



Calcined Mussel Shell from *Corbicula Sumatrana*, A Natural Preservative and Mineral Supplement of Fishmeal for Feeding Laying Quails

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Abstract | The fishmeal produced from overflowed fish by traditional steam cooking is susceptible to bacterial activity and nutrient degradation during storage due to insufficient drying and sterilization. Calcined bivalve shell had antimicrobial effects and was potentially used as a preservative ingredient to improve fishmeal's shelf life and nutritional value. The present research aimed to study the beneficial effect of calcined mussel meal (CMM) as a preservative agent to improve fishmeal's storage stability and nutritional values. CMSM was mixed with fish meal in four levels: 0, 3, 6, and 9% (w/w) and then stored at room temperature for 45 days. Samples were taken on days 0, 15, 30, and 45 and analyzed for proximate nutrients, physical properties, and bacterial colonies. Their nutritional values were evaluated by mixing the calcite-preserved fishmeal with a basal diet of 5% and feeding it to 200 laying quails for eight weeks. The feeding trial had four treatments as a basal diet combined with fishmeal containing 0, 3, 6, and 9% CMSM. Each treatment was composed of 5 replications (@ ten birds). Parameters measured included feed intake, egg production, FCR, eggshell, and tibia bone mineralization. Results found that 3-9% CMSM increased crude ash and densities of fishmeal and inhibited the increase in moisture content, protein degradation, and the development of bacteria during storage. Using fish meals containing 3 and 6% CMSM in the diet improved egg production and feed utilization efficiency of laying quails. In conclusion, the calcined lake mussel shell meal could be used 3-6% to improve fishmeal storage stability and nutritional values.

Keywords | Calcite, Fish meal, Mussel shell, Nutritional values, Physical properties, Laying quails

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INTRODUCTION

The West Sumatra province of Indonesia, located along the coastal area of the Samudra Hindian Ocean, had a

coastline of 1973.25 km and a marine exclusive economic zone (ZEE) of 51,060.23 km² (Adry *et al.*, 2018). The potential for marine fish production is about 303,105-312,550 tons/year (Adry *et al.*, 2018). Fishermen living in the coastal

areas dominated by traditional small-scale fish catching using small boats and simple mechanized fishing vessels and gears (hooks and lines, gillnet, bag nets, trap, and seines). During a good fishing season, fish caught often exceed the local market capacity, and low-price fish are challenging to sell since fish processing and fish meal manufacturing plants are not well established in this region (Khalil *et al.*, 2020). The overflowed and low-price fish are potentially processed into fishmeal to increase their economic value. Monoarfa *et al.* (2016) reported that processing overflowed fish into fish meal in Central Sulawesi increased their sale value by about 200%.

Fishmeal is a high-quality protein ingredient primarily used to balance essential amino acids in poultry, monogastric, and aquaculture diets. Fishmeal is also a good source of minerals and trace elements (Jeyasanta and Patterson, 2020).

Our previous study found that fish meal produced from the overflowed and low economic value fishes by traditional steam cooking had a considerably high content of crude protein (70-75%), low moisture (3.7-5.7%), and crude fat (1.0-3.5%) (Khalil *et al.*, 2020). The product meets the best quality (grade 1) fish meal based on the Indonesian national standard of a minimum of 60% crude protein and a maximum of 10% moisture, 8% crude fat, and 20% crude ash (SNI, 1996). However, the local fishmeal is presumably susceptible to bacterial activity during storage due to high protein content and insufficient sterilization of the traditional steam cooking and sun drying, which might negatively affect the product's microbial quality, nutrient content, and nutritional value. The traditional processing methods might not adequately eliminate bacterial contamination, posing a risk to both fish and, potentially, to humans through the food chain. This bacterial activity can further degrade the nutrients in the fishmeal, compromising its quality and effectiveness as an animal feed component. Since modern drying and sterilization techniques are not affordable for small-scale fishermen due to limited capabilities, using a preservative agent produced using locally available materials might be an appropriate alternative to maintain the microbial quality and nutritional value of stored local fish meals.

The province of West Sumatra is rich with various types of marine and freshwater shellfish, clams, oysters, and mussels. The freshwater mussel (*Corbicula sp*) lives in four prominent lakes of Maninjau (Agam district), Singkarak (Tanah Datar district), Danau Diatas, and Danau Dibawah (South Solok district). There are two types of *Corbicula sp*, namely *C. moltkiana*, which is found in Maninjau Lake, and *C. sumatrana*, which lives in Singkarak and Kembar Lake (Zeswita *et al.*, 2016). Dried lake mussel shell part, which counted about 56% intact body weight mussel, had a higher yield rate than the seashell and could be used as feed in the

form of raw coarse, raw fine, and roasted meals as calcium supplement in the livestock diet (Khalil *et al.*, 2015; Khalil *et al.*, 2018). The shells could be calcinated by burning at high temperatures to produce calcite. Calcination eliminates water and organic matter and converts calcium carbonate into calcium dioxide (CaO) (Ozer *et al.*, 2006). Calcination increased essential mineral concentration, finer particle percentage, and surface area (Ozer, 2003; Ha *et al.*, 2019), resulting in better digestibility and absorption of the essential mineral. Our previous study found that calcination of mussel shells increased calcium and zinc concentration, specific and taped densities, and enhanced feed intake and nutritional values of bivalve shells for Muscovy duck starter (Khalil *et al.*, 2021; Khalil *et al.*, 2022).

Many studies reported that the calcined oyster shell, which is mainly composed of CaO, had potent antibacterial activity (Oikawa *et al.*, 2000; Sawai, 2011; Li *et al.*, 2014; Yao *et al.*, 2014) and was potentially used as a natural preservative agent for fishmeal. The antibacterial activity of calcined bivalve shells is mainly related to the alkaline effect caused by the hydration of CaO (Oikawa *et al.*, 2000; Yao *et al.*, 2014). Oikawa *et al.* (2000) mentioned that calcined oyster shells have been used as a calcium supplement for a long time in food manufacturing. Rusdaryanti *et al.* (2020) reported that using 3.5% calcined blood cockle shell powder effectively inhibited *Escherichia coli* in food industries.

Fishmeal mixed with CMSM might have better nutritional value, especially for poultry of young or small body size and other livestock animals. It is therefore hypothesized that adding CMSM to fishmeal at a certain level is expected to prolong the shelf life of the fishmeal, provide a source of minerals, and increase the nutritional value of a diet containing calcite-mixed fishmeal for laying quail. The present research aimed to study the beneficial effect of CMSM as a preservative agent to improve fishmeal's storage stability and nutritional values for feeding laying quails.

MATERIALS AND METHODS

PREPARATION OF CALCINED OYSTER SHELL MEAL

A calcined mussel shell was prepared using a freshwater mussel shell (*Corbicula sumatrana*). Fresh mussels were collected from fishermen of Singkarak Lake, Tanah Datar district, West Sumatra, and processed into calcite according to the procedures described by Khalil *et al.* (2021). Intact mussels were washed and boiled under running water to remove the inner fleshy part. The clean shells were dried under the sun. The dried shells were crushed and calcined by burning in a modified furnace made from a metal drum for about 48 hours at an 800-900°C temperature until the shell turned white and brittle. The calcined shells were ground to pass through a 150 µm sieve into meal form to produce calcined mussel shell meal (CMSM).

Table 1: Composition, crude nutrient, and energy content of experimental diets.

Feed ingredients	Experimental diets containing fish meal mixed with CMS (%)			
	0	3	6	9
	P0	P1	P2	P3
Yellow corn	33.0	34.0	36.0	36.0
Rice bran	5.0	5.0	5.0	5.0
Fish meal+0% CMSM	5.0	-	-	-
Fish meal+3% CMSM	-	5.0	-	-
Fish meal+6% CMSM	-	-	5.0	-
Fish meal+9% CMSM	-	-	-	5.0
Soybean meal	24.0	24.0	25.0	24.0
Coconut oil	5.0	4.0	4.0	4.0
Coconut meal	10.0	9.0	8.0	8.0
Palm kernel meal	9.0	9.0	8.0	9.0
Premix ¹	0.5	0.5	0.5	0.0
Kitchen salt	0.5	0.5	0.5	0.50
Bone meal	3.0	4.0	4.0	4.0
Limestone meal	5.0	5.0	4.0	4.0
	100.0	100.0	100.0	100.0
Nutrient and energy composition				
Metabolizable Energy (kcal/kg)	2,677	2,609	2,666	2,661
Crude protein (%)	20.6	20.3	20.1	20.0
Crude fat (%)	6.5	6.2	6.4	6.4
Crude fiber (%)	4.2	4.9	4.9	4.9
Calcium (%)	3.2	3.1	3.2	3.3
Phosphorus (%)	0.7	0.8	0.8	0.9
Dry matter (%)	90.9	90.8	90.8	90.5

¹Supplied per kilogram of diet; **vitamin A:** 10,000 IU; **cholecalciferol:** 2,000 IU; **vitamin E:** 10 mg; **vitamin K3:** 2 mg; **thiamine:** 1 mg; **riboflavin:** 5 mg; **pyridoxine:** 2 mg; **vitamin B12:** 0.0154 mg; **niacin:** 125 mg; **calcium pantothenate:** 10 mg; **folic acid:** 0.25 mg; **biotin:** 0.02 mg; **BHT:** 30 mg; **selenium:** 0.1 mg; **iron:** 40 mg; **copper:** 12 mg; **zinc:** 120 mg; **manganese:** 100 mg; **iodine:** 2.5 mg; **cobalt:** 0.75 mg.

PREPARATION AND STORAGE OF FISHMEAL

Pony fish (*Leiognathus equula*), an overflowed and low-price type of fish, was used as raw material for fishmeal. The fish were obtained from the local fishermen at the fish landing center of Teluk Kabung subdistrict, Padang City, West Sumatra. The intact fish of about 160 kg were processed into fishmeal in six processing steps: chopping, steam cooking, pressing, evaporating, drying, and grinding according to the method of steam cooking for the local level described by Abowei and Tawari (2011) and Khalil *et al.* (2020). The process started by chopping and crushing the fish into porridge form. The porridge fish were placed and wrapped

in a sisal cloth, steamed continuously in a cooker for 60 minutes, and then directly squashed and compressed using two thick wooden boards to remove the water and oil. The dehydrated cooked fish were dried in the sun and ground to produce steam-cooked fish meal products. The product quality meets the best grade (grade 1) of the national quality standard for fish meal, which contains a minimal 65% crude protein, a maximal 10 % moisture, and 8% crude fat (Table 3) (SNI, 1996).

The fishmeal product of about 24 kg was divided into four equal parts (@ 6 kg). Each part was mixed with CMSM in four levels of 0, 3, 6, and 9% (w/w) and then subdivided into 12 packs (@ 500 g) labeled paper bags. The packaged products were stored at room temperature for 45 days in the Poultry Nutrition Laboratory, Faculty of Animal Husbandry, Andalas University, Padang. Samples are taken on days 0, 15, 30, and 45 for organoleptic tests and analysis of physical properties, moisture and crude nutrients, and bacterial colonies. The organoleptic test included color, aroma, and texture and was assessed by twenty trained panels. Questionnaires for the panelists were prepared using the modified 5-point hedonic scale (1-5) according to the procedure described by Obemaeta and Christopher (2012) and the Indonesian National Standard Board (SNI, 2006). The physical properties of bulk density, specific density, and angle of repose were measured according to the testing procedure described by Ogunsina *et al.* (2009) and Rutloff (1981). The proximate composition of moisture, crude ash, crude protein, and crude fat was analyzed according to the standard methods of AOAC (2000). The total bacteria colony was determined according to the cup count method Wattimena *et al.* (2020) described.

FEEDING TRIAL

A feeding trial was conducted to evaluate the nutritional value of calcite-preserved fishmeal by mixing it with a basal diet and feeding it to 200 laying quails for six weeks. There were four dietary treatments of basal diet + 5% fishmeal mixed with 0, 3, 6, and 9% CMSM, respectively. Basal diets comprise corn, rice bran, soybean meal, coconut meal, and palm kernel meal (Table 1). The experimental diets were formulated to meet the energy and nutrient requirements of laying quails based on the recommendations of the NRC (1994) and (Table 1).

The quails were divided into 20 experimental units (@ ten birds); each treatment consisted of 5 replications. The birds were kept in a battery-colony cage. They cared based on animal ethics related to holding, rearing, slaughtering, and adequately handling live animals as stated in the Republic of Indonesian Law No. 18 of 2009 (section 66). Parameters measured included feed intake, egg production, feed conversion ratio (FCR), eggshell quality, bone and eggshell mineralization, and mineral retention.

Table 2: The effects of calcined oyster addition on the organoleptic parameters of fishmeal stored for 45 days.

Parameter	CMSM level (%)	Storage period (days)				Mean
		0	15	30	45	
Aroma	0	4.17±0.18	4.22±0.13	4.02±0.45	3.72±0.23	4.03±0.03
	3	4.30±0.18	3.98±0.15	3.83±0.08	4.12±0.18	4.06±0.11
	6	4.13±0.16	4.00±0.18	3.72±0.13	3.85±0.13	3.93±0.08
	9	4.12±0.19	4.05±0.15	3.75±0.15	3.75±0.15	3.92±0.13
	Mean	4.18±0.08	4.06±0.11	3.83±0.13	3.86±0.18	
Color	0	4.35±0.13	4.32±0.20	4.15±0.13	3.67±0.13	4.12±0.05
	3	4.03±0.35	4.20±0.15	3.98±0.19	3.95±0.09	4.04±0.17
	6	4.08±0.15	4.37±0.14	4.07±0.15	3.75±0.10	4.07±0.06
	9	4.18±0.28	4.10±0.13	4.15±0.05	3.90±0.2	4.08±0.09
	Mean	4.16±0.14	4.25±0.12	4.09±0.08	3.82±0.13	
Texture	0	4.42±0.09	4.48±0.21	4.27±0.08	4.18±0.18	4.34±0.10
	3	4.27±0.18	4.23±0.15	4.32±0.18	4.15±0.13	4.24±0.12
	6	4.07±0.14	4.35±0.18	4.27±0.10	3.90±0.10	4.15±0.07
	9	4.37±0.20	4.25±0.05	4.18±0.08	4.13±0.15	4.23±0.10
	Mean	4.28±0.15	4.33±0.11	4.26±0.06	4.09±0.13	

STATISTICAL ANALYSIS

Data were statistically analyzed using the two-way analysis for the storage experiment and one-way analysis of variance (ANOVA) for the feeding trial. Duncan's method was applied to determine the differences between treatments. The effects were considered to be significant when $P < 0.05$. The results were expressed as mean, and the variance of the data was presented as the standard deviation ($\pm$ SD). Statistical analyses were performed in SPSS for Windows Release.

RESULTS AND DISCUSSION

NUTRIENT CONTENT, PHYSICAL PROPERTIES, AND TPC OF STORED FISHMEAL

Data on the effect of calcite addition on the organoleptic parameters, total plate count, and crude nutrient of fish meal stored for 45 days are presented in [Tables 2](#) and [3](#). The treatments had no significant differences in aroma, color, and texture. The mean value of the organoleptic test ranged between 3.7 and 4.3, which means the stored fishmeal revealed relatively average sensory values during the storage. As shown in [Table 3](#), adding CMSM increased crude ash. On the other hand, the fishmeal treated with CMSM showed significantly lower TPC values, moisture, crude protein, and crude fat content. The TPC value and moisture content increased during storage. Bacterial contamination in the results highlights the insufficiency of the sterilization process. The change in moisture content during storage served as a good indicator of the susceptibility of fishmeal to microbial spoilage ([Masoum et al., 2012](#); [Jeyasanta and Peterson, 2018](#)). The 5-9% moisture content was still acceptable based on the national standard of a maximum of 10% ([SNI, 1996](#)). Variations in moisture

content across different samples also reflect the inefficiency of the drying process. This inconsistency can lead to differential spoilage rates and nutrient loss in the fishmeal. This finding underscores the need for more rigorous sterilization methods and the use of a preservative agent to ensure the safety and quality of the fishmeal.

As shown in [Table 3](#), adding CMSM up to 9% reduced the moisture content from 7.0 to 6.6% and suppressed the bacterial count from 39.12 to 25.7 $\times 10^{-2}$ CFU/g DM. A preservative agent is intended to slow the moisture increase rate during storage. The present results indicate that adding calcined mussel shells inhibited the increase in moisture content and the development of bacteria in the stored fishmeal. Increasing levels of CMSM inhibited the increase of moisture and bacterial colonies. [Yang et al. \(2019\)](#) reported that the addition of calcined freshwater clam shell (*Corbicula fluminea*) (0.5, 1.0, and 1.5 g/L) as a preservative agent could effectively inhibit the pathogen, prolong the shelf life, and improve the properties of for soybean curd (tofu). Another research report from [Kim et al. \(2007\)](#) confirmed that 0.2 % oyster shell powder could reduce the total bacterial count to < 107 CFU/g, prolong the shelf life, and enhance the sensory value of tofu stored for ten days at 10°C. Sawai (2011) stated that alkaline effects caused by the hydration of CaO are considered the primary mechanism of the antibacterial activity of calcined oyster shells.

As shown in [Table 3](#), the crude protein content significantly decreased during the storage. The results may show a decline in nutrient levels over storage time, directly related to the high moisture content and bacterial activity. This degradation impacts the fish meal's effectiveness as a nutritional

Table 3: The effects of calcined oyster on crude nutrients of fishmeal stored for 45 days.

Parameter	CMSM level (%)	Storage period (days)				Mean
		0	15	30	45	
TPC (x10 ⁻² CFU/g)	0	15.79±2.09 ^f	32.53±3.63 ^{de}	46.63±2.65 ^{bc}	61.69±2.22 ^a	39.16±19.61
	3	13.40±1.25 ^f	30.02±3.63 ^e	34.56±7.78 ^{de}	47.63±0.37 ^b	31.40±14.13
	6	15.62±4.90 ^f	28.54±5.27 ^e	30.67±6.00 ^e	39.78±1.10 ^{cd}	28.65±9.96
	9	14.68±5.23 ^f	27.29±4.90 ^e	28.21±1.63 ^e	32.44±8.13 ^{de}	25.65±7.65
	Mean	14.87±1.10	29.59±2.25	35.02±8.17	45.38±12.51	
Moisture (%)	0	5.81±1.18	6.30±0.05	6.80±0.51	9.09±0.47	7.00±1.45 ^a
	3	6.50±0.16	6.23±0.28	6.62±0.18	8.86±0.26	7.05±1.22 ^a
	6	4.92±2.33	5.77±0.15	6.29±0.27	8.05±0.21	6.26±1.32 ^b
	9	6.73±1.32	5.64±0.07	6.18±0.45	7.66±0.18	6.55±0.86 ^{ab}
	Mean	5.99±0.81 ^b	5.98±0.33 ^b	6.47±0.29 ^b	8.42±0.67 ^a	
Crude ash (% DM)	0	23.58±1.22	24.31±0.66	21.88±0.38	23.13±0.39	23.22±1.02 ^d
	3	23.98±0.50	24.85±0.35	23.29±0.38	23.54±0.57	23.91±0.69 ^c
	6	25.78±0.26	26.61±0.57	24.06±0.66	25.04±0.04	25.37±1.09 ^b
	9	27.11±1.39	30.13±0.42	25.71±0.73	26.14±0.64	27.18±1.79 ^a
	Mean	25.98±1.64	24.73±2.43	24.09±1.60	25.23±1.39	
Crude protein (% DM)	0	70.45±2.94 ^a	66.71±1.67 ^{bc}	63.76±1.60 ^{cde}	59.61±2.05 ^{fg}	65.13±4.59
	3	67.71±0.99 ^{ab}	65.35±0.90 ^{bcd}	64.53±1.11 ^{cd}	63.53±1.30 ^{de}	65.28±1.78
	6	57.43±2.84 ^{ghi}	58.49±1.43 ^{gh}	57.70±1.60 ^{ghi}	56.37±0.75 ^{hi}	57.50±0.88
	9	61.39±2.71 ^{ef}	56.76±1.39 ^{ghi}	55.19±1.08 ^{ij}	53.16±0.89 ^j	56.63±3.50
	Mean	64.24±5.92	61.83±4.94	60.30±4.57	58.17±4.44	
Crude fat (% DM)	0	4.51±0.57	5.41±0.11	4.92±0.53	4.56±0.61	4.85±0.41 ^a
	3	4.16±0.73	5.05±0.43	5.86±0.32	4.23±0.83	4.83±0.80 ^a
	6	3.46±0.37	3.44±0.34	3.98±0.69	3.96±0.34	3.71±0.30 ^b
	9	3.95±0.85	3.40±0.16	4.09±0.42	4.07±0.73	3.88±0.32 ^b
	Mean	4.02±0.44	4.32±1.05	4.71±0.87	4.20±0.26	

feed component. The addition of CMSM effectively inhibited the increase in the fish meal's moisture content, bacterial colonies, and protein degradation during storage. The crude protein decreases could be reduced from 11% in the fishmeal with no CMSM to 3-4 % in the fishmeal mixed with 3-9% calcite.

Crude protein is considered the most important essential nutrient of fishmeal. The crude protein content of fish meal varies from 60.0-72.3% depending on the type of fish and the preparation method (NRC,1994). The crude protein content of the local fishmeal ranged from 53.2 to 70.5%. This range of protein content is higher than that of some fishmeal products, such as Pakistani fishmeal, as reported by Khan *et al.* (2012) and Rahim *et al.* (2017). Results from the storage experiment showed that the untreated fishmeal stored for 45 days showed the highest protein reduction. This reduction can also be attributed to the increasing activities of spoilage agents. The reduction in the crude protein of the fishmeal could be attributed to the gradual degradation of the initial crude protein to more volatile products such as total volatile bases (TVB), trimethyl amine (TMA)

hydrogen sulfide, and ammonia (Eyo, 2001) and the changes in the physical properties may be associated with the addition of calcined shell which had high densities (Khalil *et al.*, 2018). The reduction in crude protein content of the fishmeal may also have been due to a decrease in salt-soluble protein and water-soluble protein (Chomnawang *et al.*, 2005) or due to autolytic deterioration associated with the actions of endogenous enzymes and bacteria (Hultman and Rustard, 2004). According to Anderson *et al.* (1993), long-term storage of fishmeal causes the degradation of amino acids by bacterial activities and production and accumulation of biogenic amines that decrease the protein quality of the final product, and these toxic compounds adversely affect animal performance. The addition of calcined mussels slowed down the rate of protein degradation. Demarchi *et al.* (2016) and Ji *et al.* (2022) reported that proteins can be preserved for a long time by adding heated oyster shells to biominerals such as teeth, bones, and shells. Minerals can also offer protection against degradation because of the isolation and stabilization effects. The well-preserved proteins may be due to the incorporation of proteins inside the crystal lattices of nacre tablets.

Table 4: The effects of CMSM on the physical properties of fishmeal stored for 45 days.

Parameter	CMSM level (%)	Storage period (days)				Mean
		0	15	30	45	
Bulk density (g/ml)	0	0.70±0.02	0.66±0.00	0.66±0.01	0.64±0.01	0.66±0.02 ^b
	3	0.70±0.02	0.68±0.00	0.67±0.00	0.66±0.00	0.68±0.02 ^b
	6	0.70±0.01	0.70±0.01	0.68±0.01	0.67±0.00	0.69±0.02 ^{ab}
	9	0.70±0.01	0.70±0.01	0.69±0.00	0.670.00	0.71±0.01 ^a
	Mean	0.70±0.00 ^a	0.68±0.02 ^a	0.67±0.01 ^{ab}	0.66±0.01 ^b	
Specific density (g/ml)	0	1.46±0.10	1.48±0.07	1.45±0.08	1.46±0.05	1.46±0.01 ^b
	3	1.50±0.07	1.52±0.10	1.47±0.13	1.48±0.10	1.49±0.02 ^{ab}
	6	1.49±0.06	1.49±0.07	1.47±0.13	1.54±0.02	1.50±0.03 ^{ab}
	9	1.46±0.03	1.54±0.08	1.57±0.11	1.57±0.10	1.54±0.05 ^a
	Mean	1.48±0.02	1.51±0.03	1.49±0.06	1.51±0.05	
Angle of repose (°)	0	50.84±0.00	49.71±1.72	48.89±0.50	51.09±1.20	50.13±1.02
	3	49.17±0.86	49.47±0.49	49.75±0.49	51.37±0.45	49.94±0.98
	6	47.71±0.52	51.57±2.10	49.75±0.49	50.56±0.93	49.90±1.64
	9	48.31±0.00	50.57±0.47	51.09±1.20	50.02±0.83	50.00±1.21
	Mean	49.01±1.36	50.33±0.95	49.87±0.91	50.76±0.60	

Table 5: The effect of diets supplemented with fish meal mixed with different levels of CMSM on feed intake, egg production, and feed conversion ratio.

Parameter	Experimental diets contained fish meal mixed with CMS (%)				P-value
	0	3	6	9	
Feed intake					
Total feed intake (g/bird)	1354.40±55.38	1388.98±79.24	1377.18±95.32	1329.10±67.67	0.30
Daily feed intake (g/bird)	24.19±0.99	24.80±1.42	24.59±1.70	23.73±1.21	0.30
Egg production					
Egg number (egg/bird)	32.40±6.70 ^{ab}	36.50±5.63 ^a	34.76±8.36 ^a	25.10±8.28 ^b	0.05
Egg mass (g/bird)	301.66±65.89 ^{ab}	354.60±53.62 ^a	335.58±80.37 ^a	247.20±83.18 ^b	0.08
Egg weight (g/egg)	9.57±0.46	9.68±0.27	9.63±0.21	9.70±0.46	0.94
Quail-day egg production (%)	57.86±11.96 ^{ab}	65.18±10.05 ^a	62.07±14.93 ^a	44.82±14.79 ^b	0.05
Feed conversion ratio	4.70±1.20	4.00±0.75	4.27±0.89	5.92±2.19	0.17

Table 4 presents the physical properties of fishmeal. The addition of CMS significantly affected the bulk and specific density of fishmeal. As shown in Table 4, the densities increased by the increased level of CMS. Bulk density tended to decrease during the storage period. There was no significant effect of CMS addition on the angle of repose.

NUTRITIONAL VALUE OF CALCITE-TREATED FISHMEAL

Table 5 presents feed intake, egg production, and feed conversion data for quails fed diets with fish meal without and mixed with 3, 6, and 9% CMSM. Total and daily feed intake was not a statistically significant difference among the treatments. Laying quails fed diets using fish meal mixed with 3% CMSM (P1) showed the highest egg production in terms of number, egg mass, and percentage of quail-day egg production, followed by the quail that received rations

using fish meal mixed with 6% CMSM (P2). Quail in these two treatments showed the lowest feed conversion rates (4.0 and 4.27), but there was no significant difference. On the other hand, feeding the diet containing 9% CMSM-supplemented fishmeal (P3) depressed egg production and feed utilization efficiency, even lower than the control diet (fish meal without calcite addition) (P0). The birds in P3 also had significantly lower egg indexes, tibia bone diameters, and egg indexes (Tables 6 and 7).

Furthermore, as shown in Table 6, using fish meal mixed with 0-9% CMSM did not significantly affect the weight, dimensions of the egg, crude ash and mineral content, and quality of the eggshell. There was also no significant effect of CMSM addition on mineral retention, length of crude ash, and mineral composition of tibia bones of quails (Table 6). However, there is a tendency that birds fed diets con

Table 6: The effect of diets supplemented with fish meal mixed with different levels of CMSM on egg weight, egg index, and eggshell quality.

Parameter	Experimental diets contained fish meal mixed with CMSM (%)				P-value
	0	3	6	9	
Egg weight (g/egg)	9.67±0.39	9.74±0.17	9.68±0.20	9.91±0.29	0.55
Egg index:					
Egg length (cm)	3.02±0.06	3.03±0.03	3.03±0.04	3.09±0.04	0.11
Egg width (cm)	2.39±0.03	2.39±0.02	2.39±0.02	2.39±0.02	0.95
Egg index	79.23±1.16 ^a	78.77±0.49 ^a	79.03±0.95 ^a	77.57±0.72 ^b	0.02
Eggshell quality:					
Eggshell weight (g/egg)	0.72±0.04	0.73±0.03 ^a	0.73±0.03 ^a	0.73±0.04 ^a	0.05
Eggshell percentage (%)	7.48±0.22	7.53±0.25	7.60±0.29	7.42±0.35	0.08
Crude ash and mineral content of eggshell (%)					
Crude ash	51.99±0.82	51.76±0.37	52.90±1.39	52.64±0.71	0.21
Ca	31.09±0.93	30.13±1.89	31.27±2.62	32.11±0.88	0.38
P	0.76±0.24	0.84±0.28	0.60±0.08	0.75±0.24	0.52

Table 7: The effect of diets supplemented with fish meal mixed with different levels of CMS tibia bone and mineral retention.

Parameter	Experimental diets contained fish meal mixed with CMSM(%)				P-value
	0	3	6	9	
Length and diameter of tibia bone (mm)					
Length	8.52±0.16	8.67±0.10	8.52±0.18	8.59±0.24	0.52
Diameter	0.56±0.01 ^a	0.56±0.01 ^a	0.55±0.01 ^{ab}	0.54±0.01 ^b	0.02
Tibia bone weight (g/bird)					
Fresh weight	2.32±0.25	2.32±0.25	2.28±0.15	2.33±0.18	0.93
Dry weight	1.47±0.14	1.22±0.13	1.35±0.19	1.46±0.21	0.09
Crude ash and mineral content of tibia bone (%)					
Crude ash	55.77±5.86	56.16±2.94	57.13±3.40	52.94±6.68	0.64
Ca	18.17±1.89	18.55±1.50	17.87±1.29	16.96±0.93	0.46
P	5.92±1.40	6.31±1.27	6.39±2.14	5.15±2.78	0.67
Crude ash and mineral retention (%)					
Crude ash	45.48±4.79	34.51±5.24	46.92±6.24	39.28±9.20	0.06
Ca	48.55±5.35	47.47±5.91	47.91±6.21	50.87±8.79	0.88
P	75.52±8.40 ^b	83.99±3.57 ^a	77.86±3.66 ^{ab}	84.12±4.57 ^a	0.02

taining fishmeal mixed with 3 and 6% CMSM (P and P2) had better egg index, eggshell weight, crude ash, and phosphorus content of tibia bones than the control (P0) and P3.

Preserving the local fishmeal using a natural preservative is essential to supply high-quality dietary protein ingredients for the self-mixing poultry farm in West Sumatra. Local fishmeal is considered to become more critical in the future due to steadily increasing demand, limited supply, and the high cost of imported products. The use of calcite as a natural preservative could be a feasible alternative to impend moisture increase and bacterial activity. Results from the feeding trial revealed that the optimal level of CMSM addition was 3-6%. Laying quails fed diets formulated with fishmeal mixed with 3-6% calcined mussel shell tended to have higher egg production, better feed utilization effi-

ciency, egg index, and eggshell weight. The supply of fishmeal for self-mixing poultry feed is fulfilled using rejected dried salted fish due to the high cost of imported fishmeal. The fishmeal produced from rejected salted dried fish had a poor nutritional content of 22.8% crude protein, 3.4% crude fat, and 11.2% crude fiber (Hermon *et al.*, 2021). The use of rejected dry-salted fish in poultry diets negatively affects feed intake, egg production, and quality due to high salt content and heavy microbiological contamination.

CONCLUSIONS AND RECOMMENDATIONS

Results found that the local fishmeal processed by traditional simple steam cooking and sun drying was suscepti-

ble to microbial spoilage characterized by increased moisture content and bacterial colonies and decreased protein content during storage. Adding 3-9% calcined oyster shell inhibited microbial activities, increased moisture content, and protein degradation during storage meal. The use of fish meals containing 3 and 6% calcined oyster shell meal had a beneficial effect on egg production, feed utilization efficiency, eggshell quality, and bone mineralization. It is suggested that the calcined oyster shell meal could be used 3-6% to improve storage stability and nutritional values of fishmeal.

There is a need to improve the fishmeal production process. These might include adopting modern drying and sterilization techniques, implementing better moisture control, and regularly testing for bacterial contamination. Future research areas are suggested to enhance fishmeal processing further, which could involve studying the effects of different drying and sterilization methods on nutrient retention and bacterial activity in fishmeal.

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NOVELTY STATEMENT

Producing fishmeal using low-cost fish and equipment is essential for producing low-cost fishmeal in Indonesia due to steadily increasing demand, limited supply, and the high cost of imported products. However, local fishmeal produced by simple steam-cooking and sun drying might not be adequately sterilized, posing a risk of microbial contamination and nutrient degradation during storage. Using calcite from locally available bivalve shells as a natural preservative and mineral supplement effectively maintains stored fishmeal's microbial quality and nutritional values.

AUTHOR'S CONTRIBUTIONS

Conceptualization, Khalil and Andri: Data collecting and analyzing.

Ridho Kurniawan Rusli and Montesqrit: Wrote the first draft.

Khalil: Review and editing: Andri and Montesqrit.

All authors have read, commented, and agreed on the final draft of the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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