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# Supplementation of waste tea fungal biomass as a dietary ingredient for broiler chicks

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**Abstract**

The waste tea fungal biomass produced during black tea fermentation was investigated as a dietary ingredient in poultry feeds. A small portion of fungal mat was used as starter culture for the next cycle while the major portion is discarded as waste. Hence a trial study was carried out to utilize the waste fungal biomass as a supplementary diet for broiler chicks. The fungal biomass contained 179.38 g of crude protein, 120 g crude fibre, 4.82 g phosphorus, 6.56 g of calcium and 8.92 MJ metabolizable energy per kilogram of biomass. The dried tea fungus showed phytase activity of 23 IU/mg protein. The supplementation of tea fungal inclusion (TFI) at 150 g/kg concentration in poultry feed increased the feed consumption, body weight, performance efficiency factor (PEF) and the carcass characters of test broilers significantly ($P = 0.01$) over the control. The histopathological examination of liver showed no abnormalities and the mortality rate was zero.


*Keywords:* Tea fermentation; Fungal biomass; Dietary ingredient; Broiler chicks

## 1. Introduction

The increasing demand for new feedstuffs and the high prices of conventional feedstuffs have greatly stimulated the search for microorganisms capable of producing nutritive biomass that could be used in the diet of livestock and poultry. *Medusomyces gisevii*, commonly referred as 'tea fungus', can be used as a rich non-conventional source of microbial protein in poultry. Tea fungus is a consortium of two yeasts, *Pichia* sp. NRRL Y-4810 and *Zygosaccharomyces* sp. NRRL Y-4882 and a bacterium *Acetobacter* sp. NRRL B-2357 (Hesseltine, 1965). The tea fungal consortium possesses different associations varying from country to country (Chen and Liu, 2000). The tea fungus is native of Russia and it invaded all parts of the world in later periods. Consumption of fermented tea (kombucha tea) as a medical tonic by humans is in practice for a long time (Frank, 1995). A part of the fungal mat produced during black tea fermentation is used as starter culture, while the remaining goes as a waste (Hesseltine, 1965). The fungal mat is a hard cellulosic pellicle containing rich nutrients similar to the single cell protein produced by other yeasts. Supplementation of fungal phytase in broiler diet, improved the growth performance and phosphorus (Broz et al., 1994; Zyla et al., 2000; Viveros et al., 2002). Based on the biochemical constituents of the tea fungus, the present study was carried out to investigate its performance on broiler chicks as a supplementary diet.

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## 2. Methods

### 2.1. Source and maintenance of tea fungus

The tea fungus was obtained from the tribal people of Kolli hills, Tamil Nadu, India. The fungus was grown and maintained in tea medium (Hesseltine, 1965).

### 2.2. Dietary formulation

The nutrient content of the starter and the finisher broiler meal were presented in Table 1. The sun dried biomass of tea fungus was powdered and incorporated into the broiler meals at the concentrations of 0, 50, 100, 150, 200 and 250 g/kg of meal. The diets were formulated to be isonitrogenous, but differed in metabolizable energy contents. The metabolizable energy values of the experimental diets were calculated as per the recommendations of National Research Council (1984) and for tea fungus from its proximate analysis using percentage of multipliers of torula yeast as follows: ME (MJ/g) = 133.06(CP) + 232.91(EE) − 4.68(CF) + 122.77 (NFE) (Titus and Fritz, 1971).

### 2.3. Chemical analysis of the broiler diet

The proximate content of moisture, crude protein, crude fibre, crude lipid, ash, nitrogen free extractives (NFE), acid detergent fibre (ADF), neutral detergent fibre (NDF) and hemicellulose in tea fungal biomass and experimental diets were estimated by the methods of the Association of Official and Analytical Chemists (AOAC, 1990). In mineral analysis, sodium and potassium contents were estimated in flame photometer; calcium and magnesium by Jackson (1967) method; phosphorus by Dickman and Bray (1940) method; iron, copper, zinc and manganese in atomic absorption spectrophotometer (Perkin-Elmer, 5000) (Issac and Johnson, 1975). The amino acid content of the fungal biomass was analyzed by an automated precolumn derivatization with *o*-pthaldialdehyde (OPA) in reverse phase HPLC (Column: Spherisorp $C_{18}$; pump: ISCO-dual pump; detector: fluorescent; flow rate: 1.5 ml/min) (Rajendra, 1987).

### 2.4. Phytase production

#### 2.4.1. Extracellular phytase

For extracellular phytase enzyme production, the tea fungus was grown in minimal medium (Carter and Bull, 1969) containing 10 g/l of sodium phytate. Tea fungus (g/100 ml) was inoculated and incubated on an orbital shaker (120 rpm) for eight days at 28 ± 2 °C. After incubation, the cultures were filtered through glass microfibre filters. The filtrate was centrifuged at 10,000 rpm for 20 min at 4 °C and the clear supernatant was used as crude enzyme.

#### 2.4.2. Intracellular phytase

Tea fungus grown in tea medium (Hesseltine, 1965) was used for extraction of intracellular phytase. Enzyme was extracted by homogenizing 1 g of tea fungal mat in 0.1 M Tris HCl buffer, pH 7.0 for 10 min with pestle and mortar. The homogenate was filtered through glass microfibre filters and the filtrate was centrifuged at 10,000*g* for 15 min. and the supernatant was collected and designated as crude enzyme. The crude enzyme was precipitated using ammonium sulphate solution (70%) and kept overnight at 4 °C. The solution was centrifuged at 10,000*g* for 10 min and the precipitate was dissolved in 0.1 M Tris HCl buffer, pH 7.50 and used as concentrated enzyme.

#### 2.4.3. Purification

The concentrated enzyme was eluted through sephadex G-100 column (1.5 × 45 cm) using 0.1 M Tris HCl buffer, pH 7.50 with a flow rate of 5 ml/h. Protein content was estimated using the method of Lowry et al. (1951). In each fraction the phytase activity was

Table 1
Nutrient composition of experimental diets

| Ingredient | Starter meal—level of TFI (g/kg) | | | | | | Finisher meal—level of TFI (g/kg) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 |
| Tea fungus | 0 | 50 | 100 | 150 | 200 | 250 | 0 | 50 | 100 | 150 | 200 | 250 |
| Soya meal | 75 | 70 | 65 | 60 | 55 | 50 | 75 | 70 | 65 | 60 | 55 | 50 |
| Groundnut cake | 75 | 80 | 85 | 90 | 95 | 100 | 35 | 40 | 45 | 50 | 55 | 60 |
| Gingly cake | 100 | 100 | 100 | 100 | 100 | 100 | 60 | 60 | 60 | 60 | 60 | 60 |
| Fish | 120 | 120 | 120 | 120 | 120 | 120 | 100 | 100 | 100 | 100 | 100 | 100 |
| Maize | 550 | 500 | 450 | 400 | 350 | 300 | 600 | 550 | 500 | 450 | 400 | 350 |
| Molases | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Starch | 50 | 50 | 50 | 50 | 50 | 50 | 100 | 100 | 100 | 100 | 100 | 100 |
| Mineral mixture[a] | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |

Additives: Vitamin mixture (Vitamin $AB_2D_3K$): 0.1 g/kg.
[a] Fe, 0.25 mg; Cu, 5.0 mg; Zn, 2.50 mg; Mg, 0.28 mg; Co, 62.5 mg.

measured by estimating the liberated phosphorus as described below. The active fractions were pooled for the determination of enzyme properties.

*2.4.4. Phytase assay*

The incubation mixture contained in a total volume of 1 ml, the following; 0.1 M Tris HCl buffer pH 7.50, 100 μL; sodium phytate (0.1 M), 700 μL; enzyme 200 μL. The additions were made in cold and mixed thoroughly and incubated for 1 h at 37 °C. After 1 h the reaction was stopped by adding 1 ml of 0.40 M TCA (cold) and the mixture was chilled in ice. When assayed with the purified enzyme, the arrested mixture could be directly used for the determination of liberated phosphorus (Dickman and Bray, 1940).

*2.4.5. Characteristics of phytase*

The enzyme properties viz. optimum pH, temperature and substrate concentration for maximum enzyme activity ($V_{max}$ and $K_m$) were determined. The molecular weights of the enzymes were estimated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) (Laemmli, 1970) using Phast Gel gradient 10–15 medium. Pharmacia high molecular weight calibration kit proteins were used as markers. The methods proposed in Phast system™ separation technique file No. 110, Pharmacia LKB Biotechnology, Uppasala, Sweden, were followed.

*2.4.6. Animal study*

A total of 60 unsexed Vencob broiler chicks were randomly divided into six groups of ten chicks for each dietary treatment. Each group was placed and reared in a clean, disinfected pen measuring $2.65 \times 2.55$ m and a floor space of 0.225 $m^2$ per bird. The birds were fed with starter meal for the first 4 weeks and then with finisher meal up to 8 weeks of age. Food and water were supplied ad libitum. A coccidiostat and sulphamidine was administered through drinking water three times per week.

*2.4.7. Parameters analyzed*

The food intake, water intake, body weight gain, feed conversion efficiency (FCE), performance efficiency factor (PEF) and mortality rate were determined on a weekly basis for each bird of all dietary treatments. Six birds from each treatment were randomly selected, starved of food for about 18 h to empty their crops, sacrificed, defeathered and eviscerated. Carcass dressing percentage was calculated from eviscerated weight and live weight.

*2.4.8. Histopathological studies*

Four chicks from each treatment were randomly selected and killed by cervical dislocation during end of the study. The liver samples were excised and the sections were examined microscopically for cell abnormalities after staining with haematoxylin and eosin dye (Humason, 1979).

*2.4.9. Statistical analysis*

To assess the significant level of influence caused by tea fungal inclusion in broiler diet on growth performance of broiler chicks, Duncans Multiple Range Test (DMRT) was used (Duncan, 1955).

## 3. Results and discussion

### *3.1. Chemical composition of tea fungus*

Proximate analysis of the chemical constituents of tea fungus revealed that the dry tea fungal biomass contained 179.38 g/kg of crude protein, 120 g/kg of crude fibre and 44.14 g/kg lipid. The fungal biomass was rich in NFE (452 g/kg), ADF (398 g/kg) and NDF (461 g/kg). The fungal mat contained desirable levels of calcium (6.65 g/kg), phosphorus (4.82 g/kg) and potassium (13.93 g/kg) (Table 2). The metabolizable energy (ME) content was 8.92 MJ/kg. The amino acid analysis implies high values for lysine, cysteine and methionine (Table 3). The specific activity of phytase in dried fungal mat was 23 IU/mg protein.

The nutritive value of the tea fungus is in conformity with the single cell protein obtained from *Aspergillus niger* which was supplemented in the broiler diet for growth performance (Choiu et al., 2001). Since the fungal mat is composed of yeasts and bacteria, a moderate amount of crude protein, lysine, cysteine and methionine is observed. The amino acid spectrum of the different yeast strains has a pronounced effect on the protein

Table 2
Chemical composition of tea fungus

| Component | Content (g/kg dry matter) |
|---|---|
| Moisture (%) | 44.00 ± 0.05 |
| Crude protein | 179.38 ± 0.04 |
| Crude fibre | 120.00 ± 0.03 |
| Crude lipid | 44.14 ± 0.02 |
| Ash | 26.40 ± 0.04 |
| Nitrogen free extractives | 63 ± 0.02 |
| Acid detergent fibre | 398 ± 0.03 |
| Neutral detergent fibre | 461 ± 0.03 |
| Hemicellulose | 63 ± 0.02 |
| Sodium | 0.95 ± 0.03 |
| Potassium | 13.93 ± 0.01 |
| Phosphorus | 4.82 ± 0.02 |
| Calcium | 6.56 ± 0.02 |
| Magnesium | 5.75 ± 0.03 |
| Iron | 0.86 ± 0.03 |
| Manganese | 0.46 ± 0.04 |
| Zinc | 0.84 ± 0.02 |
| Copper | 0.91 ± 0.03 |
| Metabolizable energy (MJ/kg DM) | 8.92 ± 0.05 |

Table 3
Amino acid composition of tea fungus

| Component | Content (% dry matter) |
|---|---|
| Alanine | 3.30 ± 0.05 |
| Arginine | 2.28 ± 0.05 |
| Aspartic acid | 3.71 ± 0.03 |
| Cysteine | 1.42 ± 0.04 |
| Glutamic acid | 4.56 ± 0.03 |
| Glycine | 1.50 ± 0.03 |
| Histidine | 0.98 ± 0.04 |
| Isoleucine | 3.20 ± 0.05 |
| Leucine | 3.30 ± 0.02 |
| Lysine | 4.25 ± 0.03 |
| Methionine | 0.90 ± 0.03 |
| Phenylalanine | 2.00 ± 0.04 |
| Proline | 3.25 ± 0.02 |
| Serine | 1.90 ± 0.03 |
| Threonine | 1.28 ± 0.04 |
| Tyrosine | 1.60 ± 0.05 |
| Valine | 2.50 ± 0.05 |
| Tryptophan | 1.16 ± 0.04 |

efficiency ratio (Vaughan Martini et al., 1979). The increased fibre content denotes higher level of cellulose which is due to the synthesis of cellulosic pellicle by acetic acid bacteria (*Acetobacter* sp.) in the fungal mat through symbiotic interaction with the yeasts (Hesseltine, 1965).

### 3.2. Chemical composition of experimental diets

Since the feeds were prepared isonitrogenously not much variations were observed in their nutrient content (Table 4). The diets contained 12–15% moisture, 185–191 g/kg crude protein, 130–139 g/kg of crude fibre, 232–236 g/kg of crude lipid, 42–43% of NFE, 383–398 g/kg ADF, 453–470 g/kg NDF, 70–74 g/kg hemicellulose, 7.8–10 g/kg calcium, 4.0–7.0 g/kg potassium and 13 MJ/kg ME. The specific activity of phytase in 0, 50, 100, 150, 200 and 250 g/kg TFI was 0.06, 3.8, 6.5, 8.6, 11.20 and 13.65 IU/mg protein respectively. The tea fungal amendment to the broiler feed increased the fibre content and also calcium and potassium contents. Increased level of ash implies increase in mineral content due to fungal amendment to the feed.

### 3.3. Purification and properties of phytases

Extracellular (EX) and intracellular (IN) phytase purifications showed only one fraction (Fraction-8) each in sephadex G-100 column chromatography. In extracellular phytase fraction, the purification fold was 133.33 and specific activity was 36.00 IU/mg protein and the yield was 3.00%. In intracellular phytase, the purification fold was 203.13, specific activity was 32.50 IU/mg protein and the yield was 4.06%. The phytase of tea fungus had an optimum pH of 6.50 for EX and IN enzymes and temperature of 60 °C for EX and IN enzymes. The $V_{max}$ against sodium phytate was 28.10 (EX) and 25.36 (IN) IU/mg protein and $K_m$ was 2.20 (EX) and 1.98 (IN) mg/ml. The molecular weights of both the enzymes were found to be 50 kDa.

### 3.4. Growth performance of experimental chicks

In the present study it was observed that in the TFI (150 g/kg) meal fed broilers, the maximum feed consumption was 4.54 kg. The FCE and performance efficiency factor (PEF) were 1.93 and 127.76 respectively (Table 5). The body weight was found to be higher in 150 g/kg TFI. TFI up to 150 g/kg in the broiler diet increased the dressed weight (79.80–82.33%), eviscerated weight (68.65–71.03%) and the weight of liver (2.0–2.85 g/100 g), heart (0.74–0.88 g/100 g) and gizzard (2.03–3.56 g/100 g); but inclusions at higher concentrations could not improve the body weight (Table 6).

It has been reported that inclusion of single cell protein (SCP) in the broiler feed increased the feed consumption rate, body weight and performance efficiency factor (Yoshida and Hiroshi, 1980; Choiu et al., 2001).

Table 4
Chemical composition of experimental diets

| Components | Starter meal—level of TFI (g/kg) | | | | | | Finisher meal—level of TFI (g/kg) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 50 | 100 | 150 | 200 | 250 | 0 | 50 | 100 | 150 | 200 | 250 |
| Moisture (%) | 12.56 | 12.63 | 12.69 | 12.80 | 12.88 | 12.97 | 13.30 | 13.65 | 13.89 | 14.15 | 14.60 | 14.91 |
| Crude protein (N × 6.25) | 185.21 | 187.75 | 188.33 | 188.98 | 191.05 | 191.45 | 184.26 | 184.71 | 185.42 | 185.74 | 185.80 | 185.85 |
| Crude fibre | 132.28 | 133.55 | 134.30 | 135.25 | 136.40 | 138.78 | 130.15 | 131.46 | 132.64 | 135.08 | 135.97 | 136.66 |
| Crude lipid | 233.16 | 233.86 | 234.16 | 235.10 | 235.90 | 236.48 | 231.75 | 232.55 | 232.87 | 233.56 | 234.20 | 235.00 |
| Ash | 15.14 | 16.53 | 16.88 | 17.25 | 17.94 | 18.30 | 15.56 | 16.27 | 16.76 | 17.32 | 17.86 | 18.53 |
| Nitrogen free extractives | 43.44 | 42.85 | 42.64 | 42.36 | 41.88 | 41.52 | 43.83 | 43.50 | 43.23 | 42.83 | 42.68 | 42.40 |
| Acid detergent fibre | 383 | 385 | 389 | 391 | 394 | 398 | 381 | 384 | 387 | 391 | 393 | 396 |
| Neutral detergent fibre | 455 | 458 | 461 | 464 | 468 | 470 | 453 | 455 | 458 | 460 | 462 | 466 |
| Hemicellulose | 72 | 73 | 72 | 73 | 74 | 72 | 72 | 71 | 71 | 69 | 69 | 70 |
| Calcium | 7.83 | 8.15 | 8.80 | 9.23 | 9.68 | 10.08 | 7.57 | 7.86 | 8.35 | 9.14 | 9.74 | 9.96 |
| Potassium | 4.32 | 5.83 | 6.13 | 6.45 | 6.80 | 7.04 | 3.96 | 5.75 | 6.08 | 6.36 | 6.78 | 7.10 |
| ME (MJ/kg) | 13.17 | 13.14 | 13.13 | 13.12 | 13.11 | 13.08 | 13.17 | 13.15 | 13.14 | 13.11 | 13.09 | 13.07 |

Table 5
Effect of varying levels of TFI on the performance of Vencob broilers

| | Level of TFI (g/kg) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 50 | 100 | 150 | 200 | 250 | LSD (%) | CV (%) |
| Feed consumption (kg) | $4.50^{ab}$ | $4.51^{ab}$ | $4.52^{a}$ | $4.54^{a}$ | $4.52^{a}$ | $4.51^{ab}$ | 0.224 | 2.2 |
| Water intake (L) | $10.40^{e}$ | $11.20^{d}$ | $11.40^{c}$ | $11.60^{b}$ | $11.80^{ab}$ | $12.00^{a}$ | 0.529 | 1.9 |
| Weight gain (kg) | $2.30^{c}$ | $2.31^{c}$ | $2.35^{b}$ | $2.45^{a}$ | $2.38^{c}$ | $2.33^{c}$ | 0.104 | 1.8 |
| Feed conversion efficiency | $1.96^{a}$ | $1.95^{ab}$ | $1.94^{bc}$ | $1.93^{c}$ | $1.94^{bc}$ | $1.95^{ab}$ | 0.025 | 0.5 |
| Performance efficiency factor | $117.35^{bc}$ | $118.46^{b}$ | $120.10^{ab}$ | $121.76^{a}$ | $119.59^{ab}$ | $118.46^{b}$ | 0.204 | 0.1 |
| Mortality (%) | Nil | Nil | Nil | Nil | Nil | Nil | – | – |

$P = 0.01$ for all the treatments. Means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 6
Carcass characteristics of Vencob broilers fed on tea fungal meal

| | Level of TFI (g/kg) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 50 | 100 | 150 | 200 | 250 | LSD (%) | CV (%) |
| Dressed weight (%) | $79.80^{c}$ | $80.23^{bc}$ | $81.56^{ab}$ | $82.33^{a}$ | $80.45^{bc}$ | $79.63^{c}$ | 0.206 | 1 |
| Eviscerated weight (%) | $68.65^{bc}$ | $68.88^{bc}$ | $69.72^{b}$ | $71.03^{a}$ | $68.36^{c}$ | $68.10^{c}$ | 0.325 | 1 |
| Liver (g/100 g) | $2.00^{b}$ | $2.12^{ba}$ | $2.48^{ab}$ | $2.85^{a}$ | $2.72^{ab}$ | $2.50^{ab}$ | 0.458 | 2.3 |
| Heart (g/100 g) | $0.74^{a}$ | $0.78^{a}$ | $0.82^{a}$ | $0.88^{a}$ | $0.80^{a}$ | $0.73^{a}$ | 0.566 | 1.8 |
| Gizzard (g/100 g) | $2.03^{c}$ | $2.10^{bc}$ | $2.82^{abc}$ | $3.56^{a}$ | $3.21^{ab}$ | $2.98^{abc}$ | 0.624 | 2.6 |

$P = 0.01$ for all the treatments. Means followed by a common letter are not significantly different at the 5% level by DMRT.

In *Aspergillus niger* SCP supplement meal fed broilers, the feed consumption was 3.38 kg, body weight 2.02 kg and feed conversion efficiency 1.82 (Choiu et al., 2001).

It may be due to the fact that the *Acetobacter* sp. present in the consortium produces acetic and gluconic acid by symbiotic interaction with the yeasts during black tea fermentation (Chen and Liu, 2000). This combination makes the fungal mat to produce sour taste. Higher concentrations of TFI (>150 g/kg) in the diet may exert sour taste that could influence on feed consumption of broilers. The increasing weight of broilers is also due to more availability of phosphorus to the birds caused by hydrolysis of endogenous phytase upon phytate present in the diet. The monogastric animals such as poultry and pig lack or have low phytase activities in their digestive system and most undigested phytic acid is excreted in their manure. The presence of phytic acid in poultry feeds work as an antinutritional factor, since the phosphate moieties of phytic acid chelate essential minerals and possibly bind to proteins making them unavailable for absorption. Another problem is the high level of undigested phytic acid in the faecal waste that is discharged on to sewage and becomes a cause for water pollution. Phytase (D-myo-inositol hexakisphosphate hydrolase) was widely distributed in plant and animal tissues as well as in many species of fungi (Mandal et al., 1972). The extracellular phytase production from *Bacillus* sp. KHU-10 was reported by Choiu et al. (2001) and in mung beans by Mandal et al. (1972). Zyla et al. (2000) reported that *Aspergillus niger* mycelium with separate and combined effectiveness of phytase of wheat based feeds to broilers increased body weight, feed intake and phosphorus retention. Addition of fungal phytase (*Aspergillus niger*) in the broiler diet increased the growth performance whereby the apparent availability of phosphorus in broilers was markedly improved and its concentration in excreta was reduced (Broz et al., 1994). Supplementation of microbial phytase (Natuphos) in broilers on mineral utilization, growth performance and phosphorus retention was well documented (Viveros et al., 2002). Cheng et al. (1990) reported the improvement of phosphorus utilization in broilers by using phytase enzyme for the enhancement of broiler growth. Though chicks and pigs are monogastric animals the improvement of phosphorus bioavailability in pigs by yeast phytase has been well documented (Matsui et al., 2000).

The present study reveals that the waste tea fungal biomass produced during black tea fermentation process could be used as a non-toxic, energy and nutrient rich supplementary ingredient in the broiler meals. The appropriate concentration (15%) of tea fungal inclusion increases the uptake of feed and water, body weight and performance efficiency factor of the broilers. The histopathological examination showed no specific deformities and lack of mortality support the non-pathogenicity of tea fungal inclusion in broiler diets. Hence dried and powdered tea fungal biomass can be used as a good supplementary ingredient in broiler diets.

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