

## Prevention of Avian Crop Candidiasis by Dietary Supplementation of *Saccharomyces Cerevisiae*

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### Abstract

Candidiasis is a fungal disease of many avian species caused by the polymorphic yeast *Candida albicans*. These yeasts are normal flora in the upper digestive system of birds, they cause disease when the bird suffers from debilitating conditions. The yeast *Saccharomyces Cerevisiae* that is commonly used for baking and brewing has been used as an alternative for antibiotics and as a growth promoter in poultry and has been proved successful in treating oral and vaginal candidiasis in human. Our study was conducted as an attempt to find an alternative method for treating candidiasis in avian hosts using the readily available yeast *Saccharomyces Cerevisiae*. A number of 80 broilers were divided into 4 groups. Group 2 was fed a ration contained *Saccharomyces Cerevisiae*. Group 3 was challenged with oral *Candida albicans*. Group 4 treated with both organisms while group 1 was considered control and did not receive any treatment. The experiment started at one day old and continued for 9 days. Crops were examined grossly and histologically at the end of experiment. Crops appeared normal in both group 1 and 2, while in group 3 they were thickened with diphtheritic pseudomembrane grossly and fungal hyphae, pseudohyphae and spores proliferated within squamous epithelia and lamia propria histologically. In group 4 they showed markedly less macro and microscopic changes. This treatment was found effective in controlling crop candidiasis in birds.

**Keywords:** *Candida albicans*, *Saccharomyces Cerevisiae*, Broiler poultry

### Introduction

Candidiasis is a fungal disease caused by the polymorphic yeast belonging to the genus *Candida*. Although there are many candida species that cause candidiasis, *Candida albicans* is the main species that is accused for causing the disease (2). Candidiasis has been documented in many avian species including domestic and wild birds (15,19,26,31,36,37). *Candida* species are normal inhabitants in the upper digestive system of birds, and when the bird suffers from debilitating conditions it will cause a severe illness. It also occurs when there is a decrease in the number of microflora due to prolonged antimicrobial use. All these conditions will give candida a chance to replicate beyond the normal rate (28).

Candidiasis occurs mostly in young birds. Affected birds will grow slowly and will have abnormal appearance with rough feathers. Lesions are seen mostly in the crop. These lesions are patches of whitish necrotic exudates that sometimes become larger forming pseudomembranes that may cover a large portion of the mucosal lining of the crop (15). The gross lesion is usually described as crud-like or turkish towel like appearance. Microscopically, there is colonization of the crops keratinized epithelium with *Candida*'s septate hyphae (2,15).

A history of long term administration of antimicrobial therapy along with typical gross lesion can aid in the initial diagnosis of the disease. Confirmation of diagnosis is made by cultivation of the organism on Sabouraud agar but initial heavy growth is necessary for diagnosis because the organism present in small numbers in healthy birds. A wet mount preparation from the lesion can be used for the diagnosis based of visualization of budding candida cells (7,29). The disease is treated effectively by 0.05% copper sulfate in drinking water. Nystatin in feed and in drinking water is also used for treating of candidiasis (2).

The yeast *Saccharomyces Cerevisiae* that is commonly used for baking and brewing has been used as an alternative for antibiotics as a growth promoter (22,40). Other than increasing feed conversion ratio (FCR), these yeasts are used to modulate the immune system and reducing the numbers of gastrointestinal pathogens in livestock (30). In poultry production, *Saccharomyces Cerevisiae* has been proved to increase FCR in broilers and increase the quality and quantity of egg production in layers (1,3,5,6,8–11,14,18,20,21,23,24,32–35,39).

The use of some non-pathogenic microbes for the treatment of microbial infections has shown promising success in some studies (38). Examples for such studies include: treating *Clostridium difficile* infection in mice by *Clostridium scindens* and the use of *Lactobacilli* for the treatment of vulvovaginal candidiasis in women (4,12). *Saccharomyces Cerevisiae* has been proved successful in treating oral and vaginal candidiasis in human (13,16,25,27,38). Due to the lack of previous literature examining the effect of *Saccharomyces Cerevisiae* on animal candidiasis, our study was conducted as an attempt to find an alternative method for treating candidiasis in avian hosts using the readily available yeast *Saccharomyces Cerevisiae*.

## Materials and Methods

*Candida albicans* was isolated from a clinical candidiasis in a local rooster. There was diphtheritic lesion on the oropharyngeal, esophageal and crop mucosa. Swabs from lesions were streaked to Sabouraud agar containing mixture of antibiotics and incubated aerobically at 37°C for 2 days. Confirmation of the species *Candida albicans* was based on the characters described by Quinn et. al. (2011).

A number of 80 cornish cross broilers were purchased from a local poultry hatchery. They were divided into 4 groups each one consisted of 20 chicks. At the age of one day, group 2 (G2) was fed a ration contained 2% bakers yeast *Saccharomyces Cerevisiae*. This concentration was found to increase FCR in previous studies (34). G3 was challenged with oral *Candida albicans* at the dose of  $3 \times 10^9$  colony forming units (CFU) per chick at day old. This dose was based on a pilot study that was conducted prior to the experiment. G4 treated with both organisms at the same age while G1 was considered control and did not receive any treatment (Table 1).

The study was conducted at the College of Veterinary Medicine at the University of Fallujah, Iraq during the period from December 1, 2019 to March 20, 2020. The experiment continued for 9 days then a random sample from each chicks group was collected. The weight of birds was recorded and the chicks were humanly euthanized for gross and histopathological

examination. Tissue sections from the crop were prepared and stained with hematoxylin and eosin stain (HE) and Periodic acid–Schiff stain (PAS) according to Luna (1968).

*Table 1: Names of the experiment chick groups. The + sign under treatment indicate that the corresponding group has been treated with Saccharomyces Cerevisiae. The + sign under infection indicate that the corresponding group has been challenged orally with Candida albicans.*

Groups	Treatment	Infection
G1	-	-
G2	+	-
G3	-	+
G4	+	+

## Results and Discussion

### Mycology

Microbial characteristics of *Candida albicans* was typical to those described by Quinn et. al. (2011). They were isolated on Sabouraud agar. Colonies were whitish, shiny and 4-5 mm in diameter. When the organism examined directly under a light microscope a characteristics budding cells were seen. For further confirmation, germ tube formation and chlamyospores formation was tested. Germ tube formation was demonstrated after incubation in serum at 37°C for 2 hours. Germ tubes are pseudohyphae emerging from the yeast cell give the organism a sperm appearance. For the visualization of chlamyospores the organism was cultured on corn meal agar for several days. Chlamyospores are circular cells that easily distinguishable from the oval budding cells and have thicker walls ().

### Pathology

On necropsy, crops from G1 and G2 had thin walls and shiny pinkish-brown mucosae (). Histologically, the epithelium appeared normal with no inflammatory cells infiltration (). G3 crops were grossly thickened and a whitish to yellowish diphtheritic pseudomembrane covered a large portion of the mucosal surfaces (). Histological sections of these crops revealed fungal hyphae, pseudohyphae and spores proliferated within squamous epithelia and lamia propria ( and ). Tissue reaction consisted of sever inflammatory response within sub mucosa, lamina properia and musclaris mucosa (). The epithelium was ulcerated and invaded by inflammatory cells. On the margins of the ulcer there was intercellular edema, dissociation, necrosis, and exfoliation of keratinocytes (). These lesions are typical to candidiasis that were reported in many previous studies (2,7). Our finding disagree with fulan et al who reported minimal inflammatory reaction in cases of candidiasis.

Group 4 that were fed a ration containing 2% *Saccharomyces Cerevisiae* showed markedly less intensive gross lesion when compared to G3. The lesion consisted of small patches of crud-like exudate (). Histopathological examination of crop from G4 showed limited fungal

infestation of squamous layer and heavy spore accumulation within crop lumen (). The limited fungal penetration of the crop epithelium could be explained by a competitive exclusion of the *Candida albicans* by the yeast cells of *Saccharomyces Cerevisiae*. This hypothesis has also been suggested by other workers who found similar effect of this treatment to human patient suffering from oral and vaginal candidiasis (13,27).

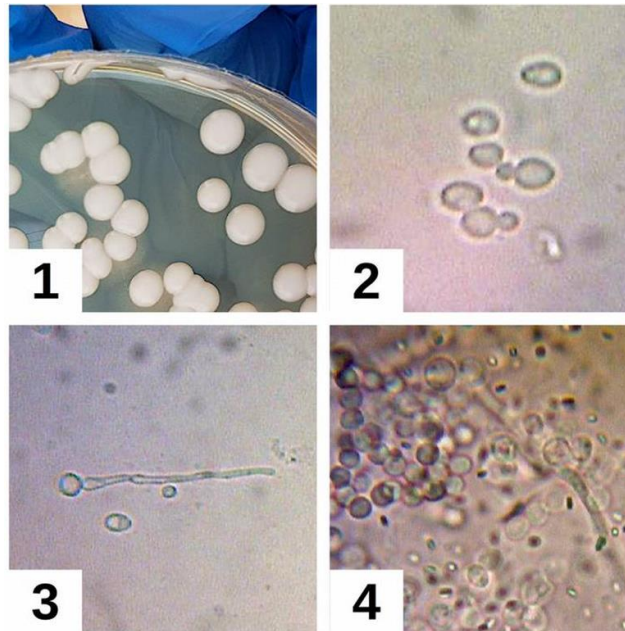
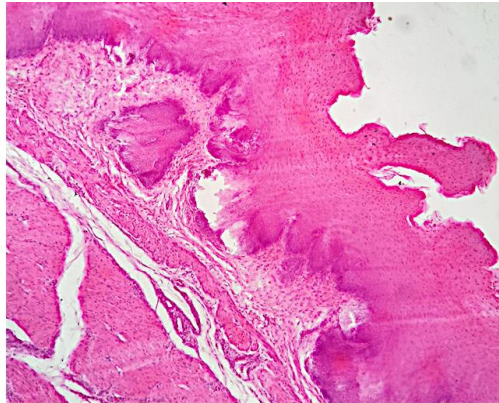


Figure 1: Mycological characters of *Candida albicans*: (1) Colonies appeared whitish, shiny and 4-5 mm in diameter on Sabouraud agar. (2) Budding cells were seen when a wet mount smear is prepared from colonies. (3) Germ tube formation was demonstrated after incubation in serum at 37°C for 2 hours. (4) Chlamydospores were seen when the organism was cultured on corn meal agar for several days.



Figure 2: A photographic image showing the normal gross appearance of a crop from control chicks of G1.

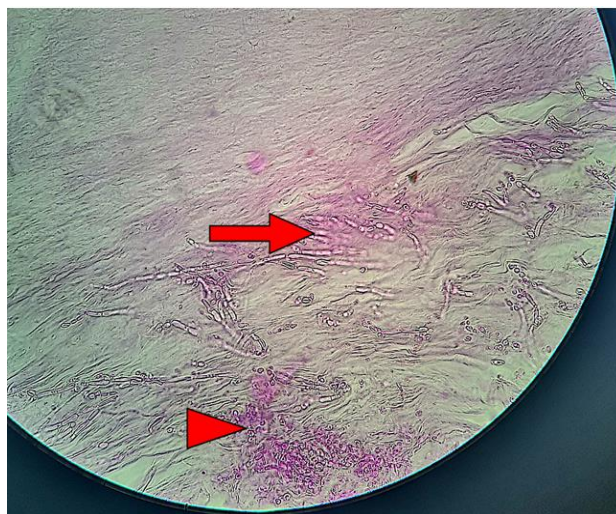




*Figure 3: Histophotographic image showed normal histological architecture of crop from control chicks of G1. HE X20.*



*Figure 4: A photographic image showing the gross lesion in a crop from chicks of G3 that are infected with candida albicans .There is increased thickness of the wall of the crop and a typical yellowish pseudomembranous diphtheritic exudate.*



*Figure 5: Histophotographic image of a crop from G3 showed fungal hyphae (arrow) and fungal spore (arrowhead) proliferated within squamous epithelia. PAS X40.*

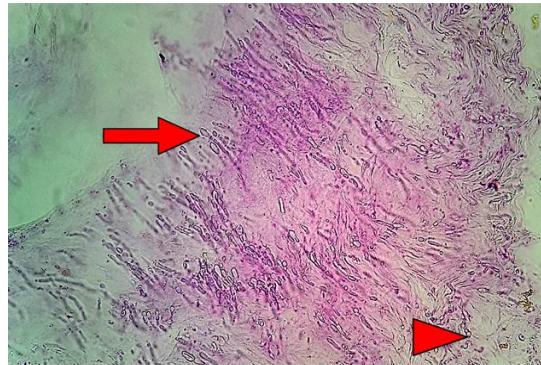


Figure 6: Histophotographic image of a crop from G3 showed fungal pseudohyphae (arrow) and spore proliferated within squamous epithelia and lamina propria (arrowhead). PAS X40.

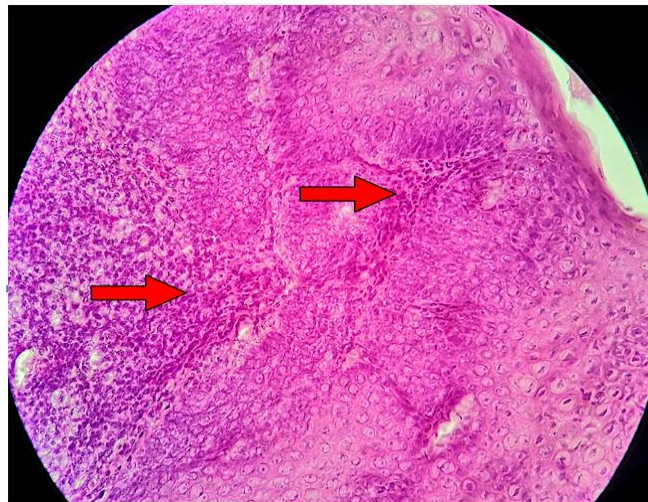


Figure 7: Histophotographic image of a crop from G3 showed severe inflammatory response within submucosa, lamina propria and muscularis mucosa. HE X40.

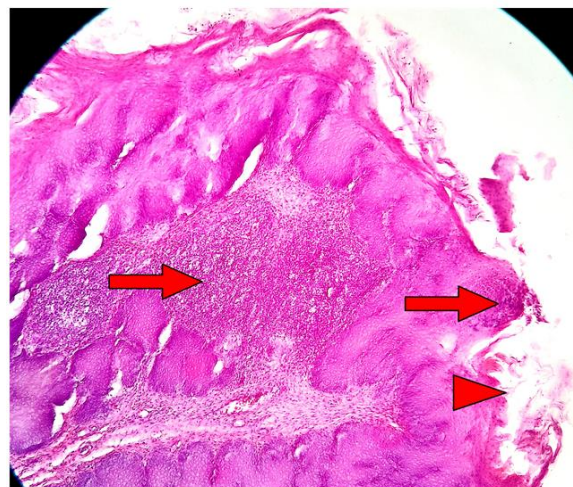
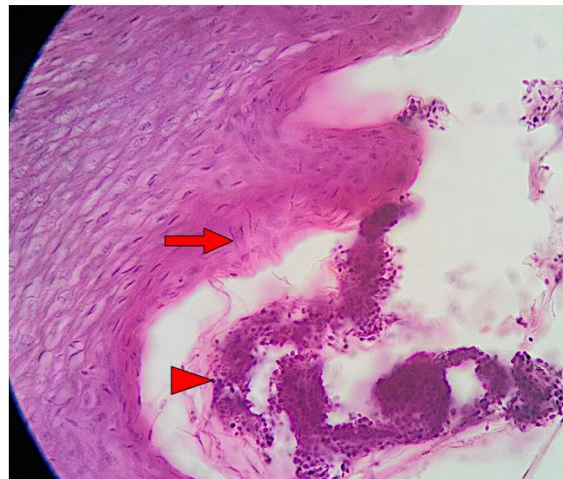


Figure 8: Histophotographic image of a crop from G3. The epithelium is ulcerated and invaded by inflammatory cells (arrows). On the margins of the ulcer there is intercellular edema, dissociation, necrosis, and exfoliation of keratinocytes (arrowhead). HE X20.





*Figure 9: A photographic image showing the gross lesion in a crop from chicks of G4 that are infected with candida albicans and treated with Saccharomyces Cerevisiae. There is increased thickness of the wall of the crop with less lesion when compared to G3.*



*Figure 10: Histopathological examination of crop from G4 showed limited fungal infection of squamous layer (arrow) and heavy spore accumulation within crop lumen (arrowhead). HE X40.*

### **Conclusions and recommendations**

This study the first experiment examining the effect of Saccharomyces Cerevisiae on avian candidiasis. This treatment was found effective in controlling crop candidiasis in birds. This method is inexpensive, has no reported side effects and can have positive effective on growth performance and FCR. We recommended adopting this treatment in clinical field as prophylactic measure to prevent candidiasis in commercial poultry. More research studies are needed to examine the effects of Saccharomyces Cerevisiae on other enteric infections.

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