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Improving the Quality of Tofu Waste by Mixing it with Carrots and Probiotics as a Feed Source of Probiotics and β-Carotene

I Gusti Nyoman Gde Bidura[1*], Ni Wayan Siti[1], Anak Agung Putu Putra Wibawa[1], Eny Puspani[1] and Desak Putu Mas Ari Candrawati[1]

[1]Faculty of Animal Science, Udayana University, Denpasar-Bali, Indonesia
***Corresponding author:** bidura_unud@yahoo.com

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ABSTRACT

The purpose of this study was to evaluate nutritional content of tofu waste mixed with carrot juice and fermented with Saccharomyces spp. (TWCF), so that it has potential as a feed supplement that is rich in β-carotene, high in protein and lysine, and contains probiotics. The laying hens (n=192, 70 weeks old) were randomly allotted into 4 diets groups. The hens were fed diets i) without TWCF as a control diets (TF0), ii) supplemented with 2% TWCF (B); iii) supplemented with 4% TWCF (C); and iv) supplemented with 6% TWCF (D). Inclusion of TWCF in diets increased nutrient digestibility (DM, OM, and CP) significantly (P≤0.05). Fermentation of TWC with YC (TWCF) can increase (P≤0.05) nutrient of TWC especially: CP, Lysine, and BC. Inclusion up to 2% TWCF can be used in laying hen diets to improve (P≤0.05) feed digestibility (DM, OM, CP), egg production, eggshell thickness, yolk color, BC, and yolk cholesterol. It is concluded that dietary supplementation of laying hens with TWCF can increase hen-day production, egg shell thickness, BC, and egg yolk color.

Key words: Carrot, Cholesterol, Tofu Waste, Yolk, Hens.

INTRODUCTION

Solid tofu waste is a by-product of tofu processing in the form of solids, and the production of tofu waste is quite high, ranging from 25-35% of the total tofu production (Kaswinarni 2007). The home industry for tofu processing in Indonesia is very large, because tofu products are becoming globally popular as a low-calorie vegetarian diet, so the solid waste tofu produced is very large, around 1,024 million tons/year (Ajijah et al. 2019).

In the tofu processing industry, solid waste generally produces around 40% of the total production capacity (Faisal et al. 2016). Tofu waste contains about 20.93% protein, 21.43% fiber, 10.31% crude fat, 0.72% calcium, 0.55% phosphorus, and 36.69% other compounds (Marlina and Askar 2004). However, the use of tofu waste as poultry feed is very limited, because it has high crude fiber and has an impact on poultry productivity (Sari et al. 2016; Witariadi et al. 2016; Nurhayati et al. 2019). Several researchers reported that the crude fiber and crude protein content of tofu waste having great variations, for example, 19.80 and 16.22% respectively reported by Nurhayati et al. (2019), and 24.03 and 14.93%, respectively reported by Mulia et al. (2015). Efforts to overcome the high content of crude fiber and to improve the quality of protein in tofu waste, especially the content of the amino acid lysine, is through a fermentation process. A low protein diet can be increased by adding the amino acid lysine (Jiang et al. 2021).

Fermentation is a simple method to increase the nutritional value of waste feed and the results are palatable (Bidura et al. 2012; Aristawati et al. 2019). As reported by Bulkaini et al. (2021), Bidura et al. (2012) and Fitasari and Santoso (2016) that the use of yeast Saccharomyces spp. or Lactobacillus plantarum in the fermentation process of waste feed (pineapple skin, rice bran, tofu waste), significantly increases the protein and lysine content in fermented feed products. Fermented feed products still contain Saccharomyces spp. as much as 107cfu/g and can act as probiotic cultures (Bidura et al. 2012). However, Mulia et al. (2015) reported that fermenting tofu waste with R. oligosporus significantly reduced crude protein and crude fat content in tofu waste. Feed supplements in general contain high protein and amino acids and must also contain high dyes (caretinoids). Beta carotene (BC) compounds, which are one of the most important carotenoids and can be converted into vitamin A, can increase antibody responses in host (Çalişlar, 2019).

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The BC content in tofu waste is low. As reported by Descalzo et al. (2012), soybean meal expeller (soybean meal) contains BC as much as 0.0mg/100g, while carrots contain BC as much as 3.2-6.1mg/100g (Yilmaz 2010). The nutritional profile of eggs can be improved by manipulating feed, because it will be very beneficial for the health of consumers (Çalişlar and Uygur 2010; Siti and Bidura 2021). For example, the enrichment of carotenoid content and yolk color can be increased with feeds that are high in BC content (Bidura et al. 2020; 2021).

This study aims to examine the nutritional contents of tofu waste mixed with carrots and fermented with Saccharomyces spp., with the purpose to evaluate its potential as a feed supplement rich in BC and lysine, and its effect, both in egg production and egg quality.

MATERIALS AND METHODS

Ethical Approval

Observations were made at the Laboratory of Microbiology and Animal Products Technology, Faculty of Animal Husbandry, Udayana University, Denpasar. While the observation of the feeding trial was carried out at the Research Station, Faculty of Animal Husbandry, Udayana University, Denpasar, in an experimental room and had been approved by the Animal Care Ethics Commission from the Faculty of Veterinary Medicine, Udayana University, Denpasar-Bali, Indonesia.

Experimental Design

A total of 192, 70-week-old Lohmann Brown hens were housed individually in open-sided cages that accommodate two rows of cages. Individual cages measuring: front 20cm; side 35cm; and 40cm high, made of strong aluminum wire. Feed was given twice a day at 07.30 and 16.30 and drinking water was available *ad libitum*. Hens weights were recorded at the beginning of the experiment and randomly distributed into 4 feed groups, and each feed group used 48 hens. Three treatments of feed containing TWCF (2, 4 and 6%), and one treatment of feed without TWCF as a control diet.

The composition of the nutrients in the diet was calculated based on the composition table of Scott et al. (1982), while the composition of TWCF was: metabolized energy (ME) 2945kcal/kg, crude protein (CP) 22.96%; lysine 2.15%; methionine 0.67%; crude fiber (CF) 17.98%; crude fat (EE) 2.65%; calcium (Ca) 0.32%; and phosphorus (P) 0.29%. All diets contained similar levels of CP: 18% and ME: 2750kcal/kg (Table 1).

Process of making flour tofu waste and fermentation

Before being fermented, fresh waste tofu (TW) was mixed with carrots in a ratio of 80:20 (g/g), then squeezed with double cloth and steamed for 30min (TWC). After cooling, TWC was then added 5% sugar+0.5% mineral mix+1% yeast culture (YC). After mixed well, then put in a plastic bag that has been given small holes and incubated for three days at room temperature. After three days, the fermented TWC (TWCF) was dried in the sun, then dried in an oven at 45°C for two days. After drying, it was then ground into fine flour and ready for laboratory analysis and implemented in feeding trials on hens. The flow chart of the TCF manufacturing process is presented in Fig. 1.

The force-feeding technique (Mustafa et al. 2004) was used to determine the digestibility of dry matter (DM), organic matter (OM), CP, and EE. All hens were fasted for 24 hours to ensure an empty digestive tract. Drinking water was available *ad libitum* during the collection period. Excreta and feed samples were collected and put into sterile tubes and then stored in the freezer for further analysis. Triplicate sample analysis was done to determine DM, OM, CP, and EE (AOAC 2005). Cholesterol in the yolk was measured by the UV-visible spectrophotometer method (AOAC 2005), and the measurement of BC in the yolk, as has been done by Bidura et al. (2020). One-way analysis of variance was done to determine the presence of significant differences (P≤0.05) and Duncan's multiple-range test was done to determine differences between treatments.

RESULTS

The content of DM, ME, CP, EE, crude fiber, minerals Ca and P, amino acids lysine and methionine, BC, and the amount of yeast *Saccharomyces spp.* in TWC fermented with YC is presented in Table 2. DM content in TWCF decreased by 0.75% lower than TWC without fermentation. ME content in TWCF was significantly 23.02% higher than TWC. However, the EE content of TWCF was 9.38% lower than that of TWC. The content of CP and amino acid lysine in TWCF increased respectively, 23.97 and 152.94% higher than TWC.

Fermentation of TWC with YC significantly (P≤0.05) increased the BC content of TWC, 127.69% higher than non-fermented TWC. In TWC, there was no *Saccharomyces spp.*, but in TWCF, the population of *Saccharomyces spp.* calculated are: 4.9x10^{7}cfu/g TWCF (Table 2).

Table 1: Nutrient content of rations for laying hens aged 70-80 weeks using TWCF

Composition ingredients, %	TWCF level in ration (%) 0	2	4	6
Yellow corn	51	49.5	46.5	47
Rice bran	8.2	14.4	15.8	16
Coconut meal	13	7.5	6.5	4.2
Soybean meal	9.54	8.5	8.5	8
Fish meal (CP: 58%)	10.84	11.65	11.65	11.65
Coconut oil	0.48	0.35	0.8	0.45
TWCF	0	2	4	6
Clamshell	6.94	6.1	6.25	6.7
Total	100	100	100	100
Nutrient (calculated)				
ME, kcal/kg	2750.64	2751.07	2752.05	2751.54
CP, %	18.01	18.01	18.12	18.02
EE, %	6.46	7.49	8.62	7.09
CF, %	4.54	4.74	5.06	5.09
Ca, %	4.24	3.89	3.97	4.19
P, %	1.93	1.8	1.83	1.92
Lysine, %	1.18	1.26	1.3	1.32
Methionine, %	0.4	0.42	0.43	0.43
Tryptophan, %	0.2	0.2	0.19	0.19

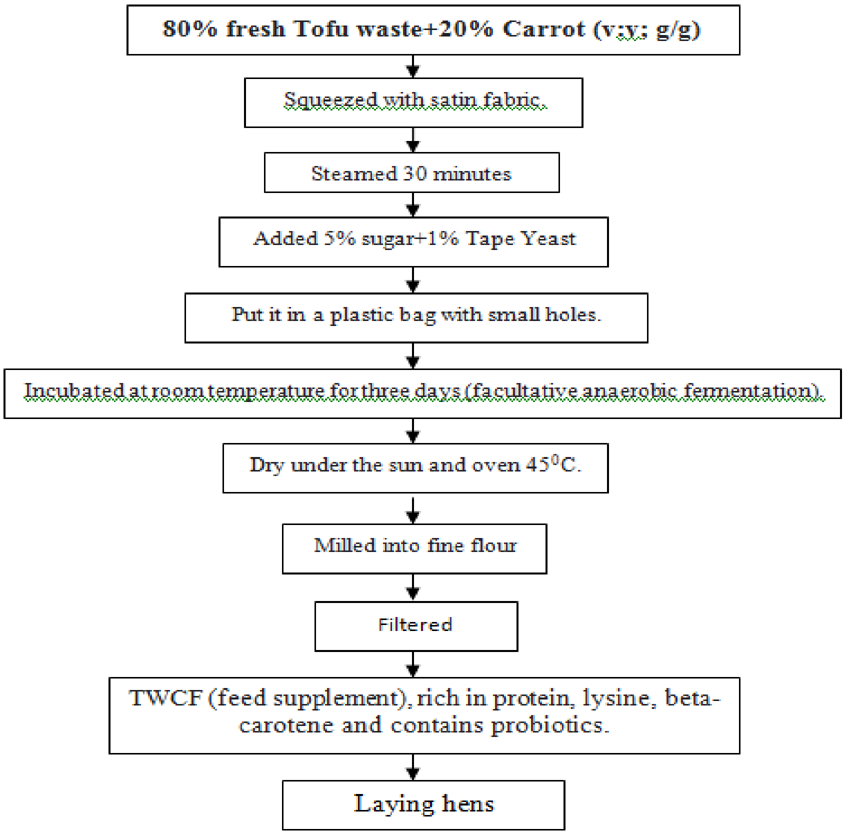

Fig. 1: Diagram of the process of making TWCF

Table 2: Nutrient content of tofu waste (TW), a mixture of tofu waste-carrot (TWC), and tofu waste-carrot fermented with *Saccharomyces spp.* (TWCF)

Nutrient	Units	TW	TWC	TWCF
DM	%	89.36±2.17	89.42±2.52	88.75±1.95
Gross energy	kcal/kg	3464.36±50.28	3325±61.35	4092±65.94
ME	kcal/kg	2472.81±72.53	2394±65.71	2945±70.92
Crude protein	%	19.13±1.39	18.52±1.05	22.96±1.37
Crude fat	%	2.18±0.017	2.24±0.031	2.65±0.015
Crude fiber	%	17.35±1.031	16.84±1.106	17.98±1.215
Calcium	%	0.25±0.001	0.37±0.001	0.32±0.001
Phosphorus	%	0.18±0.002	0.26±0.001	0.29±0.001
Lysine	%	0.96±0.015	0.85±0.021	2.15±0.014
Methionine	%	0.49±0.002	0.39±0.002	0.67±0.001
β-carotene	mg/100g	0.018±0.0001	0.065±0.0001	0.148±0.0001
Saccharomyces spp.	cfu/g	-	-	$4.9x10^7±0.13x10^6$

Table 3: Production performance of 70-80 weeks old Lohman Brown laying hens fed rations with fermented tofu waste (TWCF) supplementation

Variables	Units	Level TWCF in diets (%)				SE
		0	2	4	6	
Production performance						
Initial BW	g/bird	1757.39	1762.05	1755.94	1764.72	38.026
Final BW	g/bird	1850.15	1865.54	1870.38	1862.42	45.821
FC	g/bird/70 days	8251.20	8280.22	8324.85	8276.14	41.905
FC	g/bird/days	117.87	118.29	118.93	118.23	2.814
Egg numbers	egg/bird/70 days	56.94b	59.47a	60.33a	60.56a	0.705
Hen-day production	%	81.34b	84.95a	86.18a	86.51a	0.817
Egg weight	g/egg	55.95	56.83	57.02	57.18	0.503
Total egg weight	g/bird/70 days	3185.79b	3379.68a	3440.02a	3462.82a	49.072
FC: egg mass,	g/g	2.59a	2.45b	2.42b	2.39b	0.003
Digestibility						
DM digestibility	%	72.05b	75.83a	76.74a	76.92a	1.209
OM digestibility	%	73.18b	77.91a	78.95a	78.87a	1.108
CP digestibility	%	77.38b	79.06a	79.94a	79.69a	0.217
EE digestibility	%	78.07	77.81	77.75	77.24	1.082

The means with different alphabets within row are significantly different (P≤0.05).

Table 4: Effect of feed supplemented with TWCF on egg characteristics in laying hens aged 70-80 weeks

Variables	Level TWCF in diets (%)				SE
	0	2	4	6	
Egg shell, %	11.57a	11.52b	11.67a	11.60a	0.218
Egg yolk, %	30.09a	30.29a	30.37a	30.25a	0.506
Egg albumen (%)	58.34a	58.19a	57.96a	58.15a	0.714
Haugh unit, white height:egg weight	73.09a	73.23a	73.39a	73.46a	0.504
Egg shape, (egg width/egg length) x 100%	73.28a	73.92a	73.61a	73.75a	0.926
Specific gravity (weight:volume)	1.029a	1.031a	1.038a	1.035a	0.059
Eggshell thickness, mm	0.431b	0.468a	0.471a	0.475a	0.013
Yolk color, 1-15	8.05b	9.79a	9.68a	9.85a	0.413
β-carotene in yolk, μg/kg	1.375b	1.607a	1.638a	1.652a	0.035
Egg yolk cholesterol, mg/100g yolk	13.06a	12.19b	11.85b	11.78a	0.215

The means with superscripts ([a,b]) within row are significantly different (P≤0.05).

In Table 3, the response data of laying hens due to the administration of TWCF did not affect the feed consumption (FC), egg production, and egg weight compared to controls (without TWCF). The number of eggs during 70 days of observation in the TW1, TW2, and TW3 hen groups were (4.44, 5.95 and 6.36%) higher (P≤0.05) than control (TW0). Total egg weights for 70 days of observation in the TW1, TW2, and TW3 chicken groups were (6.09, 7.98 and 8.70%) significantly (P≤0.05) higher than control (TW0).

The average feed convention ratios (fc:egg weight; g/g) for 70 days of observation in the TW1, TW2, and TW3 group hen were (5.41, 6.56 and 7.72%) significantly (P≤0.05) lower than control (TW0).

The digestibility of DM, OM, and CP increased with the addition of TWCF (Table 3). Dry matter digestibility in the TW1, TW2, and TW3 group hen were (5.25, 6.51 and 6.76%) significantly (P≤0.05) increased compared to TW0. Likewise, the digestibility of OM in the TW1, TW2, and TW3 groups was (6.46, 7.88 and 7.78%) significantly (P≤0.05) increased compared to TW0. The digestibility of CP in the TW1, TW2, and TW3 group hen was (5.14, 5.89 and 5.57%) significantly (P≤0.05) higher than TW0 (control). However, the digestibility of EE was not significantly (P≥0.05) decreased with the addition of TWCF to the basal diet.

The inclusion of TWCF in the basal ration on the physical quality of eggs and yolk cholesterol levels in Lohmann Brown hens is presented in Table 4. The percentages of eggshells, egg whites, and egg yolks did not show any significant difference (P≥0.05) between treatment groups. Likewise, HU and BJ eggs did not show a significant difference (P≥0.05) between the treatment groups. The inclusion of TWCF in the basal diet in groups TW1, TW2, and TW3, significantly (P≤0.05) increased eggshell thickness, 8.58, 9.28 and 10.21% thicker than eggshell at TW0 (control).

The color of the egg yolks in the TW1, TW2, and TW3 hens groups was (21.61, 16.84 and 22.36%) significantly (P≤0.05) more yellow than TW0 (control). Likewise, the β-carotene contents of yolks in the TW1, TW2, and TW3 groups were (16.87, 19.13 and 20.15%) significantly (P≤0.05) higher than control (TW0). In contrast, the yolk cholesterol contents in the group: TW1, TW2, and TW3, were (6.67, 9.26 and 9.80%), significantly (P≤0.05) lower than control (TW0).

DISCUSSION

TWC fermentation with YC significantly increased the nutritional value of TWC. Similarly, Liu et al. (2015) reported that fermentation is a simple method to increase the nutritional value of feed and the results are palatable. The content of amino acids, vitamins, and nutrient digestibility increases after fermentation (Bidura et al. 2012; Bulkaini et al. 2021). According to Oboh and Elusian (2007), fermentation process leads to the biosynthesis of protein, essential amino acids, and vitamins, resulting in an increase in CP digestibility and CP quality. Suprihatin (2010) reported that the presence of enzyme activity produced by microorganisms during the fermentation process causes chemical changes to occur in organic substrates. According to Saferi et al. (2005), enzymes produced by YC can break down complex carbohydrates, such as cellulose, hemi sulolsa, and lignin, and can increase the availability of the amino acid lysine. Nurhayati et al. (2019) reported that the protein content of tofu waste increased 15.30% higher than the control (without fermentation) when fermented with 5% *Trichoderma viride* and increased by 15.80% when fermented with 2% *Saccharomyces cerevise*, on the other hand, the EE content decreased. The extracellular enzyme activity of YC in increasing the nutritional value of feed is by: (i) producing hydrolase enzymes that break down the cellulose and hemicellulose fractions, and (ii) secreting extracellular lignase enzymes by depolymerizing lignin (Perez et al. 2002). Reported by Nurhayati et al. (2019) that the crude fiber content of tofu waste decreased by 13.08% when fermented with *Trichoderma viride* and did not show any difference when fermented with *S. cerevise* compared to controls. The content of BC in tofu waste is low, whereas in carrots it is high. As reported by Descalzo et al. (2012), soybean meal expeller (soybean meal) contains BC as much as 0.03mg/100g, while carrots contain BC as much as 3.2-6.1mg/100g (Yilmaz 2010), yellow corn contains BC as much as 0.051mg/100g. Fermentation with *Trichoderma harzianum* can increase BC because this fungus is carotenoids (Jawad et al. 2016).

The addition of 2-6% TWCF to the basal ration of laying hens during the observation period (age 70-80 weeks), significantly improved egg production performance (total egg numbers, HD, total egg weight, feed efficiency, and nutrient digestibility). TWCF contains probiotic *S. cerevisiae* as much as $4.9x10^7$CFU/g. *Saccharomyces spp*. used in the TWC fermentation process can act as a probiotic in the digestive tract of hen, thereby increasing enzymatic activity and nutrient absorption (Zurmiati et al. 2014; Liu et al. 2015; Aristawati et al. 2019; Bidura et al. 2019b). Probiotic microbes can create a balance of intestinal microflora (Chang et al. 2019).

Supplementation of 2-6% TWCF to the basal diet can improve the internal quality of eggs. Eggshell percentage, yolk percentage, and shell thickness increased with the presence of TWCF in the ration. TWCF contains YC probiotics of $4.9x10^7$cfu/g TWCF. Probiotics in the digestive tract of poultry can increase nutrient digestibility, egg production, egg mass, shell thickness, Mg and Ca content in eggshells (Bidura et al. 2019ab; Bidura et al. 2021). The addition of TWCF in the feed can increase the content of CP and lysine. The increase in CP and lysine in feed greatly affects the external quality of eggs. The amino acid lysine is involved in physiological processes, particularly in the synthesis of body proteins, as well as aiding digestion and utilization of nutrients (Zeng et al. 2013; Liao et al. 2015). According to Prabowo et al. (2019), increasing the absorption of CP and mineral Ca will be able to improve protein synthesis in meat and eggs. This is evident in the research of Jiang et al. (2021), supplementation of the amino acid lysine in a low-protein diet significantly increased the synthesis of beef (breast meat and thigh) in chickens. The amino acid lysine can increase the body's protein synthesis, as well as aid digestion, nutrient utilization, and reduce nitrogen emissions (Zeng et al. 2013; Liao et al. 2015; Wang et al. 2018; van Harn et al. 2019; Jiang et al. 2021; Batool et al. 2021; Gul and Alsayeqh 2023).

The increased content of BC in TWCF will function as an antioxidant to prevent free radicals. Vitamins A and BC, have many benefits, including being useful in epithelialization of digestive cells, epithelial cell differentiation, reproduction, and the proliferation of intestinal mucosal cells, which will affect digestibility and feed efficiency. According to Çalişlar (2019), the role of BC in increasing host immunity is by increasing antibody responses and preventing acute respiratory infections in poultry. The inclusion of carrot flour in the feed significantly increased egg yolk color (Hammershoj et al. 2010; Souza et al. 2019; Bidura et al. 2021; Siti and Bidura 2021). The BC content in egg yolks ranged from 1.07-2.12g/kg (Kotrbáček et al., 2013) and 0.16-1.62 g/kg (Khan et al. 2012). Inclusion of fermented carrot leaves in the diet can increase BC concentration and yolk color (Bidura et al. 2020, 2021; Siti and Bidura 2021). Probiotics can reduce *Salmonella* infection in broiler chickens, so that chickens are healthier and nutrient absorption can be optimal (Chen et al. 2012; Yu et al. 2012; Wang et al. 2018). Chang et al. (2019) reported that feed supplementation with multi-strain probiotics enhances the gut microbiota and induces distinct cytokine expression patterns in *Salmonella* infection. According to Kogut and Arsenault (2015), *Salmonella* infection can reduce production performance and cause dysbacteriosis.

Total cholesterol and triglycerides in chicken blood serum decreased in the presence of TWCF in the diet. The BC content of fermented TWC (TWCF) was 127.69% higher than that of unfermented TWC (0.065 vs 0.148 mg/100g), as presented in Table 2. It was reported by Hsieh and Yang (2003) that BC can be increased during fermentation with *Trichoderma harzianum*, because this fungus is a carotenoid and capable of producing BC. The ability of BC in lowering cholesterol is related to the enzyme hydroxy methyl glutaryl-CoA (HMG). This enzyme plays a role in the formation of mevalonate in cholesterol biosynthesis. Cholesterol synthesis and BC synthesis together are via mevalonic and derived from acetyl CoA. If the increase in BC consumption is greater than saturated fatty acids, the biosynthesis process by the HMGCoA enzyme is directed at BC, so that saturated fatty acids are not converted into cholesterol (Nuraini 2006). High BC content in feed can reduce cholesterol levels in the serum (Bidura et al. 2020; Siti and Bidura 2021). The inclusion of fermented feed products in feed significantly reduces cholesterol content (Syahruddin et al. 2013). In addition, feed products that have been fermented by probiotics still contain probiotics, such as this TWCF feed. The use of probiotics 1.0g/kg in feed can be recommended to reduce serum and egg cholesterol levels effectively and optimal performance of chicken eggs (Ezema and Eze 2015).

The high content of hemicellulose arabinoxylan causes the use of tofu waste to be limited (Mahfudz 2006). Arabinoxylan can increase the viscosity of intestinal fluid (digestive viscosity) resulting in decreased fat and energy absorption. Dietary supplementation with carrot leaf flour has been shown to be effective in improving yolk color, lutein content, alpha-carotene, BC, and cholesterol content in yolks (Hammershoj et al. 2010; Bidura et al. 2021; Siti and Bidura, 2021). According to Çalişlar (2019), the role of BC in increasing host immunity is by increasing antibody responses and preventing acute respiratory infections in poultry.

Conclusion

Fermentation of TWC with YC (TWCF) can increase nutrients of TWC especially: CP, Lysine, and BC. Inclusion up to 2% TWCF can be used in laying hen diets to improve feed digestibility (DM, OM, CP), egg production, eggshell thickness, yolk color, BC, and egg yolk cholesterol.

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Authors' Contributions

All authors (IGNGB, NWS, AAPW and DPMAC) contributed equally to the research and writing of this article.

Conflict of Interest

There is no conflict of interest related to this research.

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