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The Role and Importance of Additional Vitamin A Supplementation in the Treatment Protocol for Crop Candidiasis in Budgerigars

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Abstract: This study evaluated the effectiveness of adding oral Vitamin A to the standard treatment for crop candidiasis in budgerigars. A total of 60 budgerigars aged 1-4 years with confirmed crop candidiasis were divided into two groups. Group 1 received Nystatin, Metoclopramide, and Enrofloxacin, while Group 2 received the same treatment along with oral Vitamin A for 14 days. Clinical scoring was used to assess symptoms pre- and post-treatment on a scale of 1 to 5. Statistically significant improvements were observed in both groups ($p<0.05$). However, post-treatment clinical scores showed a significantly greater improvement in Group 2 ($p<0.05$). The recurrence rate of the disease was 33.3% in Group 1, compared to only 13.3% in Group 2 within 365 days. These results suggest that the addition of Vitamin A to the standard treatment protocol for crop candidiasis leads to faster recovery and a lower recurrence rate. The aim of the study was the investigation of the potential benefits of incorporating Vitamin A in managing crop candidiasis in budgerigars.

Keywords: Budgerigars, candida, treatment, vitamin A.

Muhabbet Kuşlarında Kursak Kandidiazisi Tedavi Protokolüne Ek Olarak Verilen A Vitamininin Rolü ve Önemi

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Öz: Bu çalışmada muhabbet kuşlarında kursak kandidiyazı için standart tedaviye oral A Vitamini eklenmesinin etkinliği değerlendirildi. 1-4 yaş arası, kursak kandidiazis tanısı konan 60 muhabbet kuşu iki gruba ayrıldı. Grup 1, Nystatin, Metoclopramid ve Enrofloksasin alırken, Grup 2 aynı tedaviyi 14 gün boyunca oral A Vitamini ile birlikte aldı. Semptomları tedavi öncesi ve sonrası 1'den 5'e kadar bir ölçekte değerlendirmek için klinik skorlama kullanıldı. Her iki grupta da istatistiksel olarak anlamlı farklar gözlendi ($p<0,05$). Tedavi sonrası klinik skorlama Grup 2'de anlamlı derecede daha fazla iyileşme gösterdi ($p<0,05$). Hastalığın tekrarlama oranı Grup 1'de %33,3 iken, Grup 2'de 365 gün içinde sadece %13,3 idi. Bu sonuçlar, kursak kandidiazisi için standart tedavi protokolüne A Vitamini eklenmesinin daha hızlı iyileşmeye ve daha düşük bir tekrarlama oranına yol açtığını göstermektedir. Çalışmanın amacı, muhabbet kuşlarında kursak kandidiazisinin tedavisinde A Vitamini kullanımının potansiyel faydalarının incelenmesidir.

Anahtar Kelimeler: Muhabbet kuşu, kandidiazis, tedavi, A vitamin.

INTRODUCTION

There are various gastrointestinal system diseases in budgerigars (Hollwarth and Prieto, 2024). These include stasis or dilatation of the esophagus and crop, neoplasms, gastrointestinal system foreign bodies, bacterial, viral, parasitic diseases, diarrhea, constipation and cloacal prolapse.

Crop candidiasis in budgerigars is one of the common diseases of the gastrointestinal system (Terry and Campbell, 2017). Candidiasis is a mycotic disease seen in the upper digestive system (mouth, esophagus and crop) of budgies. The disease is also called moniliasis or crop mycosis. The most commonly isolated species in patients is Candida albicans (Talazadeh et al., 2022). Poor hygiene

in the bird's environment and food preparation, prolonged use of antibiotics, concurrent immunosuppressive conditions (e.g., malnutrition, debilitation), failure to clean excess formula from the skin or mouth of hand-reared chicks, high concentrations of sugar in fruit and hand-rearing formula, which provide an ideal medium for yeast growth, and alkaline crop contents, which are observed when crop stasis occurs for any reason, are reported to be predisposing factors for overgrowth of the agents (Doneley, 2018). Pigeons, turkeys, chickens, geese, pheasants, quail, parrots, budgerigars, guinea fowls, and other birds have all been found to have candidiasis (Samanta, and Bandyopadhyay, 2017). Another contributing factor is nutritional inadequacies, particularly a lack of vitamin A, which can affect the body's defense processes as a mediator of resistance against major diseases and cause candidiasis (Velasco, 2000).

The disease can occur as primary or secondary in birds, with secondary bacterial infections also accompanying the infection. In patients with candidiasis, yellow-white necrotic plaques in the mouth are seen in the form of lines (Ladds, 2009). In the chronic phase, thickening and irregularity of the crop mucosa are characteristic. Crop mucosa is covered with exudate ranging from catarrhal to mucoid. The clinical signs of the disease varies depending on the factors that cause predisposition and the pathogenicity of the agent. Crop candidiasis is usually characterized by weight loss, slow growth, crop thickening, enlargement, delayed crop emptying, regurgitation, hoarseness or abnormal vocalization (Talazadeh et al., 2022; Garces, 2023).

By detecting Candida species on a Gram, Romanowsky-type, or methylene blue stain of the excrement, crop contents, or regurgitated material, candidiasis can be diagnosed. In extreme situations where tissue invasion has taken place, the budding yeast will create hyphae that are visible in feces or scrapings taken from the throat or crop (Ibrahim, 2020).

Treatment of candidiasis includes the administration of antifungal agents. Additionally, the use of metoclopramide (0.5mg/kg for 5 days) may help promote crop motility and prevent regurgitation (Apsemidou et al., 2020; McCready, 2024). Nystatin (10 mg/kg every 12 hours for 14 days), an antifungal drug, is the most commonly used drug in treatment due to its low cost and low toxicity (Kafrashi and Babazadeh, 2022; Garces, 2023). In addition, antibiotic use is recommended against secondary bacterial infections. For this purpose, enrofloxacin can be used at a dose of 10 mg/kg body weight for 5 days. Patients may also be given vitamin A for its positive contribution to the treatment process. (Kurtdede et al., 2008).

Vitamin A is a micronutrient that is essential for supporting growth and development and maintaining the integrity of mucosal epithelial cells. Furthermore, because of its vital role in boosting immune function by controlling humoral and cellular immunological processes, vitamin A is referred to as an anti-inflammatory vitamin (Huang et al., 2018). Vitamin A is one of the antioxidant vitamins that neutralizes free radicals by reducing them in the body (Blaner et al., 2021). Oxidative stress occurs as a result of various diseases (Chatterjee, 2016). Oxidative stress reduces antioxidant levels due to increased production of reactive oxygen species (Esin et al., 2024). Therefore, in situations that create oxidative stress, the antioxidant properties of vitamin A can be used. Secretory immunoglobulin A (IgA) is an antibody that plays an important role in the immune function of mucosal membranes. It is found in all mammals and birds. It is one of the main lines of defense of our body. It is found in all mucosal membranes in our body. In vitamin A deficiency, IgA production is negatively affected. Therefore, in vitamin A deficiency, IgA production, especially in mucosal membranes, is disrupted and the immune system is negatively affected (Li and Chen, 2020). In addition, from this point of view, vitamin A will have positive effects on the healing process in diseases, especially mucosal diseases (Kurtdede et al., 2008). Vitamin A deficiency also hinders the maturation and proliferation of T and B lymphocytes, lowers the generation of natural killer cells, monocytes, and macrophages, and influences the release of cytokines and antibodies (Munteanu and Schwartz, 2022).

The aim of this study was to investigate the contribution of vitamin A added to the routine treatment protocol used in budgerigars with crop candidiasis on the healing process.

MATERIAL AND METHOD

The study material consisted of 60 budgerigars aged between 1 and 4 years old, brought to Ondokuz Mayıs University Animal Hospital with complaints of anorexia, weight loss, crop dilatation, hoarseness/abnormal vocalization, regurgitation and vomiting, and diagnosed clinically with crop candidiasis. As a result of physical examination, crops of birds suspected of crop candidiasis were gently massaged and some crop content was taken. The diagnosis was confirmed by the observation of blastospores and pseudohyphae in the native examination of smear preparations prepared from crop content, and by the observation of the agent itself or pseudohyphae in Giemsa staining of smears (Figure 1). A clinical scoring chart was made for the budgerigars with crop candidiasis included in the study. Clinical scores ranged from 1 to 5 based on symptom severity. The clinical score criteria were

based on the five most commonly observed clinical signs in crop candidiasis: anorexia, weight loss, regurgitation/vomiting, crop distension, and abnormal vocalization. (Table 1). In this study, a clinical scoring system was developed to assess clinical signs associated with crop candidiasis in budgerigars, inspired by previously established scoring systems for feather condition and sedation levels in psittacine species (Abreu et al., 2024; van Zeeland et al., 2013). Clinical scoring was performed on both groups before and after treatment.

Table 1. Clinical scoring table for budgerigars included in the study.

Symptoms	No	Yes
Anorexia	0	1
Weight loss	0	1
Regurgitation/vomiting	0	1
Crop distension	0	1
Abnormal vocalization	0	1

The study consisted of two groups consequently, treatment 1 group and treatment 2 group. Treatment 1 group received nystatin (10 mg/kg for 14 days), metoclopramide (0.5mg/kg for 5 days) and enrofloxacin (10 mg/kg for 5 days). Treatment 2 group received oral vitamin A 8.000IU/kg (Zhang et al., 2023) for 14 days in addition to these drugs. The same diet was applied to both groups during the treatment period. The treatment duration was 14 days in both groups.

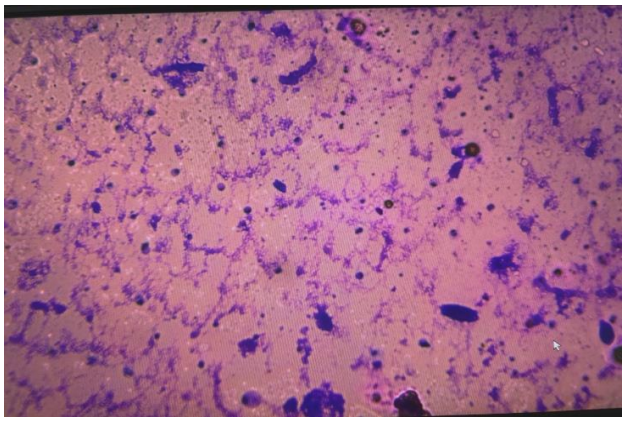

Figure 1. Microscopic appearance of Candida albicans in a smear from a budgerigar under 400× magnification (40× objective). The yeast cells appear as loosely aggregated clusters, indicative of fungal overgrowth (Black arrows).

Statistical Analysis: Clinical scores for both groups were recorded as numerical data before and after treatment. The data were analyzed using the IBM SPSS Statistics for Windows (IBM Corp., Armonk, N.Y., USA). Quantitative data were assessed for normality using the Shapiro-Wilk test. Since the data has a normal distribution, before using the independent t-test, the two groups were assessed for equality of variances. For this purpose, Levene's test was used to determine whether the standard deviation of the treatment 1 group was expected to be the same as the standard deviation of the treatment 2 group. Independent t-test was used because the data values had a normal distribution and showed variance equality. Paired sample t test the was used for comparative statistics of pre-treatment and post-treatment clinical scores within the group. For continuous variables, means and standard deviations are given. P values less than 0.05 were considered significant.

RESULTS

The statistical evaluation results of pre and post treatment scores in the groups were shown in table 2. In within-group measurements, the clinical score of budgerigars with crop candidiasis in treatment 1 group was 4 ± 0.83 before treatment, but with the decrease in symptoms after treatment, the clinical score decreased to 1 ± 0.83, and this difference was found to be statistically significant (a, b) ($p<0.05$). In within-group measurements, the clinical score of budgerigars with crop candidiasis in treatment 2 group was 3.9 ± 0.75 before treatment, while the clinical score after treatment was 0.4 ± 0.24, consistent with improvement, and this difference was found to be statistically significant (c, d) ($p<0.05$). When the measurements between the groups were examined, no statistically significant difference was found between the pre-treatment clinical scores of the treatment 1 group and treatment 2 group ($p>0.05$). However, the difference between the post-treatment clinical scores of the treatment 1 group and treatment 2 group (1 ±0.53 and 0.4 ±0.24, respectively) was found to be statistically significant ($p<0.05$).

The recurrence rate of the disease within 365 days after treatment was evaluated in both groups. In the treatment 1 group, recurrence was observed in 10 out of 30 budgerigars (33.3%), whereas in the treatment 2 group, recurrence occurred in 4 out of 30 budgerigars (13.3%). (Table 3).

Table 2. The statistical evaluation results of pre and post treatment scores in the groups.

Groups	Pre-Treatment Score	Post-Treatment Score
Treatment 1	4 ± 0.83^{a}	1 ± 0.53^{b}
Treatment 2	3.9 ± 0.75^{c}	0.4 ± 0.24^{d}
P Value	>0.05	**<0.05**

The P value in the table shows the statistical difference in measurements between groups. Different lower case letters (a, b and c, d) show the statistical difference in measurements within groups.

Table 3. Disease recurrence rates among groups within 365 days after treatment.

	Treatment 1 (n:30)	Treatment 2 (n:30)	P Value
Recurrence	10	4	**<0.05**
Percentile (%)	33.3	13.3	

DISCUSSION

Candida spp., a type of yeast, is a disease that affects almost all bird species. Symptoms of candidiasis in avian species are similar, including anorexia, weight loss, crop enlargement, hoarseness/abnormal vocalizations, regurgitation, and vomiting (Gibbons, 2020; Schmidt et al.,

2024). In our study, budgerigars with crop candidiasis showed symptoms consistent with similar publications.

In diagnosing crop candidiasis, oral, vomitus, crop, or fecal content can be used to diagnose Candida agents microscopically (Kurtdede et al., 2008; Ibrahim 2020). In addition, the diagnosis can be supported by the 'honeycomb' appearance of the crop with contrast radiography (Kurtdede et al., 2008; Krautwald et al., 2017). In addition, yellow-white necrotic plaques can be seen in the oral mucosa of birds with crop candidiasis (Hauck, 2024). The disease can be diagnosed with these diagnostic tools. In this study, candidiasis diagnose was comfirmed by observing blastospores and pseudohyphae in the natural examination and Giemsa staining of smears prepared crop content similar to previous studies in patients.

The most commonly preferred antifungal drug for avian crop candidiasis is nystatin (McCready, 2024). Nystatin binds to a compound called ergosterol in the cell membrane of Candida species. Ergosterol is a structural component of the cell membrane of fungi/yeasts and is necessary for the integrity of the fungal membranes (Rai et al., 2022). Nystatin also binds with ergosterol to create holes (pores) in the cell membrane. These holes in the cell membrane disrupt the normal ionic balance between the cell's internal and external environment. These holes cause substances contained in the cell (such as potassium, water, and other ions) to leak out. This leads to cell death or loss of growth ability (Prasad et al., 2016). As a result, nystatin prevents Candida species from multiplying in the crop, which makes it effective in the treatment of crop candidiasis. In crop candidiasis, the use of metoclopramide can help increase sluggish crop motility and prevent regurgitation. Therefore, it finds a place in the treatment protocol (Apsemidou et al., 2020; McCready, 2024).

Talazadeh et. Al. (2022), reported that, using vitamin A in crop candidiasis in birds has positive effects particularly in the immune system and potential benefits in treating fungal infections (Talazadeh et al., 2022). Vitamin A has important role associated with functions such as protecting mucosal tissues, strengthening the immune response, and healing epithelial cells (Özkanlar et al., 2016; Stephensen and Lietz, 2021). Therefore, it is thought that vitamin A supplementation may be a potential treatment tool against infections of mucosal surfaces such as the crop. Considering these effects, vitamin A was added to the treatment protocol in this study.

Vitamin A supports the regeneration of crop epithelial cells and their healthy structure. The crop mucosa is an important part of the digestive system and the epithelial cells found here play a critical role in digestion and protection from infections (Kierończyk et al., 2016). Vitamin A increases the durability of the mucosal barrier by helping cells differentiate and proliferate properly. In addition, strengthens the mucosal immune response by helping to increase the production of IgA antibodies. Vitamin A can increase the resistance of the crop mucosa to fungi (Bos et al., 2022). Diseases such as crop candidiasis, especially caused by yeasts such as Candida spp., are common in birds with weak immune systems. Adequate vitamin A intake can increase protection against such infections and can help heal mucosal damage by reducing inflammation during fungal infections (Campione et al., 2020). Probably, faster and better regression was observed in the symptoms in the vitamin A treatment group compared with the other treatment group for these reasons and the lower recurrence rate of the disease in the group using vitamin A can be explained, as well.

CONCLUSION

In this study detected that, vitamin A was added to the routine treatment protocol in the treatment of crop candidiasis, it provided faster and more effective recovery. In addition, when vitamin A was used, a significant decrease was observed in the recurrence rate of the disease. Therefore, the use of vitamin A in addition to the treatment of crop candidiasis in birds is recommended as a result of this study.

Contributions: ÇE contributed to the study's conception and design. Material preparation and data collection were performed by all authors. ÇE contributed data analysis, writing, and editing the last draft of the manuscript. The manuscript has been read and approved by all named authors.

Conflict of interest: The authors declare no competing interests.

Ethics Committee Permission: This study was approved by the Ondokuz Mayıs University Animal Experiments Local Ethics Committee (2024-49).

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