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Research Article
Effect of Tea Dregs Form and Different Fermentation Process on the Nutrient, Tannin, Saponin, flavonoid content and Antioxidant Activity

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Abstract

Background and Objective: The production of tea dregs increases every year. Tea dregs contain high levels of nutrients, bioactive compounds that are antioxidants and some antinutrients. The aim of the study was to assess the effect of fermentation on levels of nutrients, tannins, saponin, flavonoids and antioxidant activity of various forms of tea dregs. **Materials and Methods:** The present study used experimental methods and a completely randomized design. The treatment consisted of fermentation (F) comprising F0: Unfermented, F1: Fermentation using EM-4 and F2: Fermentation using *Trichoderma viride*. The fermented material was in three forms namely tea dreg-shaped leaf, granules and powders. Each treatment was replicated 4 times. The variables measured were moisture, ash, protein, fat, crude fiber, metabolizable energy, tannins, saponins, flavonoids and antioxidant activity. **Results:** The fermentation using EM-4 and *Trichoderma viride* had no significant effect ($p>0.05$) on the ash content, fat, saponin and flavonoids. However, it had a significant effect ($p<0.05$) on metabolizable energy, protein content, crude fiber and antioxidant activity. Additionally, it increased significantly ($p<0.01$) the moisture content but decreased the tannin of tea dregs. **Conclusion:** Fermentation using EM-4 and *Trichoderma viride* is effective in improving nutrient quality, flavonoid levels and antioxidant activity of tea dregs. Fermentation caused decrease in antinutrient levels in tea dregs.

Key words: Tea dregs, antioxidant activity, fermentation, tannin and saponin content, poultry feedstuff

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

INTRODUCTION

Tea dregs is one of waste product of tea industry. In 2016, tea production in Indonesia reached as much as 154,688 t[1]. The tea beverage industry in Indonesia comprises approximately 3,863 companies[2] and it produces as many as 470 t of tea dregs/year[3]. The diversity of tea processing produces tea wastes in different shapes. The tea dregs in shape depend on the kinds of tea were used by the factory. There are three kinds of tea dregs shape, namely flour, leaves and granules. Most of the tea dregs were burned, discarded as agricultural waste and used as compost.

Research on the potential of tea dregs as a feed supplement of poultry is still very rare. Each tea dregs shape contain different nutrients and bioactive compounds[4,5]. Tea dregs contain; Protein: 27.42%, crude fiber: 20.94%, fat: 2.01%, calcium: 0.2%, phosphor: 0.7%, ash: 7.83%, lignin: 6.07% and some antinutrients such as: Tannins, caffeine, the obromine, the ophylline, saponin and crude fiber[6]. Tea dregs contain bioactive compounds called polyphenol and catechins which provide antioxidants effect[7]. These antioxidants can prevent oxidation of LDL 20 times more powerfully than vitamin E[8-11] as antibacterial and photo protective agents[11,12].

Tea dregs should be fermented before it is given to poultry because tea dregs have high antinutrients content. Fermentation is a technology to improve the quality of the feedstuff. The materials used to ferment tea dregs are Effective Microorganisms-4 (EM-4) and *Trichoderma viride*. Both are very effective in fermenting crude fiber. EM-4 contains 80 genera of microorganisms comprising photo synthetic bacteria, *Actinomycetes* sp., *Lactobacillus* sp., *Saccharomyces* sp. and fungi[13]. *Trichoderma viride* is one of the fungi producing highly efficient cellulase enzymes to degrade cellulose element. According to Gunam *et al.*[14] *Trichoderma viride* produces cellulase that has complete enzyme components, i.e. cellobiohydrolase and β-glucanase. Substituted cellobiohydrolase breaks down natural cellulose and active β-glucanase into soluble dissolved cellulose. Cellulase enzymes produced by microbes can break down the cellulose structure into sugar, which is useful as a source of animal energy[15]. Crude fiber content of feedstuff decreases due to cellulase enzymes, thus the feedstuff becomes more easily digested. In general, all fermented end products usually contain simpler compounds. The simpler compounds are easier to digest and have higher nutritional value than the original ingredients[16]. The objectives of the study were to know the content of nutrients, bioactive compounds and antioxidant activity of different shapes of tea dregs before and after fermentation process.

MATERIALS AND METHODS

Materials: The tea dregs consisted of three forms, namely leaf-shape (leaves mixed with tea branch), granule and flour form. The tea dregs derived from tea factories in Pekalongan and Bekasi areas. The materials used for fermentation were EM-4, *Trichoderma viride*, molasses, water, bran, salt, MSG (monosodium glutamate), plastic bags and jars.

Methods: This experimental study used a completely randomized design (CRD). There were three treatments, i.e., F0: Tea dregs was unfermented dregs of tea, F1: Tea dregs was the fermented dregs of tea using EM-4 and F2: Tea dregs was the fermented dregs of tea using *Trichoderma viride*. The fermented material was in three forms of tea dregs namely, leaf-shape (leaves mixed with tea branch), granule and flour form. Each treatment was repeated 4 times. The observed variables were the content of nutrients, saponins, flavonoids and tannins and antioxidant activity of tea leaf fibres, granules and flour before and after being fermented.

The fermentations using *Trichoderma viride*: There are two phases fermentation using *Trichoderma viride* namely the phase of manufacturing the inoculum of *Trichoderma viride* and the phase of fermentation processing the tea dregs with *Trichoderma viride*. The method of manufacturing the *Trichoderma viride* inoculum was as follows: (1). Prepared potato extract by adding 90.1 g potato to 200 mL of distilled water, (2) Added 2 g dextrose and then transferred to an Erlenmeyer flask at a temperature of 121°C and pressures 1.5-2 atm for 15 min, (3) Added dry isolates of *Trichoderma viride* 8% of the volume of media, (4). Furthermore, incubated for 5×24 h at room temperature under anaerobic conditions. After incubation was completed, recreated the media in accordance with the way described in numbers 1-4 but using isolates previously made and (5). Added tea dregs, as much as 5% of the volume of media, for adaptation and further incubated at a temperature of 37°C. After 5×24 h, the inoculum *Trichoderma viride* was ready to use.

The fermentation process with *Trichoderma viride* was as follows: (1) Prepared materials for fermentation including inoculum, urea, molasses and tea dregs, (2) Prepared a tea dreg container and added as many as 500 g of tea dregs, (3) Urea was further added at a concentration of 1% (5 g) and molasses in a 5% dilution (5:30), (4) Then, 8% inoculum was incorporated and stirred until evenly distributed and (5) Incubated for 5×24 h under anaerobic conditions.

The fermentations using EM-4: The fermentation of tea dregs using EM-4 was as follows: (1) Prepared the materials for fermentation including tea dreg, EM-4, molasses, water and plastic, (2) Prepared 700 g of tea dregs in plastic, (3) Added 1 mL EM-4 and 1 mL of molasses diluted in 50 mL of water, (4) EM-4 solution was sprayed onto the material and mix evenly and (5) Then the material was incubated for 7×24 h under anaerobic condition at room temperature.

After the tea dregs were fermented, they were then aired for 30 min to become cool. After that, the fermented tea dregs were analyzed to determine the levels of nutrients (proximate analysis), tannin content, saponin content, flavonoid levels and antioxidant activity.

Proximate analysis: The proximate analysis was conducted at the Laboratory of Animal Feed Material, Faculty of Animal Science and University of Jenderal Soedirman.

Phytochemical screening, saponin and tannin test: Preparation of the test solution for phytochemical screening (alkaloids, saponins, tannins, steroids, triterpenoids and glycosides)was done by dissolving the extracted tea dregs in ethanol p.a. ata 1:10 ratio. Alkaloid detection was performed by reacting the test solution with Dragendorff's reagent and with Mayer's reagents. The formation of orange precipitate in the reaction with Dragendorff's reagent and yellow precipitate in the reaction with Meyer's reagent indicated the presence of alkaloids.

The saponin portion was used to observe the formation of foam after shaking. The presence of foam, 1-10 cm high, that was stable for longer than 10 min and was not lost with the addition of 1 drop of 2N HCl indicated the presence of saponins.

Tannin test was done by reacting 3 mL of test solution with 5 drops of 1% NaCl and 3 drops of gelatin solution. If a sediment was formed, then the positive extract solution contained tannin.

The antioxidant activity test: Antioxidant activity testing used the method of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) due to its ability to measure antioxidants reactions with free radicals resulting from the process of fermenting the tea dregs. A total of 10 mg of sample dissolved in methanol PA was then made into a serial concentration of 100, 200, 400, 800 ppm. As much as160 µL of extract from each series of sample concentration, plus 40 µL of DPPH solution. After 30 min at room temperature, absorbance was measured at wavelength of 517 nm. As a negative control methanol was used with the same protocol and as a blank, 200 µL methanol PA was used without additions; the solution of DPPH percent of inhibition was calculated by the equation:

$$\text{Inhibition}(\%) = \frac{(A-B)-(C-D)}{A-D} \times 100\%$$

Where

A = Absorbance of negative control
B = Blank absorbance
C = Absorbance control extract
D = Absorbance extract

The percentage data of inhibition were used to determine the value of inhibitory concentration50 (IC_{50}) in ppm. The value of IC_{50} was determined by probity analysis using Microsoft Excel 2007 software.

Testing of antioxidant activity, levels of flavonoids, saponins and tannins was performed at Tropical Crops Research Institute Bogor.

Statistical analyses: The data obtained were analysed statistically using the one-way ANOVA with SPSS (Windows version of SPSS, release 22)[17]. Significant differences between the mean was identified with honestly significance difference (HSD) test[18]. Differences of $p<0.05$ were considered statistically significant. The statistical model was as follows:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where

Y_{ij} = Response of the observation
μ = Overall mean
T_i = Treatment effect
ϵ_{ij} = Experimental error

RESULTS

Water content: The content of the leaves, granule and powder of tea dregs that were fermented by EM-4 and *Trichoderma viride* can be seen in Table 1. The water content of the leaves, granules and powder of unfermented tea dregs was 6. 25, 5.53 and 4.80%, respectively. The water content of the leaves, granules and powder of tea dregs fermented with EM-4 was14.76, 14.74 and 13.94%, respectively. The water content of the leaves, granules and powder of tea dregs fermented with *Trichoderma viride* was 27.39, 28.30 and 30.70%, respectively.

Table 1: Nutrient content of tea dregs

	Nutrient contents					
Tea dregs form	Water (%)**	Ash (%)	Protein (%)*	Fat (%)	Crude fiber (%)*	Metabolic energy (kcal kg^{-1})
Leaves form						
F0	6.25±0.03^{a}	3.92±0.10	18.83±0.87^{a}	1.44±0.88	25.64±1.54^{c}	2214.07±29.08^{a}
F1	14.76±0.03^{b}	3.05±3.37	16.89±1.77^{a}	1.28±0.95	17.37±1.20^{b}	2189.90±35.20^{a}
F2	27.39±0.05^{c}	3.47±0.05	15.76±1.82^{a}	2.11±0.50	18.22±1.98^{b}	2165.47±23.40^{a}
Granules form						
F0	5.53±0.01^{a}	3.64±0.10	26.37±0.25^{c}	1.53±0.94	21.20±2.14^{c}	2016.91±34.65^{a}
F1	14.74±0.07^{b}	3.50±0.06	24.29±0.48^{c}	2.87±0.44	13.68±0.80^{a}	2330.09±23.50^{b}
F2	28.30±0.07^{c}	3.42±0.02	21.48±0.53^{b}	2.38±1.65	11.35±2.15^{a}	2317.77±33.56^{b}
Powder form						
F0	4.80±0.02^{a}	3.54±0.12	24.94±0.13^{c}	2.72±1.02	20.10±2.33^{c}	2222.72±29.54^{a}
F1	13.94±0.02^{b}	3.77±0.08	21.01±0.22^{b}	3.48±0.86	15.26±3.18^{b}	2346.35±24.35^{b}
F2	30.70±0.02^{c}	3.10±0.03	21.19±0.83^{b}	3.01±1.02	12.26±3.78^{a}	2453.09±22.90^{c}

F0: Unfermented, F1: EM-4 fermented, F2: *Trichoderma viridae* fermented, *: Influence of fermentation had a significant effect ($p<0.05$) on nutrient contents, **: Influence of fermentation had a highly significant effect ($p<0.01$) on nutrient contents

Ash content: The ash content of the leaves, granules and powder of unfermented tea dregs was 3.92, 3.64 and 3.54%, respectively. The ash content of the leaves, granules and powder of EM-4 fermented tea dregs was 3.05, 3.50 and 3.77%, respectively. The ash content of the leaves, granules and powder from tea dregs fermented using *Trichoderma viride* was 3.47, 3.42 and 3.10%, respectively (Table 1).

Protein content: The protein content of the leaves, granules and powder of unfermented tea dregs was 18.83, 26.37 and 24.94%, respectively. For tea dregs fermented with EM-4, the protein content of the leaves, granules and powder was 16.89, 24.29 and 21.01%, respectively. The protein content of the leaves, granules and powder of tea dregs fermented with *Trichoderma viride* was 15.76, 21.48 and 21.19% (Table 1), respectively.

Fat content: The fat content of the leaves, granules and powder of unfermented tea dregs was 1.44, 1.53 and 2.72%, respectively. The fat content of the leaves, granules and powder from tea dregs fermented with EM-4 was 1.28, 2.87 and 3.48%, respectively. For the *Trichoderma viride*-fermented tea dregs, the fat content of the leaves, granules and powder was 2.11, 2.38 and 3.01%, respectively (Table 1).

Crude fiber content: The crude fiber content of the leaves, granules and powder of unfermented tea dreg was 25.64, 21.20 and 20.10% respectively. The crude fiber content of leaves, granules and powder of tea dregs fermented using EM-4 was 17.37, 13.68 and 15.26% respectively. The crude fiber content of the leaves, granules and powder of tea dregs fermented with *Trichoderma viride* was 18.22, 11.35 and 12.26%, respectively (Table 1).

Metabolic energy content: The metabolic energy content of the leaves, granules and powder of unfermented tea dregs was 2214.07, 2016.91 and 2222.72 kcal kg^{-1}, respectively. The metabolic energy content of the leaves, granules and powder of tea dregs fermented using EM-4 was 2189.90, 2330.09 and 2346.35 kcal kg^{-1}, respectively. The metabolic energy content of the leaves, granules and powder of fermented tea dregs with *Trichoderma viride* was 2165.47, 2317.77 and 2453.09 kcal kg^{-1}, respectively (Table 1).

Content of tannins: The tannins content of the leaves, granules and powder of unfermented tea dregs was 9.25, 7.67 and 10.30%, respectively. The tannins content of the leaves, granule and powder of tea dregs fermented using EM-4 was 5.32, 5.14 and 5.38%, respectively. The tannins of the leaves, granules and powder of tea dregs fermented with *Trichoderma viride* was 4.81, 5.79 and 4.74% (Table 2), respectively.

Content of saponins: The saponin content of the leaves, granules and powder of unfermented tea dregs was 0.91, 1.01 and 0.89%, respectively. The saponin content of the leaves, granules and powder of tea dregs fermented with EM-4 was 0.44, 0.38 and 0.46%, respectively. The saponin content of the leaves, granules and powder of tea dregs fermented using *Trichoderma viride* was 1.02, 0.58 and 0.79%, respectively (Table 2).

Content of flavonoids and the phytochemicals: The tea batches contained quercetin flavonoids and phytochemical substances (Table 3). The quercetin flavonoid content of the leaves, granules and powder of unfermented of tea dregs was 0.78, 0.94 and 0.90%, respectively. The quercetin flavonoid

Table 2: Levels of tannin and saponin in fermented dregs of tea

Tea dregs forms	Tannin contents (%)**	Saponin contents (%)
Leaves form		
F0	9.25±1.35[b]	0.91±0.02
F1	5.32±0.08[a]	0.44±0.03
F2	4.81±0.04[a]	1.02±0.02
Granules form		
F0	7.67±0.23[a]	1.01±0.09
F1	5.14±0.15[a]	0.38±0.04
F2	5.79±0.09[a]	0.58±0.02
Powder form		
F0	10.30±0.10[b]	0.89±0.01
F1	5.38±0.08[a]	0.46±0.14
F2	4.74±0.09[a]	0.79±0.21

F0: Unfermented, F1: EM-4 fermented, F2: *Trichoderma viridae*. fermented, *: Influence of fermentation had a significant effect ($p<0.05$) on tannins content

Table 3: Levels of flavonoid, phytochemicals and antioxidant activity of tea dregs before and after fermentation

	Quercetin flavonoid contents (%)				
Tea dregs shape	Presence of phytochemicals	Alkaloid	Steroid	Glycosides	Antioxidant activity IC_{50} (ppm)*
Leaves form					
F0	0.78±0.001[a]	+	+	+	104.06±3.24[b]
F1	0.97±0.001[b]	+	+	+	46.85±3.99[a]
F2	1.32±0.001[b]	+	+	+	30.65±2.48[a]
Granules form					
F0	0.94±0.001[a]	+	+	+	62.34±5.42[a]
F1	1.10±0.001[b]	+	+	+	38.84±3.45[a]
F2	1.07±0.005[b]	+	+	+	48.06±5.21[a]
Powder form					
F0	0.90±0.002[a]	+	+	+	66.17±1.21[a]
F1	1.28±0.008[b]	+	+	+	39.79±2.04[a]
F2	1.12±0.001[b]	+	+	+	46.89±2.00[a]

F0: Unfermented, F1: EM-4 fermented, F2: *Trichoderma viridae*. fermented. *: Influence of fermentation had a significant effect ($p<0.05$) on antioxidant activity of tea dregs

content of the leaves, granules and powder of tea dregs fermented using EM-4 was 0.97, 1.10 and 1.28%, respectively. The quercetin flavonoid of the leaves, granules and powder of tea dregs fermented with *Trichoderma viride* was 1.32, 1.07 and 1.12%, respectively. All forms of tea dregs, both unfermented and fermented with EM-4 and *Trichoderma viride* contained phytochemical substances of alkaloids, steroids and glycosides (Table 3).

Antioxidant activity: The tea dregs had antioxidant activity (Table 3). The antioxidant activity with IC_{50} of the leaves, granules and powder of unfermented tea dregs was 104.06, 62.34 and 66.17 ppm, respectively. The antioxidant activity with IC_{50} content of the leaves, granules and powder of tea dregs fermented with EM-4 was 46.85, 38.84 and 39.79 ppm. The antioxidant activity with IC_{50} content of the leaves, granules and powder of tea dregs fermented using *Trichoderma viride* was 30.65, 48.06 and 46.89 ppm, respectively.

DISCUSSION

Fermentation includes all types of metabolic processes where enzymes from microorganisms perform oxidation, reduction, hydrolysis and other chemical reactions. The chemical change of an organic substrate produces certain product. The feed fermentation process has some advantages such as improving the quality, nourishment, the digestibility and preserving of the feedstuff. Table 1 shows that the fermentation process led to changes in nutrient content of tea dregs, including reduced protein and crude fiber content, increased water content, fat and energy metabolism. Analysis of variance showed that the fermentation using EM-4 and *Trichoderma viride* had no significant effect ($p>0.05$) on the ash content. This was because microbes require minerals only slightly. According to Xing *et al.*[19] the ash content was determined by the raw materials used. The fermentation processes by EM-4 and *Trichoderma viride* did not have a high requirement for inorganic compounds, so the ash content in the tea dregs had not changed[20]. Analysis of

variance showed that the fermentation using EM-4 and *Trichoderma viride* had no significant effect (p>0. 05) on the levels of fat. This was because the energy requirement of fermentation process uses more glucose derived from complex carbohydrates of tea dregs rather than that derived from fat accumulation. Additionally, required a high C/N ratio to induce fat accumulation in the fermentation process was approximately 80:1[21]. Analysis of variance showed that the fermentation using EM-4 and *Trichoderma viride* demonstrated significant (p<0. 05) levels of metabolic energy. This was because carbohydrates dregs of tea were largely converted into glucose that can be used as an energy source by EM-4 microbes and *Trichoderma viride*. If it was burned using oxygen (O2), this process will produce gross energy (GE) in accordance with carbohydrate levels in each form of tea dregs[22].

Analysis of variance showed that the fermentation using EM-4 and *Trichoderma viride* had a significant effect (p<0.05) on protein and crude fiber levels but it had highly significant effect (p<0.01) on water content of the tea dregs. Protein levels of tea dregs after fermentation decreased, because the protein in the tea dregs is used by bacteria, fungi and molds to grow and thrive. Multiplication of cells, especially during the exponential growth of microbes requires a large quantity of protein[23-25]. Crude fiber decreased because according to Sukaryana *et al*.[26] *Trichoderma viride* and the microbes in EM-4 are microorganisms that have the ability to produce cellulase enzymes to break down cellulose into glucose. In addition EM-4 and *Trichoderma viride* can produce the enzymes protease, lipase and pectinase[27,28]. The water content of the fermented tea dregs increased very high, because water is a byproduct the breakdown of carbohydrates, proteins and organic compounds. Therefore, the water content of tea dregs after the fermentation process increases. The fermentation process of microorganisms enzyme perform hydrolysis, oxidation-reduction and other chemicals reactions, causing chemical changes in an organic substrate to produce a particular product[14]. The breaking of the substrate by *Trichoderma viride* fungus is more effective and produce higher amount of water[29,30].

Analysis of variance showed that the fermentation using EM-4 and *Trichoderma viride* has a significant effect (p<0.05) on tannin content (Table 2). Tea dregs tannin content decreased after fermentation with EM-4 and *Trichoderma viride*. This was because EM-4 and the *Trichoderma viride* fungus hydrolyze tannins to produce the extracellular tannase enzyme. It is able to hydrolyze the ester bond in tannin, glucose and gallic acid[31-34]. EM-4 and *Trichoderma viride* had a high activity to produce the tannase enzyme[35]. The decline in tannins in the study ranged from 50-60% which is greater than the decrease in tannin reported by Setiarto and Widyastuti[21] in the amount of 29.13-33.69%. *Trichoderma viride* is a cellulolytic fungus, which effectively produce cellulolytic enzymes and EM-4 also contains fungi, molds, cellulolytic bacteria that can destroy high cellulose levels and dissolve cellulose parts that are strongly bonded with hydrogen. The main components of the cellulase system are cellobiohydrolase, i.e., CBHI and CBHII, totaling 80% of the total protein produced by cellulase[36]. *Trichoderma viride* fungus can decompose the organic tannin material into gallic acid and soluble glucose[37]. Microorganisms are safe and environmentally friendly and do not require substantial energy to degrade lignin, pectin and tannins from lignocellulosic biomass[38,39]. Tannin content in the material affects the activity of protease enzymes. Low content of tannin material will increase the ability of protease enzymes to break proteins into amino acids. Tannin is a unique polyphenolic compound because it has positive and negative effects on health. Furthermore, tannin can affect the color, flavor and nutritional quality of a substance. Tannin can function as an antioxidant which binds free radicals, avoids cell damage and prevents the occurrence of various diseases[40]. In addition, tannin is an antinutrient which binds proteins to form insoluble complex compounds, thereby reducing protein digestibility. If tannins bind to the enzymes produced by the digestive system, then the activity of enzymes will decrease[41]. Variance analysis showed that fermentation using EM-4 and *Trichoderma viride* had no significant effect (p>0.05) on saponin content. This is because saponin in tea dregs decrease the effectiveness of bacteria, fungi and molds in converting saponins into sugars (glycons) and sapogenins (aglycons). Therefore, the quantitative saponin content in tea dregs does not decrease[42]. In the fermentation process, saponins effectively decrease the amount of microbes if the fermentation temperature is approximately 37°C. In addition, the surface-active compound of saponins is similar to soap. Sariri *et al*. [43]reported that fermentation using *Aspergillus niger* microbes and *Lactobacillus plantarum* can decrease saponin content in young leaves, young fruit and young trembesi fruit skin (*Albizia saman*). *Aspergillus niger* and *Lactobacillus plantarum* were not able to degrade saponins to below the tolerance limit (<0.20%).

Table 3 shows that tea dregs contained flavonoids and phytochemicals consisting of alkaloids, steroids and glycosides. All of them have antioxidant properties. Flavonoids are one of the many molecules used by cells to protect against the dangers of reactive oxygen species[44]. The three forms of tea dregs also contain alkaloids, glycosides and steroid.

Alkaloids are chemical compounds and are secondary metabolites from plants formed on the principle of mixed formation. Alkaloids are the largest group of secondary plant substances. Generally, alkaloids include alkaloid compounds containing one or more nitrogen atoms, usually in combination or as part of a cyclic system[45]. Steroids are one of the bioactive antioxidant compounds and their structure consists of 17 carbon atoms with the basic structure of 1,2-cyclopenteno hydrogenation. Analysis of variance showed that the fermentation using EM-4 and *Trichoderma viride* had a significant effect on flavonoid and antioxidant activity of tea dregs. The fermentation process increased the levels of flavonoids, because the microorganisms used for fermentation produce more β-glucosidase enzymes. This enzyme has an important role in the biotransformation process of secondary metabolite modification. This enzyme servetocut certain glucose or oligosaccharide bonds in the bran and free the phenol in the free phenolic fermentation process. Free phenol produced by the fermentation process increased antioxidant activity. The larger the volume of the phenolic content is produced, the higher the antioxidant activity[46]. Flavonoid compounds have a very high antioxidant activity. Firdiyani *et al*.[47] and Redha[48] stated that the plant has the potential of antioxidant compounds, i.e. , flavonoids, alkaloids and phenolic compounds that are polar. Gulcin *et al*.[49] and Jaya[50] stated that the tannin compounds also have antioxidant activity. Fermentation using yeast may boost antioxidant activity[51]. According to Kunaepah[52], yeast fermentation increased phenol levels that increased the antioxidant activity. The material expressed as an antioxidant is very strong if the IC_{50} value is less than 50 ppm, strong if the IC_{50} value is between 50-100 ppm, mild if the IC_{50} values is between 100-150 ppm, weak if the IC_{50} value is between 150-200 ppm and very weak if the IC_{50} value is greater than 200 ppm[53]. Additionally, Table 3 also shows that a smaller IC_{50} value means stronger antioxidant activity. The IC_{50} of the tea dregs after fermenting was smaller than the IC_{50} value before fermenting the tea dregs. This shows that the antioxidant activity of fermented tea dregs was 1.5 times higher than the antioxidant activity of unfermented tea dregs.

The implications of this study were the content of nutrients, flavonoids and antioxidant activity of tea dreg fermented are high, so tea dregs fermented can be used as poultry feed supplement. The fermentation process was able to raise the levels of water, metabolizable energy, flavonoids and antioxidant activity of tea dregs. The content of proteins, crude fiber, saponin and tannin of tea dregs were decreased after fermentation. *Trichoderma viride* ferments more effectively than EM-4 in improving the quality of nutrients content of tea dregs. Each of tea dregs shape has a different nutrient content when its fermented using EM-4 and *Trichoderma viride*. Protein content of granules shape of tea dregs which fermented by EM-4 was not decreased compare to leaves and flour of tea dregs shaped. Fermentation uses EM-4 was more effective in improving the antioxidant activity of the active compound in the dregs of tea. Tea dregs that will be used as feed of poultry supplement should be granules. The granules-shaped tea dregs should be fermented first by EM-4 probiotic.

CONCLUSION

Fermentation using EM-4 and *Trichoderma viride* is effective in improving nutrient quality, flavonoid levels and antioxidant activity of tea dregs. Fermentation caused decrease in antinutrient levels in tea dregs.

SIGNIFICANCE STATEMENT

This study discovered that the tea dregs fermented using EM-4 and *Trichoderma viride* can be used as a feed for poultry, because the antioxidant activity of the tea-dregs is very strong, the antinutrient are getting down and the protein content is quite high.

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