The effect of turmeric (Curcuma longa L.) powder addition as natural antibiotic on the quality of milk replacer for lamb during storage

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Abstract. The aim of this study was to determine the effect of turmeric (Curcuma longa L.) powder addition as a natural antibiotic, temperature, and storage time on the quality of milk replacer for lamb during storage. Turmeric powder addition equal to 0%, 0.1%, 0.2%, and 0.3% (w/v), room temperature (28°C) and refrigerator temperature (5°C) stored for 5 days were used in this study. The parameters observed were Total Plate Count (TPC), bacterial growth by Methylene Blue Reduction Time (MBRT) test, resazurin test, organoleptic test (odor and texture), pH, acid number and protein level in milk replacer. The data were analysed by analysis of variance (ANOVA) with factorial pattern 4x2x2. If there were significant difference on each parameter, Duncan test was performed. The addition of turmeric powder with different levels on E. Coli growth, TPC, acid number, organoleptic, MBRT and resazurin test showed significant difference (P<0.01), but the parameter of pH and protein level does not showed significant difference (P>0.01). Comparison with control, samples with the addition of turmeric levels that are higher can inhibit bacterial growth, more effectively, besides the effect of the storage factor shows the result that the storage of milk replacer with the best quality is at (5°C) and 0 day.

1. Introduction
Milk replacer is used for the growth of pre-existing cattle because the mother’s milk is not sufficient for their kids. The composition and consumption of nutrients and methods of giving milk replacer to pre-existing children affect performance and health of these animals. During storage of milk replacer there is usually a decrease in quality both physically and chemically will occur during storage. This happens because the high nutrient content in milk replacer causes microbes to grow well.

As we live in microbial world, there are ample opportunities for the milk to get contaminated at any stage of food chain. Microbes such as Aspergillus, Bacillus, Enterococcus, Micrococcus, Mucor, Penicillium, Rhizopus, and Streptococcus can cause spoilage of dried milk powder [1]. [2] Some of the adverse effects of the presence of pathogenic bacteria in milk can cause digestive tract disease, colitis, diarrhea, and septicemia (systemic inflammatory response syndrome). The use of synthetic antibiotics is now avoided as much as possible because many contain chemical compounds that can have negative consequences for the body and environment. The use of excessive synthetic antibiotics in milk replacer can result in microbial resistance, this results in an increase in immunity to pathogenic bacteria, diarrhea and decreased immunity in livestock. Therefore, the use of
natural antibiotics is an alternative substitute for synthetic antibiotics. The content of curcumin in turmeric has a role in inhibiting the growth of Gram positive and Gram negative bacteria so that turmeric has the potential as a natural antibiotic [3].

While [4] explains that curcumin in turmeric has the power to bind directly to various molecules that have pathogenic properties so that it will adversely affect the survival of the molecule of the pathogen. These molecules include inflammatory molecules, protein kinase, protein reductase, acetate, histone deacetylase, glyoxalase I, xanthine oxidase, HIV protease 1, ATPase, DNA methyl transferase 1, FtsZ protofilament and metal ions used by microbes to grow. Based on this, the decrease in the quality of milk replacer caused by microbial growth during storage can be solved by adding turmeric, hence the ability of turmeric here is used as research material which acts as an anti-bacterial agent added to milk replacer so that it is more durable to be stored.

2. Materials and methods

2.1. The process of making turmeric powder
Turmeric powder is made from freshly cleaned turmeric that cut to small size then drying in the oven at 50°C for 24 hours. After drying, the turmeric is mashed with a blender until it is in the form of powder, then stored in dark at 4°C [5].

2.2. Proximate analysis
Feed ingredients used for milk replacer (soybean meal, rejected skim milk, and turmeric) which have been dried in an oven at 55 °C for the next 3 days were milled and filtered with a size of 2 mm. Proximate analysis was carried out on the ingredients. The analysis of chemical composition includes levels of dry matter, organic matter, crude fat, crude protein, and crude fiber.

2.3. The activity of antimicrobial compounds against indicator bacteria (antagonistic test) was measured using the clear zone method
Bacterial inhibitor activity test procedure based on a modification of the method of Schved et al [6]. Each pathogenic bacterium is 1 ose inoculated into 10 ml NB and then incubated at 37°C until the bacterial cell count is 10^6 CFU/ml by measuring 0.1 optical density at a wavelength of 660 nm. 25 µl of E. coli bacteria was inoculated into 50 ml NB medium which was still liquid, shaken out evenly then poured 25 ml into petridish. So that the hardened medium is made of well holes with yellow sterile tips of 5 mm in diameter by 4 holes, and distilled water was used as a control. Petri discs are then incubated at 37°C for 2 days. The clear zone around the well as a zone of inhibition of turmeric against pathogenic bacteria was measured by its diameter using a ruler.

2.4. The activity of antimicrobial compounds on the growth of indicator bacteria in liquid medium
Indicator bacteria E. coli are grown in NB medium, 9 ml for each. Each treatment had three replications with the addition of 1 ml of turmeric solution which was dissolved in distilled water. Turmeric solution used concentration of 0%; 0.1%; 0.2% and 0.3% (w/v). Indicator bacteria that have been planted in several treatments was observed then for growth by spectrophotometer with a wavelength (λ) 600 nm. OD observations were carried out every hour until the stationary phase to see the inhibitory activity of turmeric against the growth of indicator bacteria.

2.5. The application of adding turmeric on milk replacer
Milk replacer is made using unused skim milk and soybean meal with a protein requirement of 24% and has dry ingredients of 20%. The treatment of turmeric is added as much as 0%;0.1%; 0.2% and 0.3%. The proportion of materials used in the treatment were provided on table 1.
Table 1. The proportion of ingredients used in each treatment

| The proportion of ingredients used | Turmeric level(%) |
|-----------------------------------|-------------------|
|  | 0      | 0.1     | 0.2     | 0.3     |
| Water (g)                         | 100.00            | 100.00  | 100.00  | 100.00  |
| Soybean meal (g)                  | 13.28             | 13.28   | 13.28   | 13.28   |
| Unused skim milk (g)              | 16.59             | 16.59   | 16.59   | 16.59   |
| Turmeric (g in as feed)           | 0.00              | 0.12    | 0.24    | 0.36    |

The treatment sample was stored within 5 days with different temperatures, there are room temperature (28°C) and refrigerator temperature (5°C). Test samples were carried out by the Total Plate Count test, organoleptic test, pH, acid number, and protein content in milk replacer on day 0 and day 5.

2.5.1. Total Plate Count (TPC)

1 ml of milk replacer sample was first done by serial dilution. This sample for the TPC test was then taken at 10, 10³, 10⁶, and 10⁹ dilutions. A sample of 1 ml was then put into a petridish containing medium MRS was still hot and flattened and waited until it solidified. Sample incubation is carried out at 37°C for 24 to 48 hours. Colonies that grow in petridishes are then calculated using a colony counter. The test was carried out on day 0 and day 5. The total colonies are then calculated with this formula below.

\[ N = \frac{\Sigma C}{(1 \times n1) + (0.1 \times n2)} \times (d) \]

N = number of product colonies, expressed in colonies per ml or colony per g
\( \Sigma C \) = number of colonies in all plates calculated
n1 = the number of plates in the first dilution calculated
n2 = the number of plates in the second calculation calculated
d = the first dilution calculated

2.5.2. Methylene Blue Reduction Time (MBRT) test

Milk replacer samples were added as much as 10 ml into a sterile hungate tube and then 1 ml of 1000 ppm methylene blue solution was homogenized. All the tubes containing the sample were incubated at 36°C and checked for color changes every hour until the sample solution in the tube changed color completely to white. Both tests were carried out on day 0 and day 5.

2.5.3. Resazurin test

The milk replacer sample was put in 10 ml into the hungate tube and then added with 1 ml of 0.1% resazurin solution and homogenized. All the tubes containing the sample were incubated at 36°C and checked for color changes every hour until the sample solution in the tube changed color completely to white. Both tests were carried out on day 0 and day 5.

2.5.4. Organoleptic test

Organoletic tests were carried out by 5 panelists who were not trained by observing milk replacer samples physically in the form of odor and viscosity in milk replacer on day 0 and day 5.

2.5.5. pH test.

The pH test was carried out by taking 5 ml of milk replacer sample homogenized with vortex then measuring its pH value with a pH meter, the test was carried out on day 0 and day 5.
2.5.6. Acid number test.
Milk replacer samples taken as much as 9 ml were put into erlenmeyer, then phenoptalein indicator was added as much as 2 to 3 drops. After that titrated with NaOH 0.1N to appear pink color. Calculation of acidity use this formula below.

\[
\text{% acid number} = \frac{\text{ml NaOH} \times N \text{NaOH} \times 0.09 \times 100\%}{\text{density} \times \text{sample volume}}
\]

2.5.7. Protein levels test by Lowry method
All previous milk replacer samples were diluted 100 times and then inserted in the tube as much as 0.5 ml and also made blank using a tube containing 0.5 ml of distilled water. Each tube was added with 2.5 mL of Lowry B solution which was a mixture of CuSO\(_4\), Na\(_2\)CO\(_3\), and Potassium tartate sodium solutions. The solution is homogenized and left for ten minutes. Lowry A solution containing folin and distilled water was added and homogenized and then left for 30 minutes, then the absorbance was read in a spectrophotometer at a wavelength of 750 nm, then included in the equation:

\[
Y = 0.569 \times + 0.101
\]

Y: product absorbance
X: protein content (µ / ml)

2.6. Data analysis
The results of the research data were analyzed using ANOVA (Analysis of Variance) with 4x2x2 factorial pattern. If the data obtained shows significant differences due two treatment, Duncan's New Multiple Range Test (DMRT). The software used for this data analysis is SPSS (Statistical Product and Service Solutions).

3. Results and discussions

3.1. The ability to inhibit turmeric against pathogenic bacteria
The results in table 2 showed that E. coli growth in each medium added with turmeric showed significantly different results (P <0.01). The growth chart in Figure 1 produces a direction coefficient which is the growth coefficient (µ). The direction coefficient describes the growth of pathogenic bacteria in each treatment given the concentration of turmeric. The higher the value of µ, the better the growth of pathogenic bacteria. This shows that the value of µ E. coli growth is getting lower with the addition of higher concentrations of turmeric.

Table 2. Direction of coefficient value (µ) on E. coli growth in exponential phase with the addition of different levels of turmeric

| Sample       | Turmeric Level (%) |
|--------------|--------------------|
|              | 0                  | 0.1               | 0.2               | 0.3               |
| First test   | 0.0499             | 0.0534            | 0.0476            | 0.0463            |
| Second test  | 0.0536             | 0.0511            | 0.0459            | 0.0457            |
| Third test   | 0.0534             | 0.0522            | 0.0508            | 0.0436            |
| Average      | 0.0523±0.002\(^b\) | 0.0522±0.001\(^b\) | 0.0481±0.002\(^a\) | 0.0452±0.001\(^a\) |

\(^a\)\(^b\) Different superscripts in the same column show significant differences(P<0.01)
**Figure 1.** Growth of *E. coli* with the addition of different level of turmeric

**Figure 2.** Turmeric clear zone with different levels of *E. coli* bacteria

A: Turmeric level 0%

B: Turmeric level 0.1%

C: Turmeric level 0.2%

D: Turmeric level 0.3%

**Table 3.** Average clear zone diameter (mm) of turmeric against the growth of pathogenic *E. coli* bacteria

| Sample       | Turmeric Level (%) |
|--------------|--------------------|
|              | 0  | 0.1 | 0.2 | 0.3 |
| First test   | 0  | 5.8 | 6.5 | 9.7 |
| Second test  | 0  | 5.0 | 6.4 | 9.7 |
| Third test   | 0  | 4.8 | 6.4 | 9.5 |
| Average      | 0±0.00<sup>a</sup> | 5.2±0.52<sup>b</sup> | 6.4±0.57<sup>c</sup> | 9.6±0.11<sup>d</sup> |

<sup>a,b,c,d</sup> Different superscripts in the same row show significant differences (P<0.01)
Based on the table 3, it can be seen that the addition of turmeric has a significant effect (P <0.01) on the growth of E. coli bacteria. Addition of turmeric with various concentrations shows different clear zone diameters. The diameter of the clear zone formed with 0% turmeric administration level, 0.1%; 0.2% and 0.3% were 0 mm; 5.2 mm; 6.4 mm and 9.6 mm respectively. This shows that the higher the concentration of turmeric added, the clearer zone diameter is wider, so the inhibition of turmeric against E. coli bacteria is higher than control. According [7] said that the clearer zone diameter is measured as weak (0-3 mm), medium (3-6 mm) and strong (> 6 mm). Based on this it is known that the inhibitory effect produced by turmeric with a concentration of 0.1% is included in the medium category, while for turmeric with a concentration of 0.2% and 0.3% it belongs to the strong category.

3.2. The quality of milk replacer for lambs
Based on the results of analysis in the laboratory, the dry matter content (DM) and crude protein (CP) of skimmed milk were 95.82% and 8.94%. The content of DM and CP for soybean meal were 88.28% and 44.58%. Formulated milk replacer has levels of DM and CP with 91.07% and 25.5% respectively.

3.2.1 Total Plate Count (TPC)
The results of testing TPC milk replacer for lamb during storage can be seen in Table 4.

| Temperature | Day | Turmeric Level (%) | Average | Average |
|-------------|-----|--------------------|---------|---------|
| 28°C        | 0   | 3.02±0.006         | 2.97±0.005 | 2.96±1.52 | 2.98±0.02 | 5.66±2.68<sup>x</sup> |
|             | 5   | 8.48±0.00          | 8.27±0.04  | 8.21±0.05 | 8.34±0.11 | 5.62±2.67<sup>y</sup> |
| 5°C         | 0   | 3.04±0.03          | 2.94±0.03  | 2.88±0.08 | 2.96±0.05 | 5.58±2.76<sup>b</sup> |
|             | 5   | 8.41±0.06          | 8.22±0.05  | 8.06±0.16 | 8.29±0.16 | 5.47±2.70<sup>a</sup> |

<sup>x,y</sup> Different superscripts in the same column show significant differences (P<0.01)
<sup>a,b,c,d</sup> Different superscripts in the same row show significant differences (P<0.01)

The highest level of addition of turmeric 0.3% resulted in the lowest TPC of 5.47 log cfu/ml. The storage treatment with refrigerator temperature (5°C) shows a lower TPC compared to room temperature storage (28°C) which is 5.62 log cfu/ml. So that treatment with refrigerator temperature storage is also able to reduce the acid value which can cause damage to milk replacer compared to room temperature storage. The best result of milk replacer storage is by adding 0.3% turmeric level and stored at refrigerator temperature.

3.2.2 Methylene Blue Reduction Time (MBRT) and resazurin test
The results of the MBRT and resazurin test on milk replacer during storage can be seen in Table 5 and Table 6.
Table 5. Duration of color change (hours) in the MBRT test on milk replacer for lambs during storage

| Temperature | Day | Turmeric Level (%) | Average |
|-------------|-----|--------------------|---------|
|             | 0   | 0.1                | 0.2     | 0.3     |        |
| 28°C        |     | 11.00±1.73         | 13.00±0.00 | 12.00±0.94 |        |
|             |     | 4.30±0.57          | 5.70±0.57 | 6.00±0.00 | 5.25±0.65 |
| 5°C         |     | 11.00±0.00         | 13.00±0.00 | 12.5±0.86 |          |
|             |     | 5.30±0.57          | 6.00±0.00 | 6.00±0.00 | 5.82±0.30 |
| Average     |     | 7.91±3.60          | 9.42±4.12 | 9.50±4.04 |         |

Superscripts in the same column does not show significant differences (P>0.05) 
Different superscripts in the same row show significant differences (P<0.01)

Table 6. Duration of color change (hours) in the resazurin test on milk replacer for lamb during storage

| Temperature | Day | Turmeric Level (%) | Average |
|-------------|-----|--------------------|---------|
|             | 0   | 0.1                | 0.2     | 0.3     |        |
| 28°C        |     | 10.00±0.00         | 13.00±0.00 | 11.57±1.34 |        |
|             |     | 5.00±0.00          | 7.00±0.00 | 6.00±1.00 |         |
| 5°C         |     | 10.00±0.00         | 13.00±0.00 | 11.50±1.50 |       |
|             |     | 5.00±0.00          | 8.00±0.00 | 6.50±1.50 |         |
| Average     |     | 7.50±2.61          | 10.25±2.89 | 9.00±3.11 |          |

Superscripts in the same column does not show significant differences (P>0.05) 
Different superscripts in the same row show significant differences (P<0.01)

Table 5 shows that adding turmeric can inhibit bacterial growth the longer the colour change of the blue solution becomes white. the bacteria that grow more slowly so the best storage result is storage at the temperature of the refrigerator and with the addition of the level of turmeric 0.2 and or 0.3%. Table 6 shows that adding turmeric can inhibit bacterial growth. The longer the colour change of the concentrated purple solution becomes white, the bacteria that grow slower, so the best storage results are storage at the temperature of the refrigerator and with the addition of levels of turmeric 0.2 and or 0.3%.

3.2.3. Organoleptic value
The results of organoleptic odor testing and viscosity of lamb milk replacer during storage can be seen in table 7 and 8.
Table 8. Results of organoleptic testing of viscosity of milk replacer for lambs during storage

| Temperature | Day | Turmeric Level (%) | Average |
|-------------|-----|---------------------|---------|
|             | 0   |                     |         |
| 28°C        | 0   | 1.00±0.00           | 1.00±0.00|
|             | 5   | 3.00±0.00           | 2.80±0.11 |
|             |     |                     | 2.95±0.00 |
|             |     | 3.00±0.00           | 3.00±0.00 |
| 5°C         | 0   | 1.00±0.00           | 1.00±0.00 |
|             | 5   | 3.00±0.00           | 2.80±0.00 |
|             |     | 2.90±0.00           | 3.00±0.00 |
| Average     |     | 2.00±1.04c          | 1.95±0.97 |
|             |     | 1.98±1.02de         | 1.91±0.95c |

Different superscripts in the same column show significant differences (P<0.01)

Based on Table 7 and Table 8 show that the temperature and duration of storage showed significant results (P<0.01). The highest concentration of turmeric (0.3%) produced the most acidic odor and had the lowest viscosity.

3.2.4. pH

The results of testing the pH value during storage can be seen in Table 9.

Table 9. Results of pH testing of milk replacer for lambs during storage

| Temperature | Day | Turmeric Level (%) | Average |
|-------------|-----|---------------------|---------|
|             | 0   | 6.49±0.07           | 6.54±0.04 |
| 28°C        | 5   | 4.03±0.07           | 4.05±0.03 |
|             |     | 4.07±0.05           | 4.05±0.01 |
|             | 0   | 6.50±0.07           | 6.57±0.06 |
|             | 5   | 6.03±0.03           | 5.99±0.04 |
| 5°C         |     | 5.97±0.02           | 6.00±0.02 |
| Average     |     | 5.76±1.06          | 5.77±1.06 |
|             |     | 5.78±1.07          | 5.81±1.08 |
|             |     | 5.77±1.06          | 5.81±1.08 |
|             |     | 5.78±1.07          | 5.81±1.08 |

Different superscripts in the same column show significant differences (P<0.01)

Based on Table 9 above, it can be seen that the addition of turmeric with different levels did not have a significant effect (P>0.01) on the pH value. But the treatment of temperature and storage time had significant differences (P<0.01). Based on the results of the TPC with the pH value generated from each treatment. It was shown that the higher the results of the TPC produced, the lower the pH value (can be seen in Table 4). [10] stated that storage time also affects the number of bacteria. Milk stored for days causes bacteria to grow in large quantities, so that CO₂ levels increase and produce carbonic acid (H₂CO₃) so that the pH value becomes lower and the number of acids becomes high.

3.2.5. Acid number. The results of testing the acidity of milk replacer for room temperature and refrigerator storage lambs on days 0 and 5 can be seen in Table 10.
### Table 10. Results of testing the acidity (%) of milk replacer for lambs during storage

| Temperature | Day | Turmeric Level (%) | Average |
|-------------|-----|--------------------|---------|
|             | 0   | 0.1                | 0.2     | 0.3 | Average |
| 28°C        | 0   | 0.32±0.09          | 0.14±0.00 | 0.13±0.03 | 0.11±0.01 | 0.17±0.08 | 0.63±0.47x |
|             | 5   | 1.16±0.10          | 1.07±0.09 | 1.07±0.05 | 0.97±0.06 | 1.06±0.06 |
| 5°C         | 0   | 0.28±0.06          | 0.13±0.01 | 0.13±0.005 | 0.11±0.00 | 0.16±0.06 | 0.24±0.12y |
|             | 5   | 0.48±0.07          | 0.27±0.01 | 0.29±0.005 | 0.26±0.02 | 0.32±0.09 |
| Average     |     | 0.56±0.37b         | 0.405±0.40a | 0.407±0.40a | 0.3±0.37a |

* Different superscripts in the same column show significant differences (P<0.01)

Based on Table 10, it can be seen that the treatment of temperature, turmeric concentration and storage time showed significant results (P<0.01). The addition of turmeric can reduce the acidity of milk replacer for lambs, making them more durable to store. This is because turmeric acts as an antibacterial so the acidity produced is lower. Based on the relationship between the TPC produced and the acidity levels produced from each treatment, it shows that the higher the TPC produced, the higher the acidity (can be seen in table 5). This is due to the large number of bacteria that grow, resulting in more lactic acid fermentation so that it is more acidic. The production of these enzymes by bacteria can be influenced by environmental factors. Production of enzymes by bacteria psychrotrophic for example, it can produce extracellular enzymes such as protease, phospholipase and lipase well in the temperature range of 20°C. The presence of bacteria can cause a decrease in levels nutrients such as protein and fat, increased levels of acidity, clumping, and decay in milk products so that the quality dairy products are decreasing. Low temperatures will inhibit the growth of these bacteria so that dairy products can take longer to stored [11].

#### 3.2.6. Protein level

The results of testing milk protein levels for room temperature and refrigerator storage lambs on days 0 and 5 can be seen in table 11.

### Table 11. Results of testing protein content (%) of milk replacer for lambs during storage

| Temperature | Day | Turmeric Level (%) | Average |
|-------------|-----|--------------------|---------|
|             | 0   | 0.1                | 0.2     | 0.3 | Average |
| 28°C        | 0   | 25.09±7.91         | 29.64±4.64 | 27.74±5.05 | 29.98±3.66 | 28.11±1.94 | 23.96±4.7ms |
|             | 5   | 20.07±3.68         | 19.24±2.00 | 20.14±2.21 | 19.83±1.01 | 19.82±0.35 |
| 5°C         | 0   | 26.74±6.37         | 27.60±6.66 | 25.58±4.90 | 28.03±4.98 | 26.98±0.93 | 24.89±2.2ms |
|             | 5   | 21.94±2.61         | 23.12±2.18 | 23.12±2.18 | 23.02±0.96 | 22.80±0.49 |
| Average     |     | 23.46±5.46         | 24.98±5.66 | 24.14±4.11 | 25.21±4.98 |

ms Superscripts in the same row and column do not show significant differences (P>0.01)

Based on Table 11, the storage temperature given did not have a significant effect (P>0.01) on protein levels, but storage time showed significant results (P<0.01). The protein content produced varied, but the treatment of adding turmeric with the highest level of 0.3% produced the highest protein content so that it did not affect the decrease in protein content. Protein levels found in commercial milk replacer...
in the study of Hill et al. [12] reached 28%. Based on this, the protein content in the research conducted is not the same as the normal range. This is due to differences in the ingredients and proportions used for making milk replacer. The research using feed ingredients that have some sources of the protein with unused milk replacer, soybean meal and turmeric are added. There are many donation factors of protein so that protein levels are high enough in milk replacer used in the study.

The level of turmeric added also does not show significant results, however when compared with control, milk replacer added with turmeric powder showed a higher protein level.

4. Conclusion
Based on the research conducted it can be concluded that the addition of turmeric can inhibit bacterial growth and can maintain the quality of milk replacer lamb during storage. The best storage for maintain the quality of milk replacer is at 5°C and 0 day.

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