a Completely Randomized Design (CRD) with nine treatments and three replicates (6 chickens/replicate). The research treatments were R0 = control/ basal feed + 0% Safflower and 0% Inositol; R1 = Basal feed + 0.5% Safflower oil; R2 = Basal feed + 1.0% Safflower oil; R3 = Basal feed + 0.5% Inositol; R4 = Basal feed + 1.0% Inositol; R5 = Basal feed + 0.5% Safflower oil and 0.5% Inositol; R6 = Basal feed + 0.5% Safflower oil and 1.0% Inositol; R7 = Basal feed + 1.0% Safflower oil and 0.5% Inositol; R8 = Basal feed + 1.0% Safflower oil and 1.0% Inositol. Basal feed was composed of corn, rice bran, soybean kernel, fishmeal, palm oil, calcium carbonate (CaCO₃), topmix, lysine, and methionine, as well as safflower oil (*Carthamus tinctorius L*) and inositol. The measured variable consists of digestive profile (the percentage of intestine weight, digesta, proventriculus, gizzard) and intestine length, crypt depth, the width and length of intestinal villi, Intestinal histology profile. The data were subjected to a statistical analysis of variance (ANOVA) continued with an Honestly Significant Difference test (HSD). The result showed that incorporating safflower oil (*Carthamus tinctorius L*) and inositol into feed did not significantly affect ($P>0.05$) the digestive profile of Male Sentul chickens. Conclusively, safflower oil and inositol up to 1% in the feed are safe for male Sentul chickens without interfering with the performance of digestive organs because it produces relatively similar intestinal weight and length, crypt depth, as well as the length and width of intestinal villi.

Keywords: safflower oil (*Carthamus tinctorius L*), inositol, intestinal villi

Abstrak. Tujuan penelitian untuk mengkaji penggunaan minyak safflower (*Carthamus tinctorius L*) dan inositol terhadap profil saluran cerna ayam sentul jantan. Materi penelitian yang digunakan adalah ayam Sentul jantan sebanyak 182 ekor umur 17 minggu yang dipelihara sampai umur 23 minggu pada kandang batere sebanyak 91 unit, dengan 6 ekor ayam/unit. Pakan basal yang tersusun dari jagung, dedak, bungkil kedelai, tepung ikan, minyak kelapa sawit, kalsium karbonat (CaCO₃), topmix, lisin dan methionine, serta minyak safflower (*Carthamus tinctorius L*) dan inositol. Metode penelitian dengan Rancangan Acak Lengkap (RAL) 9 perlakuan dan 3 ulangan, dengan 6 ekor ayam/ulangan. Perlakuan Penelitian : R₀ = Kontrol/ pakan basal dengan 0% Safflower dan 0% Inositol; R₁ = Pakan Basal + Minyak Safflower 0,5%; R₂ = Pakan Basal + Minyak Safflower 1,0%; R₃ = Pakan Basal + Inositol 0,5%; R₄ = Pakan Basal + Inositol 1,0%; R₅ = Pakan Basal + Minyak Safflower 0,5 % dan Inositol 0,5%; R₆ = Pakan Basal + Minyak Safflower 0,5 % dan Inositol 1,0%; R₇ = Pakan Basal + Minyak Safflower 1,0 % dan Inositol 0,5%; R₈ = Pakan Basal + Minyak Safflower 1,0 % dan Inositol 1,0%. Variabel yang diukur meliputi: profil saluran pencernaan (persentase berat usus, digesta, proventrikulus, gizzard) dan panjang usus, kedalaman *crypt*, lebar dan panjang vili usus) profil histologi usus. Data dianalisis secara statistik dengan analisis sidik ragam (ANOVA), dengan Uji lanjut uji Beda Nyata Jujur (BNJ). Hasil penelitian menunjukkan bahwa pemberian minyak safflower (*Carthamus tinctorius L*) dan inositol dalam pakan berpengaruh tidak nyata ($P>0,05$) terhadap profil saluran cerna ayam sentul jantan. Kesimpulan Penggunaan minyak safflower dan inositol sampai level 1% dalam pakan ayam sentul jantan dapat digunakan sebagai imbuhan pakan untuk menjaga kesehatan ayam tanpa mengganggu kerja organ saluran cerna dengan menghasilkan bobot dan panjang usus, kedalaman kript, panjang dan lebar villi-villi usus yang relatif sama

Kata kunci: minyak safflower (*Carthamus tinctorius L*), inositol, villi-villi usus

Introduction

Sentul chickens can produce 85 to 210 eggs per year (Budiman et al., 2012). Another study

reports that Sentul chickens can lay 26 eggs per period or around 150 eggs/year and they have a faster growth as well as better immune system

against disease (Mugiyono et al., 2015; Isyanto et al., 2017). An optimum egg and meat production are the performance result of the digestive tract in producing digestive enzymes so that the nutrient absorption is optimum. The contributing factors to the level of nutrient absorption include conditions of intestinal villi, crypt depth as well as villi depth and width. The improvement of digestibility and nutrient absorption is significantly affected by the quality and role of the feed. Safflower oil (*Carthamus tinctorius L*) and inositol are feed supplements for improving the nutrient metabolism to achieve an optimum performance.

Safflower oil is plant-based oil obtained from the extraction of safflower seeds (*Carthamus tinctoris L*). Safflower oil contains high unsaturated fatty acid (90.33%) and low saturated fatty acid (9.62%). The highest concentrations of unsaturated fatty acid in safflower oil are 76.22 % linoleic acid that belongs to the polyunsaturated fatty acid (PUFA) and 13.75 % oleic acid that belongs to monounsaturated fatty acid (MUFA) (Ashrafi et al., 2010; Katkade et al., 2018). Safflower oil is rich in linoleic acid (75-78%) that plays a crucial role in decreasing blood cholesterol as well as improving the performance, health status, nutritional value of starch, and the digestibility of crude fat and crude fiber. Safflower oil (*Carthamus tinctorius*) also contains omega-6 and omega-9 which, as reported by Maharani et al. (2018), exhibits secretion function in the digestion (omega-6), such as secreting acids and digestive enzymes. Safflower oil (*Carthamus tinctorius*) contributes some benefits because it contains 76.22% linoleic acids that promote nutrient digestibility. Diets with high linoleic acid cause changes in the morphology of the intestinal villi. Todorov et al. (2020) reported that mice fed a diet rich in α -Linolenic acid (ALA) showed an increase in total ALA content along with changes in villus morphology. In particular, villus length increased in mice fed

ALA-rich diets, accompanied by an increase in mucosal thickness and the number of epithelial cells. The epithelial proliferation rate as well as the proportion of goblet and Paneth cells were significantly reduced in the ALA-rich diet group. An ALA-rich diet pushes the fatty acid composition of the intestinal tissue towards an anti-inflammatory phenotype. For example, a reduction in the dietary PUFA ratio 1-6/1-3 is associated with a significant reduction in circulating levels of the pro-inflammatory marker interleukin-6 as well as non-high-density lipoprotein (non-HDL) lipoprotein cholesterol in mice deficient in low density lipoprotein receptors (LDL).

Inositol belongs to the group of vitamin B8 and one of the vital biomolecules for growth, living, and reproduction. The symptoms of inositol deficiency include appetite loss and slow growth. Inositol may play a role in increasing body weight, performing biological functions in the body, sustaining cell growth and living, lipid metabolism, and insulin sensitivity, as well as increasing the absorption of mineral phosphor. The increased *Myo-inositol* may also increase the phytase enzymes derived from the microbes of digestive tracts. The safflower is considered an immunological supplement to enhance immunity and improve productivity rather than adding growth stimulations. The combination of inositol and safflower oil as a dietary supplement could improve growth and promote the health of chickens to produce high quality meat and eggs.

Additionally, inositol also promotes body weight gain, plays vital biological functions together with tissues to synthesize and catalyze polyol. The physiological functions of particularly *Myo-inositol* include sustaining cell growth and living as well as the growth performance to increase the absorption of mineral phosphor. The research aimed to evaluate the use of safflower oil (*Carthamus tinctorius L*) and inositol on the digestive profile of male Sentul chickens.

Materials and Methods

Research Materials

This study used 91 male Sentul chickens aged 4 (four) weeks and reared for 7 (seven) weeks. The basal feed was composed of corn, rice bran, soybean kernel, fishmeal, palm oil, calcium carbonate (CaCO₃), topmix, lysine, and methionine, as well as safflower oil (*Carthamus tinctorius L*) (cold product, 100%pure, Turkey) and inositol (Myo-Inositol from Sigma-Aldrich Chemie GmbH, Steinheim, Germany). The composition and nutrient content of treatment feed is presented in Table 1 and 2. A wire cage measuring 35x35x29x56 cm was used to house two chickens. The other equipment included feed scales of 2kg/0.1g and 1200g/0.1mg.

Research Method

The research was conducted in a Completely Randomized Design (CRD) with nine treatments and three replicates (3 chickens each). The treatments were:

- R₀ = Control/ basal feed with 0% Safflower and 0 % Inositol
- R₁ = Basal feed + 0.5% safflower oil
- R₂ = Basal feed + 1.0% Safflower oil
- R₃ = Basal feed + 0.5% inositol
- R₄ = Basal feed + 1.0% inositol
- R₅ = Basal feed + 0.5% safflower oil and 0.5% inositol

R₆ = Basal feed + 0.5% safflower oil and 1.0% inositol

R₇ = Basal feed + 1.0% Safflower oil and 0.5% inositol

R₈ = Basal feed + 1.0% Safflower oil and 1.0% inositol

The feed ratio was composed on iso calorie and iso protein. The nutrient composition of feed is presented in Table 1.

Research Undertaking

Cage preparation

The cage was prepared by cleaning the whole cage and the perimeter as well as spraying disinfectant, spreading (dolomite) chalk, and fumigating with KMNO₄ and formaldehyde. The cage and food and drinking water containers were sanitized. Additionally, the cage was made into partitions and each partition was randomly numbered.

Basal Feed Formulation

The feed was composed of corn, rice bran, fishmeal, soybean kernel, palm oil, lysin, CaCO₃, methionine, and topmix. The mixture was added with safflower oil (*Carthamus tinctoriusl*) and inositol according to the level of treatment. The treatment feed was composed based on iso calorie and iso protein.

Table 1. Composition of feed for male Sentul chickens

Feedstuff	R ₀	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈
	%								
Corn	55	55	55	55	55	55	55	55	55
Ricebran	22	22	22	22	22	22	22	22	22
Soybean kernel	15	15	15	15	15	15	15	15	15
Fishmeal	5	5	5	5	5	5	5	5	5
Palm oil	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
CaCO ₃	1	1	1	1	1	1	1	1	1
Topmix	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Lysin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Methionin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100	100	100	100
Safflower oil	0	0.5	1	0	0	0.5	0.5	1	1
Inositol	0	0	0	0.5	1	0.5	1	0.5	1

Table 2. Nutrient composition of feed for male Sentul chickens

Nutrient composition	R ₀	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈
	%								
CP (%)	17.02	17.02	17.02	17.02	17.02	17.02	17.02	17.02	17.02
CFat (%)	7.96	7.96	7.96	7.96	7.96	7.96	7.96	7.96	7.96
CF (%)	4.16	4.16	4.16	4.16	4.16	4.16	4.16	4.16	4.16
ME (kcal/kg)*	2.884	2.890	2.897	2.884	2.884	2.890	2.890	2.897	2.897
Ca (%)*	1.28	1.2756	1.28	1.28	1.28	1.28	1.28	1.28	1.28
P (%)*	0.38	0.3779	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Lysin (%)*	1.11	1.1074	1.11	1.11	1.11	1.11	1.11	1.11	1.11
Methionin (%)*	0.67	0.6708	0.67	0.67	0.67	0.67	0.67	0.67	0.67

Note: Analysis result of Nutrition and Animal Feed Lab. UNSOED (2019)

*Calculated based on NRC Table (1994)

Daily Rearing of Male Sentul chickens

The maintenance included feeding in the morning and afternoon, and the water was provided ad libitum. The temperature and humidity were tracked and recorded every morning, midday, and afternoon. The male Sentul chickens were reared for two months, from eight weeks to 16 weeks old in the Experimental Farm, Animal Science Faculty, Jenderal Soedirman University, Purwokerto, Kabupaten Banyumas, Central Java. The data collection procedure was carried out by fasting the chickens for 6 hours, then each experimental unit was performed to 2 birds with body weight close to the average value. The chickens were slaughtered in accordance with Islamic law, followed by scalding, defeathering and evisceration. The internal organs were removed from the abdominal cavity, then the digestive tract was measured and observed.

Data Analysis and Measurement

The obtained data were subjected to analysis of variance (Steel et al., 1996). The Honestly Significant Difference test was conducted using SPSS ver 17.0 with a 5% error tolerance.

Digestive profile (the percentage of intestine weight, digesta, and intestine length, crypt

depth, the width and length of intestinal villi, Intestinal histology profile (Iji et al., 2001). The crypt depth, intestinal villi length and intestinal villi width were analyzed descriptively.

Results and Discussion

The digestive tract is a vital organ for livestock because it is responsible for the digestibility and nutrient absorption of feed for wellbeing, production, and reproduction. When the digestive tract undergoes a disorder, it may negatively impact livestock performance.

The present study using safflower oil and inositol in the ration of male Sentul chickens showed its impact on the digestive profile as presented in Table 3.

Table 3 shows that safflower oil and inositol in the ration of male Sentul chickens does not significantly affect ($P>0.05$) the digestive profile (intestine length and weight, digesta weight, crypt depth, the length and width of intestinal villi).

The result showed that despite the non-significant result, the treatments promoted the chickens' health and performance. Furthermore, it has quantitatively increased the digestive profile because the longer and heavier intestines would have more beneficial microbes (lactic acid bacteria) and produce more digestive enzymes. Additionally, the longer and

Table 3. Digestive profile of Male Sentul chickens consuming feed fortified with safflower oil and inositol

Treatments	IRW (%)	RWID (%)	IL (cm)	CD (μm)	IVL (μm)	IVW (μm)
R ₀	1.26±0.15	3.27±0.13	131.33± 5.51	831.73	1186.84	295.24
R ₁	1.84±0.14	3.99±0.08	161.00±21.52	854.7	2170.39	432.16
R ₂	2.30±0.40	3.33±0.11	149.00±12.77	951.46	2254.62	356.58
R ₃	2.13±0.19	3.21±0.74	156.33±12.86	631.52	1585.23	319.82
R ₄	1.80±0.06	3.16±0.40	162.00±11.36	752.15	2289.25	351.88
R ₅	1.78±0.18	3.67±0.37	145.33±5.69	1191.55	2102.84	371.62
R ₆	1.99±0.30	3.29±0.59	149.00±3.46	841.16	2321.24	395.36
R ₇	1.85±0.15	3.68±0.05	153.67±4.73	311.12	2070.40	374.56
R ₈	1.83±0.24	3.89±0.14	149.67±6.26	931.62	1608.12	314.34
Significance	ns	ns	Ns			

Note: IRW: intestinal relative weight; RWID: Relative weight of intestinal digesta; IL: Intestine length; CD: crypt depth; IVL: Intestinal villi length; IVW: Intestinal villi width

wider the villi, the more nutrient is absorbed which eventually improves chicken performance.

Feed nutrient digestibility and absorption may be affected by the surface area of intestinal epithelial, total folds, total villi, and microvilli that enlarge the absorption area, as well as the height and width of villi surface (Sugito, et al., 2007; Ibrahim 2008).

Vili enlarges the surface of small intestines and affects the process of nutrient absorption (Alfiansyah, 2011). Vili is where nutrients are absorbed, so the wider the villi the more nutrients are absorbed, thus increasing the growth of body organs and carcass (Asmawati, 2014). The increase of villi height in the small intestines is strongly correlated with the improved digestibility and absorbability because the enlargement of an absorption area expresses the smooth system of nutrient transport across the body (Awad et al., 2008).

It shows that safflower oil and inositol did not affect the profile of digestibility organ and chicken performance and did not distract the nutrient metabolism process. Safflower oil and inositol can be used as a feed supplement to promote the performance of digestive tract organs by maintaining the pH, microbial content, enzyme activity, and intestinal peristalsis. Safflower oil in the ration is harnessed as an energy source. Safflower oil is

derived from safflower seeds and its main content is unsaturated fatty acids: 13.75% oleic acid and 76.22% linoleic acid (Katkade et al., 2018).

The content of unsaturated fatty acid may inhibit the activity of lipoprotein lipase enzyme (LPL), decreasing lipogenesis, and increase lipolysis (Hidayat, 2015). Inositol belongs to B type vitamin (Indyk, et al., 2016) and provides vitamin B in the glucose/carbohydrate metabolism. Inositol has nine stereoisomers but Myo-inositol is the most prevalent one in the biological system and functions as metabolite essentials.

Inositol containing vitamin B8 and incorporated in feed plays a role in the growth where body weight gain increases with the development of tissues and organs of digestive tracts. Setiadi et al. (2011) stated body internal organs develop with the increasing body weight because these organs have to balance the increasing body demand to maintain a normal condition. Linoleic and oleic acids contained in safflower oil will be carried by lipoproteins in the blood after being absorbed by the intestinal villi, then distributed to cells throughout the body including intestinal cells and the digestive organs themselves. Utari (2010) explains that linoleic acid can prevent cell membranes from being damaged. Cells that are properly preserved can have a positive impact on tissue

development and health. The provision of inositol with vitamin B8 content has a role in growth. Corze and Soulage (2013) stated that inositol has a role as a growth promoter where it is involved as a phospholipid component of cell membranes that is related to cell growth and survival. Safflower oil and inositol supplementation plays a role in maintaining the health and growth of digestive tissues and organs.

The length of small intestines in poultry is varied according to the age, race, and type of feed. Intestine and other digestive tract is a complex organ that supports body metabolism, maintains body, skeleton, and nerve system immunity (Overland and Kjeldsen, 2002). The development and addition of total cells in the intestine are influenced by the rate at which chickens can obtain feed to fulfill the nutrient demand for their development (Geyra et al., 2001).

When feed is offered earlier, it may stimulate the development of these organs, increasing the digestive capacity and intestinal absorption. In the small intestines, peristalsis occurs, and it mixes the digesta with pancreatic and bile fluids. The contributing factors to the nutrient digestibility of small intestines are the inner surface area of intestines (folds, villi, and microvilli) and digesta transit time in the intestine (Rofiq, 2003).

Houshmand et al. (2012) reported that incorporating probiotic or organic acids into the ration of broiler chickens with low or high protein did not affect the relative weight and the length of small intestines. Similarly, Iriyanti and Hartoyo (2018) stated that supplementing symbiotics that contained *Lactobacillus sp.* did not affect the weight and the length of small intestines of Sentul chickens.

Furthermore, Hamdani et al. (2017) explained that the length and width of intestines may be affected by the surface, and

the high-fiber feed may change the digestive rate in the poultry. In this case, the higher the crude fiber, the slower the digestive rate and nutrient absorption. Additionally, bodyweight significantly affects the intestine length of poultry. According to Gumilar and Pratama (2018), the expanding surface of the cell membrane would ease the secretion of digestive enzymes and improve digestibility.

The other contributing factor to nutrient content in the feed is the fiber. The common ration for chicken usually contains a similar level of fiber, so it produces similar intestine weight. It is reported that feed with high crude fiber would affect the digestive tract condition by increasing the weight and length of the intestines (Iyayi, 2005).

Peng et al (2016) stated that feed efficiency in poultry depends on the digestive tract, particularly the villi and crypt that play a vital role in nutrient digestibility. Similarly, Laudadio et al. (2012) reported that the higher the villi, the wider the area for absorbing nutrients and potentially increases the growth and metabolism rate. Furthermore, digestive capacity and nutrient absorption may be reflected from the villi/crypt ratio: the higher the ratio, the higher the digestibility and nutrient absorption.

Intestine weight is affected by the amount and development of villi. The villi of 2-months Sentul chickens are not well developed so the absorption activity is not optimum. Sklan (2001) reported that the weight of intestinal components is affected by the number of cells and the size of villi in the components. Villi size is influenced by the rate of chicken in obtaining feed. Furthermore, the contributing factor to the metabolic products of nutrient absorption is the number of villi in the small intestines against the weight and length of small intestines in Sentul chicken (Figure 1).

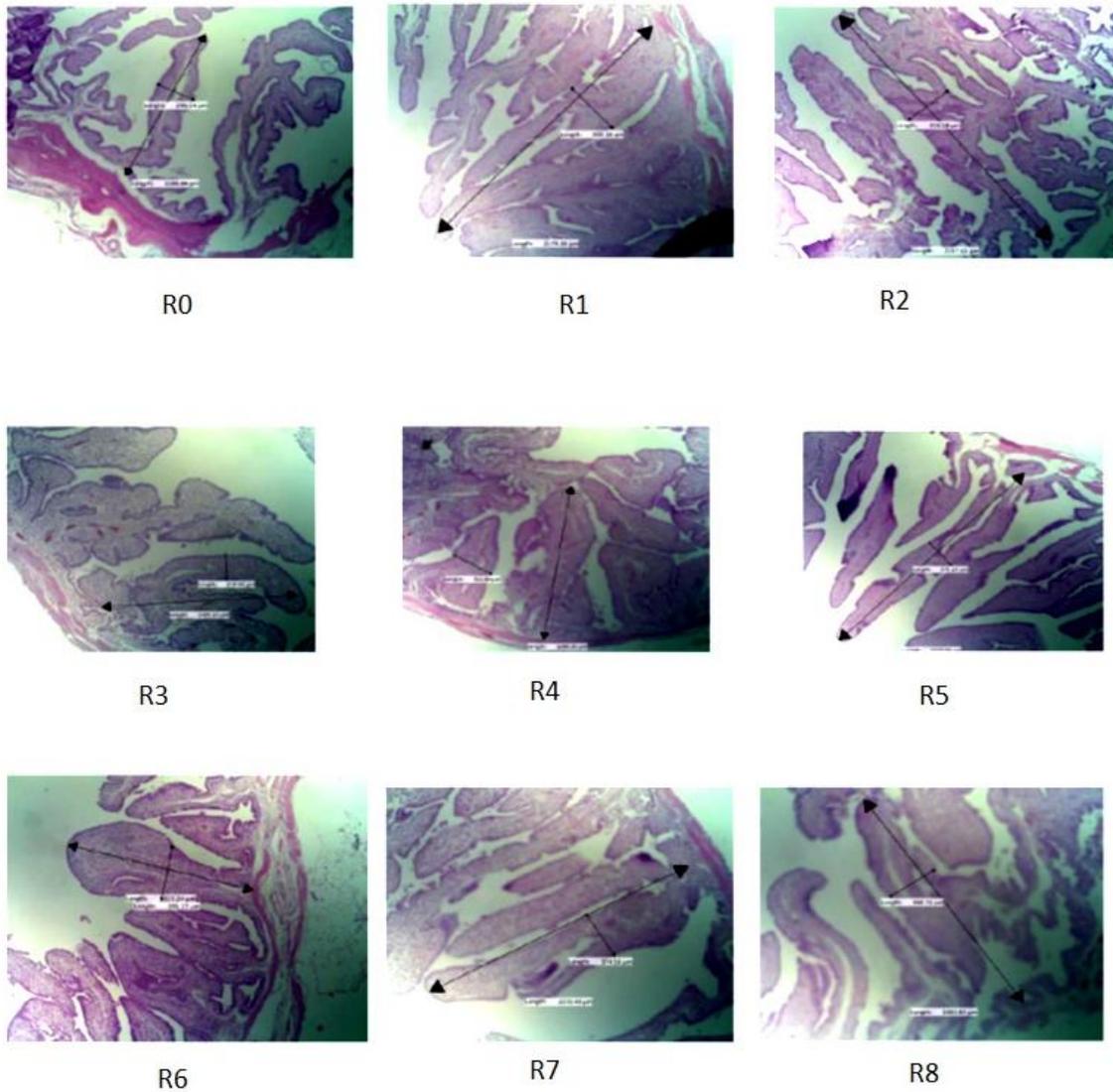


Figure 1. Length and width of intestinal villi of Sentul chicken

Nutrient digestibility of small intestines is affected by the permeability values, the inner surface area of intestines (folds, villi, and microvilli), and the transit time of digesta in the intestines. Feed with a high crude fiber would affect the digestive tract conditions, namely increasing the weight and length of the intestine (Iyayi, 2005).

According to Pardede (2018), nutrient balance in feed would affect the intestine length as well as intestine weight and body weight. Utama *et al.* (2019) reported that the more villi and goblet cells in the intestine would allow a perfect nutrient absorption because villi and goblet cells enlarge the intestinal surface

and help the absorptive cells during the absorption process.

The condition of small intestines, such as villi height describes a larger area for absorbing nutrients (Awad *et al.*, 2009). The increased height and width of villi are associated with a wider villi surface to absorb nutrition into the bloodstream (Mile *et al.*, 2006). Furthermore, Awad *et al.* (2008) stated that the increased villi height in the small intestines of broiler chickens is strongly correlated with digestibility and absorbability because the enlarged absorption area expressed the smooth system of nutrient transport across the body that benefits the host.

Conclusions

Safflower oil and inositol up to 1% incorporated into the feed of male Sentul chickens are the potential feed additives to maintain chicken health without upsetting the performance of digestive organs. The supplement produces relatively similar intestine weight and length, crypt depth, and villi length and width.

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