ISSN 1682-8356
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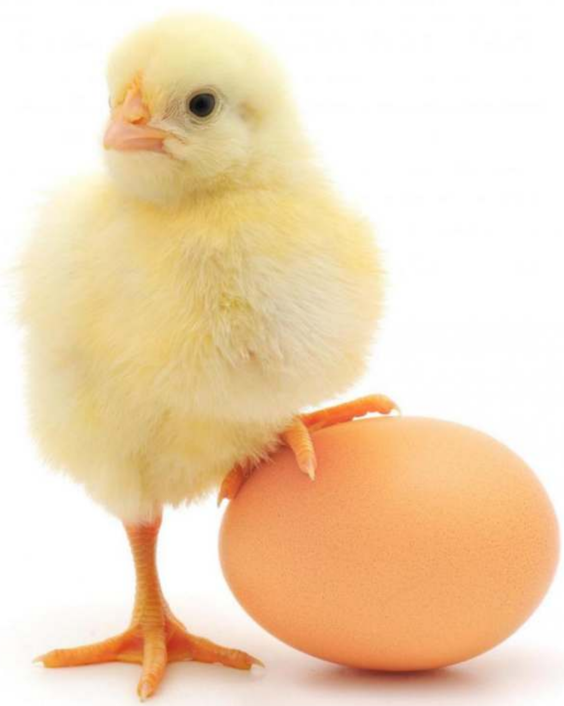

INTERNATIONAL JOURNAL OF
POULTRY SCIENCE

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E-mail: editorijps@gmail.com

OPEN ACCESS International Journal of Poultry Science

ISSN 1682-8356
DOI: 10.3923/ijps.2018.605.609

Research Article
Effect of Fermentation using *Trichoderma harzianum* and *Saccharomyces cerevisiae* on Crude Protein, Crude fibre and Zinc Content of Duckweed

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Abstract
Background and Objectives: Duckweed has a potential to be used as poultry feed, however due to its high crude fibre content, its inclusion at high levels is limited. This could be overcome by means of fermentation with microbial strains like *Trichoderma harzianum* and *Saccharomyces cerevisiae*. Therefore, the present study was designed in order to improve the nutritive value of duckweed by fermentation with *Trichoderma harzianum* and *Saccharomyces cerevisiae*. **Materials and Methods:** There were five treatments, each having 4 replicates, thus making it a total of 20 experimental units. The treatments consisted of P1: (Fermentation using *Trichoderma harzianum* for 1 day followed by *Saccharomyces cerevisiae* fermentation for 9 days), P2: (*Trichoderma harzianum* for 3 days followed by *Saccharomyces cerevisiae* fermentation for 7 days), P3: (*Trichoderma harzianum* for 5 days followed by *Saccharomyces cerevisiae* fermentation for 5 days), P4: (*Trichoderma harzianum* for 7 days followed by *Saccharomyces cerevisiae* fermentation for 3 days) and P5: (*Trichoderma harzianum* for 9 days followed by *Saccharomyces cerevisiae* fermentation for 1 day). **Results:** The fermentation of Duckweed using *Trichoderma harzianum* and *Saccharomyces cerevisiae* microbes was found helpful in modifying the nutritive value of duckweed. The best combination that improved the nutritive value of duckweed in terms of increasing the crude protein (33.88%) and zinc (88.6%) content and decreasing the crude fibre (8.16%) content was P2 in which duckweed was fermented with *Trichoderma harzianum* for 3 days followed by *Saccharomyces cerevisiae* fermentation for 7 days. **Conclusion:** Fermentation with *Trichoderma harzianum* for 3 days followed by *Saccharomyces cerevisiae* fermentation was found effective in improving the nutrient composition of duckweed for poultry feeding.

Key words: Duckweed, fermentation, nutrient quality, *Saccharomyces cerevisiae*, *Trichoderma harzianum*

Citation: Hendi Setiyatwan, E. Harlia, D. Rusmana, Tubagus Benito and Lovita Adriani, 2018. Effect of fermentation using *Trichoderma harzianum* and *Saccharomyces cerevisiae* on crude protein, crude fibre and zinc content of duckweed. Int. J. Poult. Sci., 17: 605-609.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Duckweed has been known for a long period of time as a potential source of food for humans and animals. It is a water plant that lives floating on the surface of the water. It is proliferated vegetatively[1] with the total biomass production reaching 1.76-2.34%[2,3]. It contains 22.4% crude protein and 10.16% crude fiber[2]. Moreover, it has amino acids {such as lysine (6.9%), methionine (1.4%) and histidine (2.7%)}[4] and high content of minerals and vitamin A[5]. It has been used as an animal feed[6-8]. The use of Duckweed in poultry nutrition is limited to 5% level due to high crude fiber content, unbalanced essential amino acids and low Zn content.

To overcome the problem of limited use of Duckweed, fermentation is a viable option. Fermentation can increase the nutrient availability and reduce the amount of anti-nutrients[6]. Fermentation also increases the content of proteins, essential amino acids, vitamins and bioactive compounds[9]. Thapa and Tamang[10] also reported that fermentation process increased the content of vitamin B12, vitamin B complex and C, lysine and tryptophan and various minerals. Furthermore, Astuti *et al.*[11] reported that fermentation improves the content of folic acid, niacin, riboflavin, nicotinamide and pyridoxine.

The use of *Trichoderma harzianum* for fermentation has been reported to have positive effect on lowering of crude fiber. Ghosh and Chattopadhyay[12] reported that the crude fiber of palm kernel flour decreased after fermentation because of the use of *Trichoderma harzianum*. Moreover, Setiyatwan[7] indicated that the crude fiber content of duckweed decreased by 76.06% after fermentation by *Trichoderma harzianum* for 6-9 days. *Saccharomyces cerevisiae* is a yeast that can produce heterologous proteins[13]. The fermentation using *Saccharomyces cerevisiae* yeast can enrich the material with amino acid dl-methionine and inorganic Zinc (Zn) mineral. Zn is an important element in the growth and metabolism of *Saccharomyces cerevisiae*. In addition, zinc is involved in the structure and function of protein and nucleic acids[14]. *Saccharomyces cerevisiae* can combine Zn ions of origin substrate. The amount of Zn absorbed depends on the Zn source, cell physiology, specific surface properties of the organism and the physicochemical influence of the environment[15]. The concentration of Zn in the substrate affects the ability of *Saccharomyces cerevisiae* to form metal bonds[16].

Duckweed quality can be improved by fermentation process using *Trichoderma harzianum* and *Saccharomyces cerevisiae*. The secondary metabolic pathway of *Trichoderma harzianum* and *Saccharomyces cerevisiae* are synergistic. *Trichoderma harzianum* can provide nitrogen for the growth of *Saccharomyces cerevisiae* and subsequently used as a raw material for the formation of metallic bonds and amino acids. The activity of both microbes produces complementary effects for better results, thus imitates a natural microbial growth medium. Duckweed fermentation process using two microbes (co-culture) is expected to produce functional complementary enzymes due to the mutual expression of metabolic pathways in substrate utilization. However, the research on the quality improvement of duckweed through fermentation using *Trichoderma harzianum* and *Saccharomyces cerevisiae* has not been carried out. Therefore, the purpose of this research was to enhance the nutritive value of Duckweed by fermentation process using two types of microbes.

MATERIALS AND METHODS

Materials and research tools: The research material used was duckweed, potato dextrose, PDA agar, Aquadest, $ZnCO_3$, dl-methionine, rice, tripton, cotton, *Trichoderma harzianum* and *Saccharomyces cerevisiae*. The equipments consisted of analytical scales, technical scales, measuring flask 1000 mL, Erlenmeyer 1000 mL, stirrer mugs, reaction tubes, Oase needles, spirtus lights, Erlenmeyer glasses, sterilisator, beaker glass, etiquette paper, electric oven, refrigerator, autoclave and fermentor.

Research procedure: Substrate consisted of a mixture of duckweed and selective medium. The mixture was boiled in the water for 60 min at a temperature of 115°C and pressure of 1.1 atmospheres. *Trichoderma harzianum* microbes were incubated in each treatment @ 3×10^7 spores/100 g of the substrate for 1, 3, 5, 7 and 9 days at room temperature. After first stage fermentation, $ZnCO_3$ (186 ppm) and dl-methionine (286 ppm) were added. Afterwards, substrate was fermented using *Saccharomyces cerevisiae* @ 3×10^7 spores/100 g of substrate for 9, 7, 5, 3 and 1 day. Thereafter, the product was dried in the oven.

Experimental design: The experimental design used was Completely Randomized Design (CRD). There were five treatments, each having 4 replicates, thus making it a total of 20 experimental units. The treatments consisted of:

- **P1:** Fermentation using *Trichoderma harzianum* for 1 day followed by *Saccharomyces cerevisiae* fermentation for 9 days
- **P2:** *Trichoderma harzianum* for 3 days followed by *Saccharomyces cerevisiae* fermentation for 7 days

- **P1:** *Trichoderma harzianum* for 5 days followed by *Saccharomyces cerevisiae* fermentation for 5 days
- **P4:** *Trichoderma harzianum* for 7 days followed by *Saccharomyces cerevisiae* fermentation for 3 days
- **P5:** *Trichoderma harzianum* for 9 days followed by *Saccharomyces cerevisiae* fermentation for 1 day

Parameters recorded

- **Crude protein:** Crude protein content (based on Dry Matter) was determined by Wendee method. The proximate analysis was carried out by Kjeldahl method. It consisted of destruction, distillation and titration stages
- **Crude fiber:** The analytical method used to determine crude fiber (based on Dry Matter) was by proximate analysis using Wendee method. It involves dissolving the soluble ingredients in strong acids and bases. The material is then filtered out leaving the remaining residue. This residue is crude fiber comprising of cellulose and slight lignin and pentosan
- **Zinc content:** The Zn content was analyzed by Atomic Absorption Spectrophotometer (AAS)

Statistical analysis: Data collected were subjected to one-way analysis of variance (ANOVA) as per Steel and Torrie. If there were differences in means, they were compared using Duncan's multiple range test with 5% significant level[17].

RESULTS AND DISCUSSION

Crude protein content of fermented duckweed: The fermentation of Duckweed using *Trichoderma harzianum* and *Saccharomyces cerevisiae* was helpful in increasing the protein content of duckweed (Table 1). The results of the present study are in-line with other studies, such as Omwango *et al.*[18] and Ezekiel and Aworh[19] who improved the protein content of Pineapple waste (up to 45%) and Cassava peel by various microbes.

The highest Duckweed protein content was observed in the group in which Duckweed was fermented using *Trichoderma harzianum* for 3 day followed by *Saccharomyces cerevisiae* fermentation for 7 days (P2). This was followed by the groups P1, P3 and P4. The significantly ($p<0.05$) lowest Duckweed protein content was observed in the group in which Duckweed was fermented using *Trichoderma harzianum* for 9 day followed by *Saccharomyces cerevisiae* fermentation for 1 day (P5). This is in line with the study of Kuswardani and Wijajaseputra[20] who stated that the short duration fermentation using *Saccharomyces cerevisiae* decreases the protein content. The longer fermentation with *Saccharomyces cerevisiae* was found to be more useful in increasing the Duckweed protein content. This is in agreement with the results of Kustyawati *et al.*[21] who reported that the addition of *Saccharomyces cerevisiae* can be used as a duckweed modification agent to increase its protein content and solubility.

Crude fiber content of fermented duckweed: The effect of fermentation on crude fibre content in duck weed is given in Table 1. The lowest crude fibre content in duckweed was observed in the group in which duckweed was fermented using *Trichoderma harzianum* for 3 days followed by *Saccharomyces cerevisiae* fermentation for 7 days (P2). This was followed by the groups P3, P4 and P5. Highest crude fibre content in duckweed was observed in the group in which duckweed was fermented using *Trichoderma harzianum* for 1 day followed by *Saccharomyces cerevisiae* fermentation for 9 days (P1).

Dietary fibre is the part of plant material consisting mainly of cellulosic and non-cellulosic polysaccharides and a non-carbohydrate component, lignin. These components are highly resistant to hydrolysis by alimentary enzymes and therefore cannot be digested or absorbed in the blood stream. So, high crude fibre is not desirable. Hatta *et al.*[22] stated that *Trichoderma* sp. can reduce lignocellulose. Furthermore, Nsereko *et al.*[23] suggested that *Trichoderma harzianum* can break the bonds of lignin and cellulose because it produces cellulase, xylosidase and xylanase enzymes. Shaikh *et al.*[24] reported *Saccharomyces cerevisiae* decreased the crude fibre content of cottonseed cake.

Zinc content of fermented duckweed: The effect of fermentation on zinc content in duck weed is given in Table 1. The highest zinc content in duck weed was observed in the

Table 1: Crude protein, crude fiber and Zn content of duckweed fermented with *Trichoderma harzianum* followed by *Saccharomyces cerevisiae*

	Treatments				
Parameters (%)	P1	P2	P3	P4	P5
Crude protein	31.84 ± 0.76^{b}	33.88 ± 0.85^{c}	31.90 ± 0.82^{b}	31.01 ± 0.82^{b}	29.73 ± 0.89^{a}
Crude fiber	16.27 ± 0.82^{c}	8.16 ± 0.87^{a}	10.27 ± 0.84^{b}	10.25 ± 0.82^{b}	10.33 ± 0.83^{b}
Mineral Zn	83.08 ± 1.43^{a}	88.60 ± 0.96^{c}	86.60 ± 2.46^{b}	84.60 ± 0.80^{ab}	84.60 ± 1.72^{ab}

Means in the same column with different superscript differ significantly ($p<0.05$)

group in which duck weed was fermented using *Trichoderma harzianum* for 3 days followed by *Saccharomyces cerevisiae* fermentation for 7 days (P2). This was followed by the groups P3 in which duck weed was fermented using *Trichoderma harzianum* for 5 days followed by *Saccharomyces cerevisiae* fermentation for 5 days. The lowest zinc content in duck weed was observed in the group in which duck weed was fermented using *Trichoderma harzianum* for 1 day followed by *Saccharomyces cerevisiae* fermentation for 9 days (P1).

The difference in the zinc content of duckweed among various treatments was observed due to the fact that the nutrient content of duckweed depends on the environment where it is grown[25]. Zinc in the feed is in the form of inorganic mineral salts, such as Zn oxide[26]. Schneider *et al.*[27] stated that Zn is the essential element and acts as a co-factor for many proteins and enzymes.

CONCLUSION

Fermentation with *Trichoderma harzianum* and *Saccharomyces cerevisiae* was found effective in modifying the nutrient composition of duckweed. The best combination that improved the nutritive value of duckweed in terms of increasing the crude protein and zinc content and decreasing the crude fibre content was *Trichoderma harzianum* fermentation for 3 days followed by *Saccharomyces cerevisiae* fermentation for 7 days. Hence, this combination is recommended for improving the nutritive value of duckweed for poultry feeding.

SIGNIFICANCE STATEMENT

This study discovers the methods to modify the nutrient quality of duckweed for its incorporation in poultry feed. This study will help the researchers to study the effect of fermentation in improving the nutritive value of duckweed by using various microbial strains which many researchers were not able to explore earlier. Thus, a new theory on the improvement of quality of duckweed through fermentation using a combination of microbes may be arrived at.

ACKNOWLEDGMENT

The authors would like to thank the honorable Rector of Padjadjaran University, Ministry of Research, Technology and Higher Education of Padjadjaran University who supported this research. The research was carried out with the financial support from DIPA Fund of Universitas Padjadjaran no. 855/UN6.3.1/PL/2017 dated March 8th, 2017.

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