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Research article

Effect of substituting crude palm oil with saponified black soldier fly larvae (*Hermetia illucens l.*) oil on performance and digestive tract characteristics of broiler chickens

Ega Anggi Lestari[1] and Chusnul Hanim[2,*]

[1]Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia.
[2] Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia.

Abstract

Several studies have shown that black soldier fly larvae (BSF-L) oil is an effective substitute for the use of crude palm oil (CPO) as an energy source in broiler chicken feeds. CPO contains several long-chain fatty acid (LCFA), such as palmitic and oleic acid, while BSF-L oil contains various medium-chain fatty acid (MCFA), namely lauric acid. In addition, lauric acid can function as an antimicrobial and immunomodulator. As an antimicrobial, it can inhibit the activity of enzymes that play a role in energy production and nutrient transport. Lauric acid is also an immunomodulator that preserves the integrity of the intestinal barrier by increasing the permeability of tight junctions. Therefore, this study aimed to evaluate the effect of substituting CPO with saponified BSF-L oil on broiler chicken performance and digestive tract characteristics. There were 280 male broiler chickens of the New Lohmann Indian River strain (MB 202 Platinum) in the experiment. CPO and saponified BSF-L oil were administered at different ratios, namely 3:0 (0% saponified BSF-L oil in feed), 2:1 (1% saponified BSF-L oil in feed), 1:2 (2% saponified BSF-L oil in feed), and 0:3 (3% saponified BSF-L oil in feed). Each treatment ratio consisted of 7 replications, which were administered to 10 chickens. In this study, the test animals were reared for 35 days, with 3 maintenance phases, including starter, grower, and finisher. The formulated feed was applied in the grower phase, followed by the removal of digestive tract organs to assess various parameters, such as length, weight, and pH of the digesta. The jejunum was also analyzed to obtain the histomorphology of intestinal villi and tight junctions gene expression. The data obtained were analyzed for variance with a one-way pattern and continued with the Duncan Multiple Range Test due to differences. The results showed that the substitution of CPO with saponified BSF-L oil had a significant effect ($P<0.05$) on feed intake (FI), index performance (IP), duodenal length, ileal pH, and JAM-2 and OCLN gene expression. The treatment also had a significant impact ($P<0.01$) on body weight (BW), average daily gain (ADG), and ZO-1 gene expression. In addition, the 2:1 treatment could improve the performance of broiler chickens and the ileal digesta's pH value, as well as reduce the length of the duodenum. As the level of saponified BSF-L increased, tight junctions gene expression also increased. However, it did not affect the histomorphology of jejunal villi or the relative weight of the digestive organs.

Keywords: Digestive tract characteristics, Performance of broiler chickens, Saponified BSF-L oil, Tight junction

[*]**Corresponding author:** Chusnul Hanim, Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada, Jl. Fauna No. 3 Bulaksumur, Yogyakarta 55281, Indonesia. E-mail: c.hanim@ugm.ac.id.

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INTRODUCTION

With the expanding population and improving quality of life, the demand for food naturally increases. This trend is closely associated with the importance of an adequate food supply and maintaining proper nutrition. In this context, broiler chickens meat is a highly sought-after animal protein source, known for its affordability. To meet the significant demand, it is essential to prioritize and maintain high productivity in broiler chickens farming. Animal feedshas been reported to be essential for providing livestock with the energy required for proper growth and development. Since broilers require a lot of energy in their feed, depending only on grain is inadequate in providing the needed nutrients. Consequently, oil is often incorporated into feed to ensure it contains a high-calorie content, which enhances its palatability for the chickens as well as facilitates overall growth and better intake.

In line with previous studies, crude palm oil (CPO) is often used as a source of feed energy for chickens and can be substituted with black soldier fly larvae (BSF-L) oil, resulting from defatting in maggot flour production. CPO typically contains high levels of long-chain fatty acid (LCFA), including 44.02% palmitate (C16) and 39.15% oleate (C18:1) (Basiron, 2005). Meanwhile, BSF-L oil contains medium-chain fatty acid (MCFA), namely 43.10% laurate (C12) (Jayanegara et al., 2020). There are differences in the metabolic processes of MCFA and LCFA compounds in the body, where MCFA are often transported into the liver through the portal vein without stimulating triglyceride production. The metabolic process continues after absorption to produce ketone bodies, which join the citric acid cycle to supply energy via adenosine triphosphate (ATP). MCFA can pass into mitochondria without the need for carnitine-dependent transport, hence, it enables rapid oxidation and ATP formation (Juby et al., 2023).

Lauric acid in BSF-L oil has been demonstrated in numerous studies to have antibacterial and immunomodulatory properties. Moreover, monoglycerides and MCFAs can inhibit feed pathogen growth, support intestinal health, and have antibacterial and immunomodulatory qualities (Jackman et al., 2020). Lauric acid works by affecting alterations in the fluidity of the membranes surrounding bacterial cells. These modifications may enhance the compound's ability to enter cells and hinder the activity of enzymes involved in the synthesis of energy and the movement of nutrients (Sulastri et al., 2016). A particular type of MCFA known as lauric acid has a potent ability to destroy bacterial membranes by forcing fluid inside the cells and allowing hydrogen ions to enter more easily (Kim and Rhee, 2016). Tight junction permeability can be enhanced by decanoic acid (C10) and lauric acid (C12), which activate protein kinase C and myosin light-chain kinase (MLCK). According to Shimazaki et al. (1998), MLCK enhances paracellular permeability by contracting the peri-junctional actomyosin ring. Tight junctions, which are multi-protein complexes that close the paracellular gap between adjacent epithelial cells, regulate the permeability of the intestinal barrier (Awad et al., 2017). In this case, disturbance of the integrity and structure of tight junctions directly leads to immune cell activation and prolonged tissue inflammation (Suzuki, 2020). Decreased integrity of the intestinal barrier allows the passage of harmful substances, which can induce inflammation as an immune response (Yang et al., 2016).

Feed ingredients in the form of oil easily experience a rancidity process during storage for an extended period. To prevent the rancidity process, saponification using calcium is an effective method for extending shelf life. Saponified oil is often easier to store and mix in feed without using tools because it is in solid form (mash). Therefore, this study aimed to determine the effect of substituting CPO with saponified BSF-L oil in feed. The feed ingredient substitution is expected to have a positive effect by increasing the characteristics of the digestive tract to improve the performance of broiler chickens.

MATERIALS AND METHODS

Ethical clearance

All animal operations used were authorized by the Faculty of Veterinary Medicine Universitas Gadjah Mada Study Ethics Committee with number 00149T/EC-FKH/Ex./2021.

Experimental design

This study used a complete randomized design (CRD) with a one-way pattern, consisting of 4 treatments and 7 replications, with each replication consisting of 10 chickens. Substitution of CPO using saponified BSF-L oil was given in different amounts. Comparison of giving CPO and saponified BSF-L oil, including 3:0 (0% saponified BSF-L oil in feed), 2:1 (1% saponified BSF-L oil in feed), 1:2 (2% BSF-L oil saponified in feed), and 0:3 (3% saponified BSF-L oil in feed) was carried out.

The feeding was carried out based on the predetermined protocol, where the starter, grower, and finisher contained a crude protein (CP) of 22%, 21%, and 19%, respectively. In addition, feed treatments were applied when the chickens were 11 days old. The composition of the rations and the fatty acid composition provided were detailed in Tables 1 and 2.

Preparation of Saponified BSF-L Oil

Saponified BSF-L oil was prepared through physical and chemical processes. The physical process was conducted by heating, while the chemical process was carried out by adding NaOH and CaCl2 using 7:1.5:1.5 composition. Furthermore, the manufacturing process started by heating the BSF-L oil to a temperature of 80°C, then adding the NaOH solution and stirring to obtain a granular solid. The granules were added to the CaCl2 solution and stirred to obtain dry sample. The supplement was left in the air, at normal temperature. Subsequently, it was crushed with a blender and filtered to obtain the same size. The final result of the supplement formed a mash-shaped solid with a yellowish-white color. Following the procedure described by Mjøs (2003), stating that saponified BSF-L oil was evaluated for fatty acid composition using Gas Chromatography (GC-Ageilent Technologies 7890B). The results of the fatty acid composition analysis were presented in Table 3.

Table 1 Feed components and nutrient content of grower feed (11-21d) used in research

Feed components (%)	Ratio CPO and saponified BSF-L oil (%:%)			
	3:0	2:1	1:2	0:3
Corn	50.00	51.44	52.85	54.30
Soybean meal	34.00	34.00	34.00	34.00
Rice bran	6.49	5.10	3.70	2.25
Meat bone meal	3.00	3.00	3.00	3.00
Saponified BSF-L oil	0.00	1.00	2.00	3.00
Crude palm oil	3.00	2.00	1.00	0.00
Limestone	1.20	1.15	1.13	1.07
Dicalcium phosphate	0.88	0.88	0.89	0.95
Mineral mix[1]	0.30	0.30	0.30	0.30
Vitamin mix[2]	0.04	0.04	0.04	0.04
NaCl	0.33	0.33	0.33	0.33
Toxin binder	0.20	0.20	0.20	0.20
L-lysine	0.22	0.22	0.22	0.22
DL-methionine	0.20	0.20	0.20	0.20
L-threonine	0.05	0.05	0.05	0.05
Choline chloride	0.09	0.09	0.09	0.09
Content of nutrient (%)				
Crude protein	21.60	21.55	21.50	21.44
Crude fiber	4.03	3.91	3.79	3.66
Ether extract	5.95	5.47	4.99	4.51
Lysin	1.36	1.35	1.35	1.34
Threonin	0.89	0.89	0.88	0.88
Methionine	0.53	0.53	0.52	0.52
Calcium	0.87	0.87	0.87	0.87
Available phosphate	0.44	0.44	0.44	0.44
ME (Kcal/kg)	3,169.00	3,166.00	3,162.00	3,158.00

[1] : supplied per kg of diet= Mn 40 g, Zn 32 g, Fe 32 g, Se 0.1 g, Cu 6.050 g, I 0.404 g.
[2] : supplied per kg if diet= Vitamin A 50,000,000 IU, Vitamin B1 10 g, Vitamin B2 30 g, Vitamin B3 225 g, Vitamin B5 62 g, Vitamin B6 10 g, Vitamin B9 5 g, Vitamin B12 0.1 g, Vitamin D3 10,000,0000 IU, Vitamin E 80 g, Vitamin C 20 g, Vitamin K3 10 g, Vitamin H 0.1 g.

Broiler Chickens Maintenance

This study used 280-day-old chicks (DOC) male broilers of the New Lohmann Indian River strain (MB 202 Platinum). All broilers received the Newcastle disease (ND) 1, Gumboro, as well as ND-2 vaccines, and were reared for 35 days. In addition, the colony cage was made by installing iron partitions with a size of 1 m^2, also water and feed were provided ad libitum. Maintenance was divided into 3 phases such as starter at 1 to 10 days, grower at 11 to 21 days, and finisher at 22 to 35 days of age. DOC weighing was carried out at the beginning of maintenance to determine the initial weight. In addition, weighing assessed the chickens' increase after each raising period. The delivered feed and the residual feed for each colony cage were weighed after each maintenance phase to calculate the amount consumed. Chickens mortality was observed every day from each colony pen. Meanwhile, body weight and feed were measured to estimate performances such as FI, BW, ADG, feed conversion ratio (FCR), and IP. The broiler management adhered to the guidelines of the Indian River Broiler Management Handbook by Aviagen (2018).

Table 2 Feed components and nutrient content of finisher feed (22-35d) used in research

Feed components (%)	Ratio CPO and saponified BSF-L oil (%:%)			
	3:0	2:1	1:2	0:3
Corn	56.42	57.85	59.27	60.37
Soybean meal	28.50	28.50	28.50	28.50
Rice bran	5.90	4.48	3.07	2.00
Meat bone meal	3.00	3.00	3.00	3.00
Saponified BSF-L oil	0.00	1.00	2.00	3.00
Crude palm oil	3.00	2.00	1.00	0.00
Limestone	1.07	1.06	1.00	0.98
Dicalcium phosphate	0.76	0.76	0.81	0.80
Mineral mix[1]	0.30	0.30	0.30	0.30
Vitamin mix[2]	0.04	0.04	0.04	0.04
NaCl	0.32	0.32	0.32	0.32
Toxin binder	0.20	0.20	0.20	0.20
L-lysine	0.20	0.20	0.20	0.20
DL-methionine	0.17	0.17	0.17	0.17
L-threonine	0.03	0.03	0.03	0.03
Choline chloride	0.09	0.09	0.09	0.09
Content of nutrient (%)				
Crude protein	19.75	19.70	19.64	19.61
Crude fiber	3.96	3.84	3.72	3.62
Ether extract	5.99	5.51	5.03	4.57
Lysin	1.20	1.20	1.19	1.19
Threonin	0.80	0.79	0.79	0.79
Methionine	0.47	0.47	0.47	0.47
Calcium	0.79	0.79	0.79	0.79
Available phosphate	0.40	0.40	0.40	0.40
ME (Kcal/kg)	3,226.00	3,222.00	3,218.00	3,212.00

[1] : supplied per kg of diet= Mn 40 g, Zn 32 g, Fe 32 g, Se 0.1 g, Cu 6.050 g, I 0.404 g.
[2] : supplied per kg if diet= Vitamin A 50,000,000 IU, Vitamin B1 10 g, Vitamin B2 30 g, Vitamin B3 225 g, Vitamin B5 62 g, Vitamin B6 10 g, Vitamin B9 5 g, Vitamin B12 0.1 g, Vitamin D3 10,000,0000 IU, Vitamin E 80 g, Vitamin C 20 g, Vitamin K3 10 g, Vitamin H 0.1 g.

Chickens slaughtering and sample preparation

During the 35-day raising process, one chickens was taken based on the body weight of the average in the colony (total of 28 birds) from each replication in each treatment. Chickens were necropsied after slaughter, then samples of the digestive tract of broiler chickens were prepared. A total of 2 cm of the small intestine from the jejunum was taken and placed in a cream pot containing 10% formalin buffer solution to be preserved, and then histomorphological analysis of the villi intestine was performed. Furthermore, 1 cm of the jejunum's small intestine was also taken twice and placed in a microtube, which was immersed in liquid nitrogen for tight junction gene expression analysis.

Table 3 Fatty acid composition of feed used in research

Fatty acid composition (%)	Ratio CPO and saponified BSF-L oil (%:%)							
	Grower				Finisher			
	0:3	2:1	1:2	0:3	0:3	2:1	1:2	0:3
C10:0 (Decanoic)	-	0.21	0.21	0.56	-	0.18	0.26	0.38
C12:0 (Lauric)	0.52	8.77	12.34	17.50	0.67	5.13	10.42	14.33
C14:0 (Myristic)	0.84	2.97	3.84	5.00	0.85	1.98	3.15	4.17
C16:1 (Palmitoleic)	32.26	28.62	25.66	22.08	30.60	29.68	25.33	20.25
C17:0 (Heptadecanoic)	0.29	0.88	0.87	1.44	0.27	0.57	0.95	1.25
C18:0 (Stearic)	-	-	-	0.11	-	-	-	0.12
C18:1 (Oleic)	4.08	3.99	4.02	4.01	3.97	4.11	3.83	3.68
C18:2 (Linoleic)	34.16	29.78	26.51	25.28	33.45	29.94	26.82	23.14
C18:3 (Linolenic)	1.19	1.30	1.44	1.40	1.20	1.22	1.46	1.73
C18:3 (Gama-linoleic)	23.94	21.81	21.32	20.51	26.87	24.96	26.25	28.31
C20:1 (Eicosanoic)	0.54	0.42	0.17	0.34	0.51	0.46	0.14	0.35
C21:0 (Heneicosanoic)	-	0.20	0.29	0.30	0.28	0.24	0.19	0.23
C23:0 (Tricosanoic)	-	0.11	0.19	0.11	0.20	0.18	0.16	0.14
C24:0 (Lignoceric)	-	-	-	-	-	-	-	0.11
C24:1 (Nervoic)	-	-	-	-	-	0.19	-	-
MUFA	36.88	33.03	29.85	26.43	35.08	34.44	29.30	24.28
PUFA	59.29	52.89	49.27	47.19	61.52	56.12	54.53	53.18
SFA	1.65	13.14	17.74	25.02	2.27	8.28	15.13	20.73

MUFA=Monounsaturated fatty acid, PUFA=Polyunsaturated fatty acids,SFA=Saturated fatty acids.

Measurement of digestive organs

The digestive organs were carefully measured for length using a tape measure, weight, and the pH of the digesta. Measurements started by separating each part of the digestive organ. Each organ was weighed using a digital scale after the organs had been removed from the feed digesta. The organs that were measured for length and weight included the proventriculus, ventriculus, duodenum, jejunum, ileum, cecum, and large intestine. The pH levels were checked using a pH meter that had been set up correctly beforehand. The parts of the digestive system checked for pH were the proventriculus, ventriculus, ileum, and cecum.

Histomorphological analysis of the small intestine

The jejunum samples kept in 10% formalin were examined under a microscope. These preserved intestine samples were prepared and stained with hematoxylin and eosin (HE). The structure of the intestinal villi in the jejunum was observed using a microscope at 4 times magnification. The measurements taken included the height, width, crypt depth, area, and the ratio of villi height to crypt depth.

Tight Junction Gene Expression Analysis

A total of 5 samples of Real-time polymerase chain reaction (RT-PCR) were used to analyze the expression of genes. The analysis consisted of 3 stages namely RNA preparation and purification, reverse transcription to complementary DNA (cDNA), as well as DNA amplification process. Small intestine samples from the jejunum were subjected to RNA extraction using a Quick-RNA Miniprep plus kit (Zymo study, USA). The total RNA obtained was reverse transcribed to obtain cDNA using ReverTra AceTM qPCR RT Master Mix with gDNA Remover (Toyobo,

Japan). The DNA amplification used the Quantstudio RT-PCR System (Thermo Fisher Scientific, United States) and THUNDERBIRDTM SYBRTM qPCR Mix (Toyobo, Japan). Each stage was conducted according to protocol instructions. The next stage was a melting curve analysis where mRNA expression of β-actin was used as a control. All samples were duplicated, and their average was used to adjust the cycle threshold (Ct) value. The comparison of the Ct value method determined the concentration of the target gene β-actin. The $2^{-\Delta\Delta C(t)}$ technique was utilized (Livak and Schmittgen, 2001) to calculate relative changes in gene expression based on RT-PCR. The primer pairs used were shown in Table 4.

Table 4 Fatty acid composition of saponified BSF-L oil and BSF-L oil

Fatty acid composition (%)	Saponified BSF-L oil	BSF-L oil
C10:0 (Decanoic)	0.79	1.13
C12:0 (Lauric)	30.38	38.96
C14:0 (Myristic)	8.34	8.53
C15:0 (Pentadecanoic)	0.19	0.15
C16:0 (Palmitic)	21.02	17.41
C16:1 (Palmitoleic)	2.99	2.27
C17:0 (Heptadecanoic)	0.17	0.14
C18:0 (Stearic)	-	2.56
C18:1 (Oleic)	0.16	16.48
C18:2 (Linoleic)	30.62	9.16
C18:3 (Gama-linoleic)	0.16	-
C18:3 (Linolenic)	0.91	0.90
C20:1 (Eicosanoic)	0.70	1.04
C20:3 (Eicosatrienoic)	0.10	0.18
C20:4 (Eicosatetranoic)	-	0.21
C21:0 (Heneicosanoic)	0.15	-
C22:0 (Decosanoic)	-	-
C24:0 (Lignoceric)	0.16	-
C24:1 (Nervoic)	0.15	0.20
MUFA	4	20.20
PUFA	31.79	10.24
SFA	61.20	68.88

MUFA=Monounsaturated fatty acid, PUFA=Polyunsaturated fatty acids,SFA=Saturated fatty acids.

Research Data Analysis

The data obtained was analyzed for variance using a one-way pattern. Further tests were conducted using the Duncan Multiple Range Test when there were significant differences. The analysis used SPSS version 25.

RESULTS

Broiler Chickens Performance

The results of measuring the broiler chickens performance with the CPO substitution treatment with saponified BSF-L oil were detailed in Table 5. During the grower phase, the substitution of CPO with saponified BSF-L oil in a ratio of 2:1 could increased BW and ADG. However, the results significantly decreased at a ratio of 0:3 ($P<0.01$). During the finisher phase and the total grower (11-35 days),

the substitution of CPO with saponified BSF-L oil in the ratio of 0:1 and 2:1 could increased BW and ADG, while the results significantly decreased ($P<0.01$) at a ratio of 0:3. The IP increased without any substitution and significantly decreased with an increased in the number of substitutes ($P<0.05$). FI in the total phase with a comparison of CPO substitution with saponified BSF-L oil 0:1 and 2:1 increased BW and ADG, but the results significantly decreased in the 0:3 ratio ($P<0.05$). Substitution of CPO with saponified BSF-L oil did not affect FCR and depletion. Overall, the substitution of CPO with saponified BSF-L at a ratio of 0:1 and 2:1 resulted in the best performance.

Table 5 Primer pairs used in RT-PCR of tight junction gene expression

Gene	Pimer sequence (5'→ 3')	Orientation	Reference	Amplification temperature
CLDN 1	GGTGAAGAAGATGCGGA TGG TCTGGTGTTAACGGGTGT GA	Forward Reverse	Proszkowiec-Weglarz et al. (2020)	58°C
JAM 2	CTGCTCCTCGGGTACTTG G CCCTTTTGAAAATTTGTG CTTGC	Forward Reverse	Proszkowiec-Weglarz et al. (2020)	56°C
OCLN	GATGGACAGCATCAACG ACC CTTGCTTTGGTAGTCTGG GC	Forward Reverse	Proszkowiec-Weglarz et al. (2020)	56°C
ZO-1	GCCAACTGATGCTGAACC AA GGGAGAGACAGGACAGG ACT	Forward Reverse	Proszkowiec-Weglarz et al. (2020)	58°C
β - Actin	ATGAAGCCCAGAGCAAA AGA GGGGTGTTGAAGGTCTCA AA	Forward Reverse	Xie et al. (2019)	59°C

Length of Digestive Organs

The results of measuring the length of the digestive organs using the substitution treatment of CPO with saponified BSF-L oil were presented in Table 6. Substitution of CPO with saponified BSF-L oil with a ratio of 3:0 yielded the best duodenum length results. Furthermore, duodenal length significantly decreased with the addition of CPO substitution level with saponified BSF-L oil ($P<0.05$). Substitution of CPO with saponified BSF-L oil did not impact the length of the proventriculus, ventriculus, jejunum, ileum, cecum, and large intestine.

Relative Weight of Digestive Organs

Data on the results of measuring the relative weight of digestive organs using the substitution treatment of CPO with saponified BSF-L oil was shown in Table 7. Substitution of CPO with saponified BSF-L oil did not influence the relative weight of the digestive organs of broiler chickens.

Table 6 Performance of broiler chickens with different levels of CPO substitution with saponified BSF-L oil

Broiler chickens performance	Ratio CPO and saponified BSF-L oil (%:%)				SEM	p-value
	3:0	2:1	1:2	0:3		
Grower phase (11-21 d)						
FI (g)^{ns}	869.00^{yz}	0887.57^{ab}	853.22^{ab}	846.50	6.01	0.063
BW (g)	987.14^{yz}	1005.93^{z}	973.73^{yz}	948.43^{y}	6.74	0.013
ADG (g/day)	057.80^{yz}	0059.55^{z}	056.57^{yz}	054.31^{y}	0.61	0.013
FCR^{ns}	001.37^{yz}	0001.36^{ab}	001.37^{ab}	001.42	0.01	0.228
Depletion (%)^{ns}	000.00 ^{yz}	0000.00^{ab}	001.43^{ab}	000.00	0.36	0.410
IP^{ns}	344.87 ^{yz}	0353.53^{ab}	333.33^{ab}	319.05	4.87	0.060
Finisher phase (22-35 d)						
FI (g) ^{ns}	1795.86^{y}	1764.71^{ab}	1740.51^{ab}	1587.52	30.39	0.063
BW (g)	2030.07^{zy}	1995.67^{zb}	1944.62^{yz}	1815.72^{y}	22.60	0.001
ADG (g/day)	0074.50^{zy}	0070.69^{zb}	0069.35^{yz}	0061.95^{y}	1.40	0.007
FCR ns	0001.72 ^{y}	0001.79^{ab}	0001.80^{ab}	0001.83	0.02	0.228
Depletion (%)^{ns}	0002.86 ^{y}	0002.86^{ab}	0004.29^{ab}	0001.43	1.01	0.820
IP	0328.03^{by}	0310.81^{ab}	0296.82^{ab}	0279.98^{a}	6.39	0.043
Total phase (grower and finisher)						
FI (g)	2664.86^{by}	2652.29^{b}	2593.74^{ab}	2434.02^{a}	33.19	0.043
BW (g)	2030.07^{zy}	1995.67^{z}	1944.62^{yz}	1815.72^{y}	22.60	0.001
ADG (g/day)	0066.71^{zy}	65.34^{z}	0062.78^{yz}	0058.37^{y}	0.87	0.001
FCR^{ns}	0001.60^{y}	1.63	0001.65^{ba}	0001.67	0.01	0.354
Depletion (%)^{ns}	0002.86 ^{y}	2.86	0005.71^{ab}	0001.04	0.36	0.544
IP	0353.61^{by}	341.80^{ab}	0316.96^{ab}	0307.01^{a}	6.52	0.031

^{ns} : not significantly different
$^{a, b}$: significant variances are indicated by distinct superscripts on the same row ($p<0.05$)
$^{y, z}$: significant variances are indicated by distinct superscripts on the same row ($p<0.01$)

Table 7 Length of digestive organs of broiler chickens with different levels of CPO substitution with saponified BSF-L oil

Length of digestive organs (cm)	Ratio CPO and saponified BSF-L oil (%:%)				SEM	p-value
	3:0	2:1	1:2	0:3		
Proventriculus ^{ns}	05.14^{b}	05.07^{b}	05.14^{b}	04.86^{b}	0.09	0.658
Ventriculus ^{ns}	05.87^{b}	06.06^{b}	05.86^{b}	05.91^{b}	0.07	0.788
Duodenum	36.14^{b}	31.64^{a}	32.14^{a}	30.23^{a}	0.75	0.026
Jejunum ^{ns}	81.14^{b}	76.43^{b}	82.29^{b}	74.36^{b}	1.42	0.150
Ileum ^{ns}	67.29^{b}	59.14^{b}	66.71^{b}	59.36^{b}	1.64	0.128
Cecum ^{ns}	39.50^{b}	37.97^{b}	42.14^{b}	38.29^{b}	0.71	0.149
Colon ^{ns}	21.86^{b}	20.79^{b}	25.86^{b}	21.29^{b}	0.80	0.087

^{ns} : not significantly different
$^{a, b}$: significant variances are indicated by distinct superscripts on the same row ($p<0.05$)

Digesta pH Value of Digestive Organs

The measurement of the pH value of the digestive organs with the substitution treatment of CPO with saponified BSF-L oil was detailed in Table 8.

Substituting CPO with saponified BSF-L oil in a ratio of 1:2 significantly increased the pH value of digesta in the ileum organ (P<0. 05). The smallest pH value was obtained without any substitution of CPO with BSF-L. Substitution of CPO with saponified BSF-L oil did not impact the pH value of the digesta of the proventriculus, ventriculus, and cecum.

Table 8 Relative weight of digestive organs of broiler chickens with different levels of CPO substitution with saponified BSF-L oil

Relative weight of digestive organs (%)[ns]	Ratio CPO and saponified BSF-L oil (%:%)				SEM	p-value
	3:0	2:1	1:2	0:3		
Proventriculus	0.37	0.32	0.38	0.40	0.02	0.329
Ventriculus	1.32	1.32	1.35	1.41	0.04	0.825
Duodenum	0.60	0.55	0.51	0.55	0.02	0.532
Jejunum	1.05	0.94	0.99	0.95	0.02	0.408
Ileum	0.70	0.63	0.64	0.64	0.02	0.618
Cecum	0.33	0.34	0.40	0.36	0.01	0.280
Colon	0.17	0.18	0.21	0.52	0.80	0.371

[ns] : not significantly different

Histomorphology of the Jejunum

Data on the measurement of the histomorphology of the jejunum with the substitution treatment of CPO with saponified BSF-L oil could be seen in Table 9. Substitution of CPO with saponified BSF-L oil did not affect its histomorphology.

Table 9 pH value of digesta of broiler chickens digestive organs with different levels of CPO substitution with saponified BSF-L oil

Digestive pH value of digestive organs	Ratio CPO and saponified BSF-L oil (%:%)				SEM	p-value
	3:0	2:1	1:2	0:3		
Proventriculus [ns]	4.41[b]	4.43[b]	4.59[ab]	4.50	0.07	0.807
Ventriculus [ns]	3.34[b]	3.37[b]	3.64[ab]	3.36[bb]	0.07	0.317
Ileum	4.60[a]	5.01[b]	4.80[ab]	4.73[ab]	0.06	0.058
Cecum[ns]	5.76[b]	5.49	5.81[ab]	5.59[ab]	0.07	0.383

[ns] : not significantly different
[a, b] : significant variances are indicated by distinct superscripts on the same row (p<0.05)

Tigh Junction Gene Expression

The results of tight junction gene expression by substitution treatment of CPO with saponified BSF-L oil were analyzed in Table 10. The expression of tight junction genes in the jejunal region of the intestinal villi, specifically in the JAM-2 and OCLN genes (P<0.05) and ZO-1 (P<0.01), may be markedly elevated by substituting saponified BSF-L oil for CPO. The CLDN-1 gene was unaffected when saponified BSF-L oil was substituted for CPO. In the intestinal villi of the jejunum, replacing CPO with saponified BSF-L in a 0:3 ratio produced the best outcomes for tight junction gene expression.

Table 10 Histomorphology of broiler chickens jejunum with different levels of CPO substitution with saponified BSF-L oil

Histomorphology of jejunum (mm) [ns]	Ratio CPO and saponified BSF-L oil (%:%)				SEM	p-value
	3:0	2:1	1:2	0:3		
Villi height (H)	1.39	1.34	1.39	1.54	0.05	0.502
Villi width (W)	0.22	0.20	0.18	0.21	0.01	0.215
The area of the villi (A)	0.26	0.24	0.23	0.28	0.01	0.627
Crypt depth (C)	0.22	0.20	0.23	0.21	0.01	0.708
H/C	6.17	7.06	6.65	7.48	0.39	0.698

[ns] : not significantly different

Table 11 Expression of tight junction genes of broiler chickens jejunum with different levels of CPO substitution with saponified BSF-L oil

Tight junction gene	Ratio CPO and saponified BSF-L oil (%:%)				SEM	p-value
	3:0	2:1	1:2	0:3		
CLDN-1[ns]	1.04[a]	1.13[a]	1.63[aa]	2.02[a]	0.15	0.068
JAM-2	1.08[a]	0.96[a]	1.45[ab]	1.83[b]	0.13	0.057
OCLN	1.06[a]	0.59[a]	0.88[aa]	2.69[b]	0.30	0.040
ZO-1	1.16[y]	1.20[y]	1.74[yz]	2.83[z]	0.21	0.006

[ns] : not significantly different
[a, b] : significant variances are indicated by distinct superscripts on the same row. ($p<0.05$)
[y, z] : significant variances are indicated by distinct superscripts on the same row. ($p<0.01$)

DISCUSSION

When broiler chickens were given a 2:1 ratio of saponified BSF-L to CPO, the birds performed better overall. Performance may suffer if feed substitution levels are raised to greater than 1%.. This occurred because BSF-L oil contained a lot of MCFA compounds, which were more easily absorbed and metabolized by the body than LCFA compounds, so the energy was produced swiftly. Furthermore, MCFA compounds served as an instant energy source rather than as a fat reserve (Takeuchi et al., 2008). The higher the MCFA contained in the ration, the faster the chickens's energy needed was met so that the chickens could quickly stop eating. However, other compounds needed, such as protein, had not been met. Chickens consumed rations to meet the energy needed for basic living, growth, production, and reproduction. When its energy was filled, the chickens stopped eating (Rizal, 2000) which increased the level of substitution using saponified BSF-L oil in the feed resulting in a paler and dustier feed color, resulting in decreased feed palatability. Palatability was influenced by the shape, smell, taste, texture, and temperature of the food served. According to Nastiti, (2010), broiler chickens preferred brightly colored food ingredients. The addition of oil to feed was useful for helping the solubility of fat-soluble vitamins and reducing the dusty nature of feed (Franz et al., 2010).

The length of the duodenum was reduced by the substitution of CPO with saponified BSF-L oil. The duodenum was a place for feed degradation with the help of enzymes originating from the pancreas. The shorter the duodenal canal, the shorter the enzyme penetration to ensure that the feed was not degraded optimally. This result was not in accordance with previous study teams who reported that the

digestive system's health could be improved by the addition of lauric acid, which was characterized by enhancing the digestive tract. Larvae containing, in particular, lauric acid are reported to have a modulating effect on lactic acid bacteria (LAB) (Chaklader, 2021). As the LAB population rises, the helpful bacteria in the small intestine flourish, and the pathogenic bacteria's ability to thrive will be inhibited by the extra short-chain fatty acids being produced (Kamal, 2016). A lengthy digestive tract that has developed in a broiler chicken's digestive system to maximize nutrient absorption is a sign of a healthy digestive system (Pertiwi et al., 2017).

The relative weight of organs was not affected by the substitution of CPO with saponified BSF-L oil. This finding was not in agreement with the opinion of other studies who stated that lauric acid could improve digestive tract health. The results of the study indicated that giving BSF-L had a significant effect on reducing the percentage of pancreatic weight and increasing the percentage of weight of the duodenum and colon (Rio et al., 2023). The growth of the relative weight of the digestive system organs to maximize food absorption is a sign of a healthy digestive tract in broiler chickens (Pertiwi et al., 2017).

The substitution of CPO with saponified BSF-L oil in a ratio of 2:1 increased the pH value of the ileum. The lauric acid content in BSF-L oil can modulate LAB growth. Chaklader (2021) stated that larvae containing, in particular, the modulating effects of lauric acid on LAB have been observed. Widodo et al. (2015) stated that LAB basically naturally exists in the chicken’s gastrointestinal tracts. The LAB requires suitable environmental conditions, one of which is pH in the range of 2 to 6.5. The health of broiler chickens can be improved by increasing the amount of LAB, which will result in better nutrient absorption from feed.

The histomorphology of the jejunum was not affected by the substitution of CPO with saponified BSF-L oil. This result did not follow other researchers who specified that lauric acid could improve villous health by increasing villous size. Glycerol monolaurate (GML) showed strong growth-promoting capacity and antimicrobial activity. In contrast, compared to the control group, the administration of 1 GML g kg-1 enhanced the jejunal muscle thickness and villi height (Amer et al., 2020).

Tight junction gene expression was greatly influenced by the substitution of CPO with saponified BSF-L oil. Increasing the level of substitution causes increased expression of tight junction genes. Lauric acid can modulate tight junction gene expression, resulting in increased cell permeability. Sodium salts of some MCFA, especially capric (C10) and lauric (C12) acids, can increase absorption by altering intestinal tight junction barrier function (Coyne et al., 2000). MCFA compounds can modulate permeability based on their effect on the main structural components of tight junctions. Additionally, compounds C10 and C12 cause actin to redistribute, which has been demonstrated to interact with tight junctions through its interaction with ZO-1 directly (Coyne et al., 2003).

Apart from its effect on livestock performance, feed component cost is also become an important factor to take into account, especially since saponified BSF-L oil is more expensive than other feed stuffs. For the growth phase, the control group's cost per kilogram of feed was 10,360 IDR, and for the finisher phase, it was 10,325 IDR. Meanwhile, the cost per kilogram of feed for broilers in the grower phase under CPO with saponified BSF-L oil treatment, with ratios of 2:1, 1:2, and 3:0, is 10,578, 10,792, and 11,047 IDR, respectively. As for the finisher phase under the same treatment, the costs are 10,540, 10,758, and 10,958 IDR, respectively. With the cost rise and broiler performance under consideration, the ideal level of saponified BSF-L oil was found to be at the 1% level, which corresponded to a CPO such as a BSF-L oil ratio of 2:1. The 1% inclusion level had an optimal impact on broiler performance without substantially increasing feed costs, which was the basis for this decision.

CONCLUSIONS

In conclusion, the substitution of CPO with saponified BSF-L oil in a ratio of 2:1 improved the performance of broiler chickens and the ileal digesta's pH value, however, the length of the duodenum was reduced. As the level of saponified BSF-L increased, the expression of tight junction genes increased. However, the histomorphology of the jejunal villi and the relative weight of the digestive organs was not affected. Based on this study, the substitution of CPO with saponified BSF-L oil in a ratio of 2:1 yielded the best results in improving the performance and characteristics of the digestive organs.

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AUTHOR CONTRIBUTIONS

The authors confirm contribution to the paper as follows: study conception and design: Chusnul Hanim.; draft manuscript preparation: Chusnul Hanim and Ega Anggi Lestari; data collection: Ega Anggi Lestari; analysis and interpretation of results: Chusnul Hanim and Ega Anggi Lestari.; and All authors reviewed the results and approved the final version of the manuscript.

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