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Chemical and Microbiological Quality of Broiler Meat Supplemented Fermented Pursalane *(Portulaca oleracea L.)* Flour in Commercial Diets

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ABSTRACT

The aim of this research was to know the effect of supplemented fermented purslane flour (FPF) in diet on the chemical quality, and microbiology of broiler chicken meat. One hundred DOC were used in this research. This research used a completely randomized design with 5 treatments and 4 replications. The treatments were K0 = commercial diet without FPF, K1 = commercial diet + 2.5% FPF, K2 = commercial diet + 5% FPF, K3 = commercial diet + 7.5% FPF, and K4 = commercial diet + 10% FPF. The variables studied were water content, protein content, and cholesterol levels of meat and contamination of *Salmonella* sp. Data were analyzed using Analysis of Variance (ANOVA). The results showed that the supplemented fermented purslane flour in diet significantly affected the water content and protein content of meat, but it did not significantly affect the cholesterol meat levels and contamination of *Salmonella* sp. It was concluded that the supplemented of fermented purslane flour in broiler chickens with a level of 10% was the best level. At this level, chicken meat is produced with the lowest water content while the highest protein content.

Keywords: Chemical quality, Meat, Microbiology, Pursalane fermented

Introduction

The number of poultry meat consumption has been undergoing increment year by year. Data from Ditjen PKH (2016) revealed that capita consumption of broiler chicken meat in 2016 was 5,110 kg, a 6.52% rise compared to consumption in 2015 which was 4,797 kg. Moreover, the capita broiler meat consumption from 2011 to 2017 were 3,650 kg; 3,494 kg; 3,650 kg; 3,963 kg; 4,797 kg; 5,110 kg, and 5,683 kg respectively. The high demand of broiler meat should be followed by the improvement of meat quality either chemically and microbiologically.

One challenges faced by farmers in producing high quality broiler meat is the increasing cholesterol content that increases as the chicken grow older. Saidin (2000) reported broiler meat contains cholesterol as many as 110 mg/100 gram fresh meat. The high cholesterol intake can lead to cardiovascular disease and stroke.

Furthermore, another challenge in broiler meat production is that the high nutrient content enables various microorganism to grow well – spoiling and reducing the meat quality. Dewi *et al.*, (2016) stated that meat belongs to high nutritious animal-based food that beneficial for human growth and health. It is also a good medium for microorganism. According to BSN (2009), animal-based food, including broiler meat should be free from microorganism such as *Salmonella sp.*

The aforementioned challenges can be alleviated by providing feed additive containing active compound that able to reduce the cholesterol level and limit the pathogen microorganism growth that can impair the meat quality. Unsaturated fatty acid such as omega 3 and 6 are known to have positive health outcomes. Tuminah (2009) stated that unsaturated fatty acid can lessen the blood's low density lipoprotein (LD) without affecting its high density lipoprotein (HDL) level. Meanwhile, antibacterial compounds such as saponin, flavonoid, and tannin can be used to hamper the bacteria growth. Moreover, tannin can act as growth inhibitor for various microorganism (Hendradjatin, 2009).

All chemical compounds contained by purslane (*Portulaca oleracea L.*). purslane is known as one of weeds that possesses great potency as animal feed. It contains high concentration of omega 3 and 6 that provide health benefits. Simopoulus *et al.* (1995) reported that purslane contains linoleic acid (in mixture: 704 to 18,245 ppm; within seed: 67,686 ppm),

linoleate acid (in mixture: 3,221 to 64,315 ppm; within seed: 7,226 ppm), alpha-linoleate acid (4,000 to 80,000 rpm), oleic acid (in mixture: 16 to 2,160 ppm; within seed: 49,935 ppm), omega 3 (30,000 ppm). Polyunsaturated fatty acids (PUFA) consumption, specifically omega 3 plays vital roles in prevent coronary heart disease (PJK) that is related to triglyceride and very low density lipoprotein (VLDL) level in blood plasma (Supari, 2000). Furthermore its its reported that omega 3 consumption as much as 0.35 gram/day is related with the reduction of death cases caused by PJK by 29% in 2 years. Purslane also contains antibacterial agents such as saponin, flavonoid, and tannin. A study done by Batari (2007) revealed that purslane contains 4.05 mg/100 gram of flavonoid.

Although purslane is potential to be used as animal feed, it has high crude fiber as limiting factor. purslane has 17% of crude fiber. To overcome this limiting factor, purslane is required to be fermented. Fermentation process can be carried out by using probiotics (Hafsah, 2003), among of them is Effective Microorganism-4 (EM-4). EM-4 is one of affordable probiotic that can be easily found in the market. EM-4 comprises 90% of *Lactobacillus* sp (lactic acid bacteria), phosphate solvent, photosynthetic bacteria, *Streptomyces sp*, and cellulose-decomposer fungi. Per one liter, EM-4 contains 1.5×10^6 cfu/mL *Lactobacillus casei*, 1.5×10^6 cfu/mL *Saccharomyces cerevisiae*, and 1.0×10^6 cfu/mL *Rhodopseudomonas palutris*.

Numerous studies have reported that the use of purslane as animal feed. Manafe *et al*. (2017) substitute purslane flour in broiler chicken feed to evaluate its effects on the chicken performance. The study found that substitution of purslane flour as many as 10 to 15% reduced the broiler chicken performance. It was associated with the increasing crude fiber content by by 5.89 and 6,52% increment for 10% and 15% of substitution respectively. Suciani *et al*. (2011) stated that broiler chicken are not able to digest high content of crude fiber as it will lead to the poor efficiency of nutrient utilization.

Tulanggalu *et al*. (2017) fed broiler chicken with purslane flour to evaluate its effects on the physical meat quality. Mulik (2016) also conducted a study using purslane flour as feed additive for broiler chicken to evaluate its effects on the level of cholesterol, omega 2, and omega 6 on the meat. Karlina *et al*. (2013) evaluated the efficacy of purslane extract as an antibacterial agents for *Staphylococcus aureus* and *Escheria coli.*

Unfortunately, there are small information circulated regarding fermented purslane (TKT) supplementation as feed additive and its effects on chemical and physical broiler meat quality. Thus, this study was carried out for the cause.

Materials and Methods

Material

This study used 100 day-old chicks (DOC) of CP 707 broiler chicken (unsexed), obtained PT Charoen Pokphand Oefafi. EM-4 was purchased from agricultural store in Kupang. Purslane used on this study was obtained from agricultural fields surround the Tesabela village, Pantai Baru municipality, Roto Ndao regency. The chemical composition of pre- and post- fermentation of purslane flour is presented on Table 1, while the nutrient value of feed used on this study is shown on Table 2.

Equipment used on this study include knife, cooling box, petri dish, glass tube, volumetric pipet, colony counter, scissor, tweezers, inoculation needle, bunsen burner, pH meter, vortex, incubator, autoclave, sterile cupboard, analytical balance, filter paper, Soxhlet apparatus, oven, blender, Kjeldahl apparatus, erlenmeyer, and spectrophotometer. Chemical reagents used on this study were plate count agar (PCA), buffered peptone water 0.1% (BPW), trysote (TB), reagenkovac, triple sugar irogan agar (TSIA), Salmonella-shigella agar (SSA), methyl red-voges proskauer (MR-VP), urea broth and simmons citrate agar (SCA), petroleum ether, H_2SO_4, distilled water, boric acid, bromo cresole green indicator, red methyl indicator, $NKH(IO_3)2$, alcohol, chloroform, and acetate anhydride.

Method

Grinding purslane into flour. Purslane wan taken out and separated from its roots. Its stems and leaves were cut into fine forms wits its particle size approximately 3 cm, then dried under sunlight for 6 days. The dried purslane was then ground by using grinder into flour.

Purslane fermentation. (a) Starter preparation (Winedar *et al*., 2006), was made by mixing EM-4 with molasses (1:1 v/v; 50 ml EM-4 and 50 ml molasses added with water). (b) Fermentation process (Winedar *et al*., 2006), was applied to purslane flour (40 ml : 60 g). The mixture was then covered with plastic cover and placed avoiding direct sunlight. Fermentation process was carried out for 4 days and mixed

Tabel 1. Chemical composition of purslane flour before having a fermentation and after having a fermentation

Nutrient content	purslane flour	Fermented purslane flour
Dry matter (%)	87.72	80.28
Organic matter (%)	74.93	80.28
Crude protein (%)	9.61	13.79
Crude fat (%)	3.03	0.81
Crude fiber (%)	15.90	14.48

Source: Chemical analysis at Laboratory of Feed Chemistry Universitas Nusa Cendana (2018).

Tabel 2. Chemical composition or nutrient value of diet

Diet	Chemical composition (%)						Metabolizable energy (Kkal/Kg)
	Dry matter	Organic matter	Crude protein	Crude fat	Crude fiber	Nitrogen free extract	
K0	91.99	92.08	19.33	5.03	4.83	62.88	2,800
K1	94.00	94.09	19.67	5.05	5.22	64.13	2,865
K2	96.00	96.09	20.02	5.07	5.60	65.38	2,900
K3	98.01	98.10	20.36	5.09	5.99	66.63	2,985
K4	99.80	99.90	20.71	5.11	6.30	67.88	3,000

Note: K0 = Basal diet (commercial), K1 = basal diet + 2.5% fermented purslane flour, K2 = basal diet + 5% fermented purslane flour, K3 = basal diet 7.5% fermented purslane flour, K4 = basal diet + 10% fermented purslane flour.

every 24 hours. After getting fermented, the mixture was then dried without direct sunlight for 2 days. The product was then added to basal diet accordingly.

Animal rearing. The temperature of the animal housing were recorded as follow: morning (29°C), noon (31°C), and night (28°C). During rearing period, chicken was vaccinated with ND vaccine. After getting brooded for one week, DOC were fed accordingly based on the treatment groups. Feed and drinking water were provided ad libitum. After 4 weeks of rearing, chicken were slaughtered (2 chicken for each group of treatments).

Experimental design. The study was performed by using completely randomized design that consisted of 5 treatments with 4 replications. The treatment groups were K0 = Basal diet (commercial), K1 = basal diet + 2.5% fermented purslane flour, K2 = basal diet + 5% fermented purslane flour, K3 = basal diet + 7.5% fermented purslane flour, K4 = basal diet + 10% fermented purslane flour. The treatments were based on study reported by Mulik (2016) that used purslane flour as many as 2.5%, 5%, and 7.5%. The study found out that purslane supplementation up to 7.5% still reduced the cholesterol content of meat. Thus, to explore the efficacy of purslane flour in reducing cholesterol content, the treatment on this study was adjusted to 10%.

Variables observed on this study were chemical composition (water content, protein content, and cholesterol content of meat), microbiological aspect (Salmonella sp) of broiler chicken aged 1 month. Meat sample was obtained from breast muscle. All data were then subjected to ANOVA analysis and Duncan Test if there is significant differences between groups.

Result and Discussion

Effects of treatments on the water content of broiler chicken meat

Table 3 illustrates the average of water content of broiler chicken meat on this study. The highest water content was observed on K0 group in which it contained 75.25% water, while the lowest was observed on K4 group (73.23%). The fermented purslane flour fermentation reduced water content of the meat significantly by 10% (P<0.05%).

Fermented purselen flour supplemented as many as 10% (K4) resulted in the highest water content on meat as the water content started to increase on K3, K2, K1, and K0 group, which the last one resulted the poorest quality. The reduction of water content on meat from K4 group might be caused by the fiber content on fermented purselen flour. The fermentation process was intended to reduce the high fiber content of the flour. However, as the supplementation was increased to higher level, the amount of fiber content would follow to increase. As shown on table 2, the fiber content of K0 was 4.83%, while K4 diet contains 6.30% fiber. This crude fiber content played role in reducing the water content of broiler chicken meat.

It is unknown yet, how the mechanism of fiber in lessening the water content of broiler chicken meat. However, previous study reported that feeding broiler chicken with feed containing fiber produced less water content on the meat. Poedjiadi (2005), cellulose which makes up the cell wall of plant has ability to absorb water, thus can reduce the water content on meat.

The water content reduction on broiler chicken meat was also caused by the protein content of the meat itself which able to bind water, especially the free water. Free water is available outside the meat, thus, it is easier to loss. Moreover, the water available in dynamic shape is in labile state that easy to move if subjected to any changes (Afrila and Santoso 2011). It is supported by Lawrie (2003) who stated that meat protein can bind meat water. The high meat protein content enable the increment of water retention on the meat. Thus, it reduces the number of free water, and vice versa.

Water content of meat on this study ranged from 73.23% to 75.25%. This finding tends to be lower than other previous studies, such as one that reported by Estancia et al. (2012) in which water content on broiler chicken meat ranged from 74.75% to 75.98%.

Effect of treatments on the protein content of broiler chicken meat

Table 2 shows that the average of total cholesterol found on the broiler chicken meat on this study was 80.77 g/100 gram as the highest (KR1) and 60.64 mg/100g as the lowest (K4). The statistical analysis revealed that the supplementation of fermented purslane flour did not have any significant effects on reducing the cholesterol level of the meat (P>0.05). However, empirically the supplementation resulted in the tendency on the reduced cholesterol level of the meat.

Tabel 3. Chemical and microbiological composition of broiler chicken meat supplemented with fermented purslane flour in commercial diet

Variables	Treatments					SEM	P value
	K0	K1	K2	K3	K4		
Water content (%)	75.25[b]	75.20[ab]	73.83[ab]	73.74[ab]	73.23[a]	0.33	0.02
Protein (%)	17.96[a]	18.43[ab]	18.56[ab]	18.66[ab]	19.11[b]	0.474	0.05
Cholesterol (mg/100g)	80.77	81.32	75.03	71.80	60.64	3.32	0.22
Salmonella sp. contamination (Cfu/g)	Negative	Negative	Negative	Negative	Negative	-	-

[a,b] different superscript on the same row indicates significant difference between groups (P<0.05)
Note: K0 = Basal diet (commercial), K1 = basal diet + 2.5% fermented purslane flour, K2 = basal diet + 5% fermented purslane flour, K3 = basal diet + 7,5% fermented purslane flour, K4 = basal diet + 10% fermented purslane flour.

Supplementation of fermented purselene flour on broiler chicken feed had not been able to deliver significant effects on meat cholesterol level. It might be resulted from the possibility of omega 3 and 6 losses during the fermentation process. According to Suharyanto et al. (2006), the increment of temperature during fermentation can avert the desaturase enzyme activity. Thus, reducing the unsaturated fatty acids formation. As fermentation take place longer, the fatty acid content will keep decreasing. This proposed explanation is supported by Sudaryatiningsih and Supyani (2009) who reported that 6 hours fermentation by using *Rhizopus oryzae* give the best result in linoleic and linoleate fatty acid formation. If the fermentation undergoes longer than 6 hours, those 2 fatty acids levels will decrease along with the fermentation time.

The aforementioned condition caused the proportion of omega 3 and 6 was not on best ratio to be able to reduce the cholesterol content. Mulik (2016) reported that supplementation 7.5% of purslane flour on broiler chicken diet produced meat containing omega 3 and 6 with 1:5 ratio. At that point, cholesterol level of the broiler chicken meat was able to be reduced by 24%.

Simopulus et al. (1995) reported that linoleic acid contains linoleic acid (in mixture: 704 to 18,245 ppm; within seed: 67,686 ppm), linoleate acid (in mixture: 3,221 to 64,315 ppm; within seed: 7,226 ppm), alpha-linoleate acid (4,000 to 80,000 rpm), oleic acid (in mixture: 16 to 2,160 ppm; within seed: 49,935 ppm), omega 3 (30,000 ppm). Unfortunately, the omega 3 and 6 content that were expected to have optimum effects was not seen on this study.

Effects of treatments on the *Salmonella sp* contamination on broiler chicken meat

Table 3 shows that purslane flour supplementation resulted meat with similar quality, in which *Salmonella* sp contamination was not observed among all groups (negative). Thus, this study confirmed that broiler chicken receiving either purslane flour supplemented or unsupplemented feed did not have any difference in terms of the microbial contamination on the meat.

The finding indicated that there were other external factors other than chemical compounds on fermented purslane flour that cased the similar meat quality among groups. The negative value of Salmonella sp contamination on this study was caused by the fact that meat sample were directly observed after slaughtering (0 hour). On this case, Salmonella sp. commonly has not grown on the meat. This is supported by Riwa (2015) who showed that purslane flour supplementation on broiler chicken produced meat that free from Salmonella sp contamination if the observation was performed right after the slaughtering process (0 hour). Moreover, the increasing number of Salmonella sp will occur along the storage time (12 hours to 24 hours in room temperature).

The uncontaminated meat on this study was also a result of rearing process that carried out hygienically on all groups – indicating the high microbiological quality of the meat. Kusumaningrum et al. (2013) stated that the microbe sources on the meat are commonly from animal body, respiratory tracts microbes, or digestive tracts. Animal products contaminated with feces from digestive tract have greater potency to be contaminated with Salmonella sp. (D'Aoust, 2000; Sams, 2001). However, with good management and standardized process, Salmonella are rarely to be found on animal products (Siagian, 2002).

Conclusions

The supplementation of fermented purslane flour on broiler chicken at 10% level gave the best result. At that level, the meat contained the lowest water content and highest protein content. The cholesterol level of meat and Salmonella sp contamination on the meat were not affected by the supplementation.

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