Single and combined effects of formic acid and *Saccharomyces cerevisiae* on breast meat quality of the Indonesian indigenous crossbred chickens

Lillah Zuhrotul Ukhro\(^1\), Sugiharto Sugiharto, Teyser Adi Sarjana, Turrini Yudiarti, Endang Widiastuti, Hanny Indrat Wahyuni, and Anang Mohamad Legowo

\(^1\)Department of Animal Science, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java, Indonesia.

E-mail: lillahzuhrotul@gmail.com

**Abstract.** The study was conducted to evaluate the effect of formic acid and/or *Saccharomyces cerevisiae* on breast meat characteristics of the Indonesian indigenous crossbred chickens. A total of 200 day-old chicks were randomly distributed to four experimental groups (with five replications each), including T0 (chicks fed control diet), T1 (chicks fed diet supplemented with formic acid at 2g/kg), T2 (diet supplemented with *S. cerevisiae* at 3g/kg) and T3 (diet supplemented with formic acid at 2g/kg and *S. cerevisiae* at 3g/kg). At week 9, the chicks were slaughtered and from which the samples of breast meat were collected. Our results showed that total protein was higher \((p < 0.05)\) in breast meat of T3 than that in other groups. The moisture content and water holding capacity were lower \((p < 0.05)\) than that in other groups. The breast meat from T0 showed higher \((p < 0.05)\) \(L^*\) (lightness) values than other meats. The fat content, \(\text{pH}\), \(a^*\) (redness) and \(b^*\) (yellowness) values of meats were not substantially affected by the treatments. In conclusion, dietary supplementation of a combined mixture of formic acid and *Saccharomyces cerevisiae* was effective in improving the breast meat quality of the Indonesian indigenous crossbred chickens.

**1. Introduction**

Nowadays, the poultry industry is one of the most prospective business opportunities in Indonesia, as it supplies animal protein needed by people [1]. Besides broiler chickens, the Indonesian indigenous crossbred chicken, which is the crossbreed between male Indonesian native chicken and female modern laying hens [2], has attracted consumers interest considerably. These types of chickens have unique meat tastes, and thereby many people prefer to consume the meat from these chickens. One of the poultry industry challenges is the prohibition of antibiotics as feed additives in poultry feed by the Indonesian authorities. Meanwhile, the purpose of adding antibiotics in the diet is actually to increase growth rates and help fighting pathogenic bacteria causing diseases. However, this strategy has some negative effects, such as antibiotic resistance and residual deposits in chicken meat [2]. For this reason, researchers and nutritionists are now encouraged to find an effective alternative to in-feed antibiotics that are friendly to human health and well-being [3].

The expected way to replace the role of antibiotics in poultry diets is utilization of organic acid and probiotic. Formic acid is one type of organic acids that has been used as an antimicrobial agent and have beneficial effect in improving the intestinal morphology, thereby maintaining optimal digestibility and growth performance of poultry [4,5]. *Saccharomyces cerevisiae* is another example of bioactive compounds that may be expected to substitute the use of in-feed antibiotics in poultry rations. Some previous studies demonstrated that *S. cerevisiae* gives a positive impact on poultry health and production traits [6,7]. Study showed that the combinations of organic acid and other bioactive compounds such as probiotics may result in better responses in chickens [8]. Furthermore, the information of the effect of a mixture of formic acid and *S. cerevisiae* in terms of the breast meat quality of the Indonesian indigenous crossbred chickens are still limited. The study was therefore...
conducted to evaluate the effect of formic acid and/or *S. cerevisiae* on breast meat characteristics of the Indonesian indigenous crossbred chickens.

## 2. Materials and Methods

### 2.1. Bird and Diets

The study was conducted on the Indonesian indigenous crossbred chickens from a local breeding farm. A total of two hundred one-day-old chicks were used and randomly placed in different pens and housed in an open house system. Chick were divided into 4 experimental groups which contain 5 replications (10 chicks/replicate). The experimental group including T0 (chicks fed control diet), T1 (chicks fed diet supplemented with formic acid at 2g/kg), T2 (diet supplemented with *S. cerevisiae* at 3g/kg) and T3 (diet supplemented with formic acid at 2g/kg and *S. cerevisiae* at 3g/kg). Ingredients of the chicks diet were shown in Table 1. Diets and drinking water were provided *ad-libitum*.

### 2.2. Sample Preparation

One male bird from each replication was randomly slaughtered, scalded, and defeathered on the week 9 of feeding experiment. The breast meat was deboned from the bird and the visible skin was removed. The Indonesian indigenous crossbred chicken breast meat was homogenized for analyses of proximate composition, water holding capacity, pH, and color values.

### 2.3. Proximate Composition

The proximate composition such as moisture, crude protein, and crude fat was chemically determined using specific technique [10]. Moisture contents on breast meat measured by drying 2g samples by oven for 6 h at 105°C. Crude protein content was measured by micro Kjeldahl refers to Legowo et al. [10]. Breast meat crude fat contents was measured using Soxhlet extraction system.

### 2.4. Water holding capacity, pH, and colour values

The value of water holding capacity (WHC) in breast meat was determined using the procedure described by Hamm using filter-paper press method [11]. A 0.3 g of sample were placed on filter paper and covered with other filter paper on top then the samples were pressed until the dry and wet area formed, then calculated total of H₂O were out of the sample.

### Table 1. Ingredients of the experimental chicks diet (%)

| Ingredient              | Starter  | Finisher |
|-------------------------|----------|----------|
| Yellow corn             | 54.75    | 58.50    |
| Meat bone meal          | 4.75     | 2.00     |
| Soybean meal            | 35.75    | 32.75    |
| Soybean oil             | 1.50     | 3.50     |
| DL-Methionine           | 0.30     | 0.30     |
| L-Lysine                | 0.20     | 0.20     |
| Limestone               | 0.50     | 0.50     |
| Dicalcium phosphate     | 1.50     | 0.10     |
| Premix                  | 0.50     | 0.50     |
| NaCl                    | 0.20     | 0.20     |

Analysed composition:
- Crude protein: 20.90 20.60
- Crude fiber: 7.06 6.87
- Crude fat: 0.94 1.46
- Metabolizable Energy¹ (kcal/kg): 3314.99 3474.03

¹ME was calculated based on Balton formula = 40 \{0.87 \left[\text{crude protein} + 2.25 \text{crude fat} + \text{nitrogen-free extract}\right] + 2.5\} [9]
The value of pH in breast meat was measured using digital pH meter. The cathode calibrations, then pricked in the sample and left up to that number printed on a digital measurement are stabilize. Cathode pH meter, rinsed with distilled water and dried before used again. The colour value of breast meat was measured using colorimeter according to method of Commission International d’Eclairage (CIE) with Hunter colour value L* a* b* unit [12]. The L* value represents the colour brightness, a* value represents the level of green-red colour, b* value represents the colour level blue-yellow.

2.5. Statistical analyses
The obtained data were analysed using statistical program SPSS 25.0 (Statistical Package for the Social Sciences) for windows. The differences between treatments were subjected to Analysis of Variance using the probability level of 0.05. Duncan’s multiple range test was conducted when the significant difference was found.

3. Results and Discussion
3.1 Proximate Composition
The proximate compositions of breast meat of the Indonesian indigenous crossbred chicken are presented in Table 2. Supplementation of formic acid and S. cerevisiae in single or combination showed significant effect ($p < 0.05$) on crude protein and moisture content of meats. The crude protein content of meats significantly increased ($p < 0.05$) in chicks fed basal diet supplemented with combination of formic acid and S. cerevisiae (T3) compared to that in T0, T1 and T2 chicks. The moisture content was lower ($p < 0.05$) in T3 than that in other chicks. Meanwhile, no significant differences was detected on crude fat content across the chicken meats.

Table 2. Proximate compositions (%) of the Indonesian indigenous crossbred chicken breast meat

| Parameter     | Group |       |       |       |       | p Value |
|---------------|-------|-------|-------|-------|-------|---------|
| Moisture      | T0    | T1    | T2    | T3    |       | 0.004   |
|               | 76.77±0.50* | 76.82±0.20* | 76.37±0.11<sup>b</sup> | 75.69±0.10<sup>b</sup> |       | 0.004   |
| Protein       | 21.15±0.30<sup>b</sup> | 21.15±0.50<sup>b</sup> | 21.44±0.45<sup>b</sup> | 22.16±0.43<sup>a</sup> |       | 0.000   |
| Fat           | 0.65±0.11 | 0.73±0.14 | 0.68±0.10 | 0.72±0.15 | 0.724 |         |

T0 = control diet (basal diet), T1 = basal diet plus formic acid at 2g/kg diet, T2 = basal diet plus S. cerevisiae 3g/kg diet, and T3 = basal diet plus formic acid at 2g/kg diet and S. cerevisiae 3g/kg diet. *<sup>a</sup>Means within the same row indicating different superscripts are significantly differ.

3.1.1 Protein Content
In this study, crude protein content was higher in T3 meat than in other chicken meats. Increasing meat protein levels might be related to the activity and performance of digestive enzymes that determine protein synthesis and protein accumulation in meat (protein deposition) as reported by Samanta et al. [13], in which the addition of organic acids in feed could increase proteolysis activity and increase amino acid digestibility due to the well-balanced gastrointestinal tract environment. Moreover, Altaf-ur-Rahman [14] in a previous study stated that in several specific conditions S. cerevisiae could help high-performance and activity of protease enzyme in the gastrointestinal tract. Indeed, the high amino acid digestibility could substantially induce protein deposition in meat and might result in the increased protein content of meats.

3.1.2 Moisture Content
In the current study, supplementation of S. cerevisiae or combination of S. cerevisiae and formic acid decreased the moisture content of meats. In such case, the decreased water content in meats seemed to be related to the decrease in the number of free water molecules in the meats. Balakumar and Arasaratnam [15] pointed out that treatment using yeast S. cerevisiae increased the osmotic pressure resulting in a decrease of free water molecules. Meanwhile, Salah et al. [16] reported that supplementation using organic acids affected the hydrophobic amino acid content in meat protein. According to Pearce et al. [17] the amino acid groups formed a protein and thereby determine its ability to bind and form type of water molecules. In this study, the moisture content was calculated
using oven drying method that could only measure an evaporated water [10]. According to Park [18] the evaporated water is composed of free-water and weakly-bound water molecules. It could therefore be assumed that the moisture content of meats highly depends on the types of water molecules in meats. This is in line with Warner’s demonstrating that the amount of water content of meat is influenced by the presence of water molecules in tissue that are arranged in the form of free water molecules and weakly bound water molecules [19].

3.1.3 Fat Content

Our study showed that the addition of formic acid and S. cerevisiae showed no effect on the fat content of Indonesian indigenous crossbred chicken meat. This condition was presumably due to the low-amount of treatment compound that might not able to affect changes in blood cholesterol and triglycerides forming process. The number of feed intake and nutrients obtained by the chicken body is important since it will be used for metabolic process such as fat formation. This was in line with the report of Soeparno [11] that the amount of nutrient content in the ration given to livestock can modify the mechanisms of fat formation or lipogenesis that can be also related to meat fat content. The other possibility was that high-protein content in meat might be lead to inhibit fat enzyme to work optimally. As reported by Syakir et al. [20] the high-amount of protein in body can suppress the activity of fatty acid synthase enzymes which results in inhibition of lipogenesis in the body.

Table 3. Water holding capacity (WHC), pH, and colour values of Indonesian indigenous crossbred chicken breast meat

| Parameter | T0 | T1 | T2 | T3 | p Value |
|-----------|----|----|----|----|---------|
| WHC       | 39.98±0.84<sup>a</sup> | 37.80±0.78<sup>b</sup> | 37.91±1.27<sup>b</sup> | 37.67±1.67<sup>b</sup> | 0.023 |
| pH        | 6.3±0.50 | 6.32±0.40 | 6.36±0.18 | 6.29±0.95 | 0.267 |
| L*        | 37.55±2.19<sup>a</sup> | 35.55±4.46<sup>ab</sup> | 31.00±1.54<sup>b</sup> | 36.80±4.90<sup>b</sup> | 0.044 |
| a*        | 22.40±5.80 | 23.15±2.95 | 27.40±7.46 | 20.20±6.20 | 0.300 |
| b*        | 37.15±2.07 | 29.95±16.48 | 32.65±2.43 | 37.55±3.07 | 0.452 |

T0 = control diet (basal diet), T1 = basal diet plus formic acid at 2g/kg diet, T2 = basal diet plus S. cerevisiae at 3g/kg diet, and T3 = basal diet plus formic acid at 2g/kg diet and S. cerevisiae 3g/kg diet. <sup>ab</sup>Means within the same row indicating different superscripts are significantly differ.

3.2 Water Holding Capacity, pH, and Colour Value of Meats

The data on Table 3 presents the effect of dietary treatments on WHC, pH, and colour values (CIE; L*, a* and b*) of the Indonesian indigenous crossbred chicken breast meats. In the present study, no significant differences (p > 0.05) were detected on pH, and a* and b* values of breast meat among the groups of chickens. However, water holding capacity (WHC) and L* value of meats show significant difference (p < 0.05).

3.2.1 Water Holding Capacity

The addition of formic acid and S. cerevisiae had a significant effect (p < 0.05) on decreasing of the WHC of the Indonesian indigenous chicken meat. The decrease in WHC was thought to be caused by structural and chemical changes in the muscle. El-Senousey et al. [21] found that the incorporation of organic acids resulted in a decrease in muscle glycogen concentrations. The small amount of glycogen causes actomyosin bonds to form, which speeds up the rigor mortis process. This is consistent with Soeparno [11], who claims that the production of actomyosin during rigor mortis causes room for water molecules to shrink, resulting in fluid secretion in muscles and a decrease in WHC. Meanwhile, the probiotic supplementation increased meat’s endogenous protease activity [22]. According to Schreurs [23], the amount of reactive myofibrillar protein groups and their ability to bind water can be affected by proteolytic enzyme activity. The increase in proteolytic activity is believed to have contributed to the decrease in meat WHC observed in this research.
3.2.2 pH Values of Meats

Data on the pH of meats showed that the addition of formic acid and \textit{S. cerevisiae} had no effect on the pH value of Indonesian indigenous crossbred chicken meats. This condition might be caused by the low-doses of formic acid and \textit{S. cerevisiae} administrated that was not able to affect the muscle buffer capacity to alter. Muscle buffer capacity is key in determining the potential for muscle glycogen in the glycolysis process to produce a maximum pH changes. This is in line with Asghar and Henrickson [24] showing that the potential of glycogen in the glycolysis process for the decreasing pH mechanism could be different which caused by the buffer capacity in muscles. Furthermore, high and low pH values of meat determine the appearance of the colour that will be resulted in meat. A higher pH value of meat will form the appearance of the colour of the meat to be dark [25].

3.2.3 Colour Values of Meats

Our findings showed that the treatment had a significant effect \((p<0.05)\) on \(L^*\) values, but had no significant effect on \(a^*\) and \(b^*\) values of meats. In this study, the \(L^*, a^*\) and \(b^*\) values of the Indonesian indigenous crossbred chicken meat ranged from 31.00 to 37.55; 20.20 to 27.40 and 29.95 to 37.55, respectively. The previous study reported that \(L^*, a^*\) and \(b^*\) values of the crossbred chicken breast meat were 58.64 to 59.98, 4.35 to 4.88 and 10.18 to 11.38, respectively [2]. Whereas, according to Wattanachant et al. [26], broiler chicken meat has a \(L^*\) value of 32.53-38.79 and a meat pH of 5.93 - 6.62, indicating a high pH value that affects the presence of a darker meat colour.

The variation in colour values that resulted were most likely due to a number of reasons, including the use of feed additives, the age at which the chicken was slaughtered, and the variety of chicken used. The \(L^*\) colour value in breast decreased as a result of our study findings, which we believe was caused by an increase in protein meat content, which affected myosin activity and influenced meat colour appearance. Breast meat with more white fiber muscle, high glycogen content, and low ATPase activity is well established [27]. These conditions resulted in a fast conversion of piruvate to lactate, which then influenced the reaction between myoglobin amino acid sequence and other biomolecules, affecting meat colour stability and shift [11].

4. Conclusion

In conclusion, dietary supplementation of a mixture of formic acid and \textit{S. cerevisiae} was effective in improving the breast meat quality of the Indonesian indigenous crossbred chickens.

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