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ORIGINAL ARTICLE

Effect of Dietary Yeast Supplementation on Growth Performance and Colonization of *Salmonella enteritidis* in Japanese Quails

Ahmed M. HASSAN[1] Manal M.A. MAHMOUD[2] Dalia M. HAMED[3] Omnia E. KILANY[4]

[1] *Suez Canal University, Department of Animal and Poultry Hygiene, Ismailia, Egypt*
[2] *Suez Canal University, Department of Nutrition and Clinical Nutrition, Ismailia, Egypt*
[3] *Suez Canal University, Department of Poultry and Rabbit Medicine, Ismailia, Egypt*
[4] *Suez Canal University, Department of Clinical Pathology, Ismailia, Egypt*

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SUMMARY

The present research studied the capacity of *Saccharomyces cerevisiae* to enhance performance of Japanese quails and to antagonize *Salmonella enteritidis* colonization in the intestinal tract. Two hundred and forty unsexed quails were assigned into four treatment groups (T1-T4). Groups T1 and T2 served as non-infected and infected controls received basal diet, while T3 and T4 served as non-infected and infected groups received basal diet supplemented with 3 g yeast /kg (*Saccharomyces cerevisiae* 8x10^9 cells l/gram). Obtained results revealed significant elevated performance of treated quails with yeast as compared to controls starting from second week till the end of experimental period. Also, yeast supplementation to infected group alleviated the drastic effect of infection on performance. Cecal colonization was significantly reduced in infected group treated with yeast as compared to infected group received basal diet in both 7 at 21 days post infection. Yeast supplementation significantly reduced lactose fermenter and non-lactose fermenter as well as total bacterial counts in both time intervals. Supplementation of birds with yeast led to a trend toward increase in both erythrogram and leukogram (T3) as compared to T1, and alleviated the drastic effect of infection (T4) as it was significantly increase from T2. Hemoglobin and Packed cell volume were significantly reduced due to infection at 21 days old (14 days post infection), yeast supplementation restored this effect. Infected non treated group (T2) showed leukocytosis at the end of the experimental period, lymphocytosis then lymphopenia, and heterophilia. Yeast supplementation significantly alleviated the drastic effect of infection. Generally, Yeast supplementation improved bird's growth and blood parameters and reduced intestinal colonization with pathogenic microorganisms.

Key Words *Yeast, Japanese Quail, Salmonella enteritidis, Colonization, Probiotics, Immune Organ Indices*

Bıldırcınlarda Yemlere Maya Katılmasının Büyüme Performansı ve *Salmonella enteritidis*'in Kolonizasyonu Üzerine Etkileri

ÖZET

Bu çalışmada Japon bıldırcınlarının büyüme performansını arttırmak ve sindirim sistemimde *Salmonella enteritidis* kolonizasyonunu engellemek amacıyla *Saccharomyces cerevisiae*'nın etkinliği araştırılmıştır. İki yüz kırk cinsiyeti belirsiz bıldırcın dört çalışma grubuna (T1-T4) ayrıldı. T1 ve T2 grupları enfekte olan ve olmayan gruplar olarak bazal diyet alırken T3 ve T4 grupları enfekte olmayan ve olan gruplar olarak 3 g maya / kg (*Saccharomyces cerevisiae* 8x10^9 hücre l / gram) ilave edilmiş bazal diyet almışlardır. Elde edilen sonuçlara göre ikinci haftadan itibaren çalışma sonuna kadar maya uygulanan bıldırcınlar kontrol grubuna kıyasla önemli ölçüde yüksek performans göstermişlerdir. Ayrıca, enfekte gruba maya takviyesi enfeksiyonun gelişme performansına olan ağır etkisini hafifletti. Bazal diyet alan enfekte gruba kıyasla maya ile tedavi edilen enfekte grupta enfeksiyon sonrası 7. ve 21. günlerde sekal kolonizasyon anlamlı ölçüde azalmıştır. Maya takviyesi laktozu fermente eden ve etmeyen toplam bakteri sayısını her iki zaman aralığında önemli ölçüde azaltmıştır. Maya ile yem takviyesi T1 ile karşılaştırıldığında T3 grubunda hem erythrogram ve hem de leukogram'da artış eğilimi gösterirken, T4 grubunda enfeksiyonun köklü etkisi T2'ye göre önemli ölçüde hafiflemiştir. Enfeksiyona bağlı olarak hemoglobin ve total hücre hacmi 21 günlük (enfeksiyon sonrası 14 gün süreyle) hayvanlarda önemli ölçüde azalırken maya ilavesi bu etkiyi onardı. Enfekte olup tedavi edilmeyen grupta (T2), deneme süresi sonunda lenfositoz ardından lenfopeni ve heterofili gözlendi. Maya takviyesi enfeksiyonun şiddetli etkisini önemli miktarda hafifletti. Genellikle, maya takviyesi kanatlıların büyümelerini ve kan parametrelerini düzeltirken patojen mikroorganizmaların intestinal kolonizasyonunu azalttı.

Anahtar Kelimeler *Maya, Bıldırcın, Salmonella enteritidis, Kolonizasyon, Probiotikler, İmmun organ indeksleri*

Corresponding author: Ahmed M. HASSAN
Suez Canal University, Department of Animal and Poultry Hygiene, Ismailia, Egypt. e-mail: ahmed_hassan@vet.suez.edu.eg

INTRODUCTION

Quails could be considered a good and economical source of animal protein. The edible parts of its carcass are higher as compared to those of other poultry species (Saleh, 1988). Therefore, countries having shortage in animal protein, such as Egypt can depend on intensive production of quail to compensate a part of this shortage.

Stress due to intensive production conditions to enhance the bird's performance creates imbalance of intestinal microflora and also lower the body defense mechanisms. Birds may get stressed from different factors such as overcrowding, unfavorable ambient temperature, feed intake, and vaccination. (Taksande et. al. 2009).

Salmonella is an enteric pathogen that colonizes the intestinal tract of poultry and humans, and accounts for millions of cases of gastroenteritis and food-borne illness each year (Van Look et al., 2000). *Salmonella enteritidis* can be transmitted to humans through the food production chain, and undercooked or row eggs and poultry meat are a particularly high risk for humans (Gillespire et al., 2005), it represented a major health problem worldwide in the last few decades (Herikstad, 2002).

Nowadays many growth promoters are being used including probiotics, which have helped to improve feed utilization, microbial balance and growth rate of birds. Probiotics have the advantages over the antibiotics as the antibiotics usage result in common problems such as development of drug resistance and drug residues. Yeast is a common probiotic used in poultry production as it has the ability to stimulate digestion and aid in maintaining microbial equilibrium in the gut. Live yeast, such as *Saccharomyces cerevisiae* contains numerous enzymes that could be released into the intestine and aid existing enzymes in the digestive tract in the digestion of feed. Also, yeast contains vitamins and other nutrients that may produce beneficial production responses (Kornegay et al., 1995). Moreover, yeast supplementation can inhibit pathogenic bacteria and increase the number of anaerobic and cellulytic bacteria (Abdel Azeem, 2002) Also Satin et. al., 2003 revealed that yeast can improve immune response in birds.

The main objective of this study was to evaluate the effect of dietary yeast supplementation on performance of Japanese quails, and its effect on competitive exclusion of *Salmonella enteritidis* after experimental infection.

MATERIALS and METHODS

Experimental quails and management

A total of 240, one week old unsexed Japanese quails purchased from the agricultural technological center, Faculty of Agriculture, Cairo University, Egypt were assigned randomly to four treatment groups having three replicates (A- C) (20 chicks each). Birds were kept in battery cages for 35 days. Feeds were formulated to meet the nutritional requirements as suggested by the NRC, 1994 as shown in Table (1). Birds were given the basal diet (T_1) or basal diet and infected with *Salmonella enteritidis* (T_2). The third treatment group (T_3) was given the same diet supplemented with 3 g yeast /kg (Saccharomyces cerevisiae $8x10^9$ active live yeast cells/gram, Tonilisat® China Way Corporation, Taiwan). While, the fourth treatment (T_4) was supplemented with yeast and infected with *Salmonella enteritidis*.

Vit. A 12 mIU, vit. D_3 2 mIU, vit. E 1000mg, vit. k_3 1000mg, vit. B_1 1000mg, vit. B_2 5000mg, vit. B_6 1500mg, vit. B_{12} 10mg, biotin 50mg, pantothenic acid 10000mg, nicotinic acid 30000mg, folic acid 1000mg, manganese 60000mg, zinc 50000mg, iron 30000mg, copper 4000mg, iodine 300mg, selenium 100mg, cobalt 100mg, carrier($CaCO_3$) to 3kg. (Golden premix- Selim Pharm Elasher, Egypt. patch No. 8181, production 3-2010).

Vit. A 15 mIU, vit. D_3 2 mIU, vit. E 1000mg, vit. k_3 1000mg, vit. B_1 1000mg, vit. B_2 5000mg, vit. B_6 1500mg, vit. B_{12} 10mg, biotin 50mg, pantothenic acid 10000mg, nicotinic acid 30000mg, folic acid 1000mg, manganese 60000mg, zinc 50000mg, iron 30000mg, copper 4000mg, iodine 300mg, selenium 100mg, cobalt 100mg, carrier($CaCO_3$) to 3kg. (Golden premix- Selim Pharm Elasher, Egypt. patch No. 8181, production 3-2010)

Table 1. Ingredient and chemical composition of experimental basal diet

Ingredients	%
Ground yellow corn	57.83
Soya bean meal (45%)*	32.94
Fish meal (60.05)*	3.50
Corn gluten (62)*	3.48
Dicalcium phosphate	0.33
Limestone	1.16
DL-Methionine	0.09
Lysine	0.07
Iodized sodium chloride	0.30
Minerals and vitamins premix**	0.30
Calculated composition	
Crude protein (%)	24.00
ME (kcal/kg)	2900.00
Calorie/protein ratio (C/P)	120.83
Calcium (%)	0.80
Phosphorus (%)	0.30

*. Determined according to AOAC, 1990.

** Each 3 kg contain the following vitamins and minerals

Salmonella entretidis infection

A serotype of *Salmonella enteritidis* resistance to novobiocin-nalidixic acid (NO 25 µg/ ml, /NA 20 µg /ml) was selected and maintained on SS agar. Challenge inoculums for intra crop were prepared in sterile phosphate-buffered saline. The viable cell concentration of the inoculums was determined by colony counts on SS agar plates and inoculums contained $1.00x10^7$ CFU/ml was used to challenge birds at the age of two weeks (Guard et.al, 2010).

Parameter Measured

A- Performance parameters

The body weight and feed consumption of birds per replicate were recorded on individual basis at weekly intervals. Feed conversion ratio was also calculated weekly (Quigley et.al., 1997). Immune organ indices for spleen, bursa of Fabricius and thymus were formulated as: immune organ weight (g)/body weight (kg) (Bobyntsev, 2005).

B- Determination of Salmonella enteritidis

Infected birds were observed for clinical signs and mortalities for 3 weeks. At 14 and 21 days post *SE* challenge, 3 birds from each group were picked up randomly and sacrificed. Samples from intestine, liver and

tests/ovary were aseptically removed and examined for *S. enteritidis*; the caecal count was performed quantitatively according to (Bolder and Palmu, 1995). Clinical signs and post mortem examinations were recorded for all treatment groups. At the end of observation period dead and sacrificed birds were subjected to post mortem lesion scoring according to (Whitney et.al. 2006) (Table 5).

C- Haematological picture

Parameters of the erythrogram were determined according to standard techniques described by Coles, (1986). Blood films were stained by Giemsa stain for differential leukocytic count (Feldman et al., 2000).

Statistical analysis

Results are expressed as means ± SE for each group. Groups were tested for differences by performing the ANOVA and fisher's least protected significance test using the Stat view 4.53 software, (Abacus Concepts Inc., Berkeley, CA). Differences were considered statistically significant at $p<0.05$.

RESULTS and DISCUSSION

Supplementing yeast to the basal diet significantly improved weight gains in quails starting from the second week till the end of the experimental period (Table 2). Meanwhile, in the infected groups, it was clear from Table 5 that infection dramatically reduced body weights and yeast supplementation restored this effect with a significant elevation at third week post infection. Increased body weights in non-infected supplemented group might be due to increase of the brush border membrane enzymes including sucrase-isomaltase, lactase, maltase-glucoamylase, α-glucosidase and alkaline phosphatase which have a positive influence on nutrient degradation and absorption (Buts et al., 86 & John et al., 96). Also, *S. cervisiae* cells contain high level of polyamines that may contribute to rise in expression of intestinal enzymes (Buts et al., 94). Similar findings were reported by Taksande et al. (2009) and Kumararaj et al., (1997) who showed significant improvement in average live body weight of quails supplemented with *S. cerevisiae* and probiotics, respectively.

There was no significant difference in average daily gain (except 2nd week) (Table 3). This result is in agreement with Ayanwale et al. (2006).Conflicting reports regarding the effect of yeast on broiler growth rate, on one hand body weight gain was increased (Ghasemi et al., 2006), on the other hand (Oboh and Akindahunsi, 2005 and Chumpawadee et al. (2008) observed that supplementation of cassava yeast to broiler diets did not improve growth rate The reason of variation might be related to the strain of yeast, concentration and form of yeast used (Chumpawadee et al. 2009).

Table 2. Effect of yeast supplementation on weekly body weights in grams (Mean±SE)

Body weight	Control	Yeast supplemented
Initial	30.33±1.20	29.33±0.88
1 st week	72.00±2.88	70.33±2.96
2 nd week	105.33±2.96	121.67±5.48*
3 rd week	169.00±4.04	189.00±6.65*
4th week	214.67±1.45	232.00±4.16*
5th week	255.67±3.84	269.67±1.45*

*Means are statistically different at $p<0.05$.

The data illustrated in Table 3 indicated that there was significant differences in feed consumption only at the 4th week, whereas yeast supplementation significantly reduced feed consumption, though the body weight was significantly higher, meanwhile feed conversion ratio showed significant improvement due to yeast supplementation only at the second week of the experiment. These results are in agreement with Chumpawadee et al. (2009) on Japanese quail also are in agreement with previous studies in broilers (Chumpawadee et al., 2008; Karaoglu and Durdag, 2005) who observed that feed intake was not affected by yeast inclusion in the diet. The present findings are in agreement with Ergun et al. (2000). Some studies showed that probiotics supplementation in the feed of chickens improve the feed conversion ratio (Day, 1997). The reason for the variable effect of biological additives may be confounded by variations in gut flora and environmental condition (Mahdavi et al., 2005).

Table 3. Effect of yeast supplementation on average body weight gain (g/day/bird) feed consumption, and feed conversion.

Week	Average body weight gain		Feed consumption		Feed conversion	
	Control	Yeast	Control	Yeast	Control	Yeast
1st	5.95±0.31	5.86±0.54	10.22±0.14	11.23±0.05	1.73±0.06	1.95±0.17
2nd	4.76±0.33	7.33±0.39*	16.55±0.24	17.00±0.50	3.52±0.30*	2.33±0.08
3rd	9.09±0.33	9.62±0.17	23.76±0.95	24.09±0.18	2.59±0.14	2.51±0.05
4th	7.00±0.43	6.71±0.84	26.65±0.36*	24.25±0.50	3.85±0.30	3.61±0.06
5th	5.62±0.58	5.40±0.70	22.12±0.11	22.62±0.67	4.02±0.38	4.33±0.57

*Means are statistically different at $p<0.05$

Immune organs/body weight ratios results were in accordance with performance results as there was statistically significant increase in thymus/body weight ratio in yeast (T3) supplemented group as compared to non-treated controls (T1). Meanwhile, there was a trend toward increase in bursa/bodyweight ratio as a result of yeast supplementation (Table 4). Obtained results are in agreement with findings of satin et. al., 2003 who stated that yeast supplementation improved immune response.

Table 4. Effect of yeast supplementation on the immune organs/body weight ratios

Measures	Control	Yeast supplemented
Thymus	0.14±0.02	0.32±0.05*
Bursa	0.09±0.01	0.16±0.03
Spleen	0.22±0.11	0.11±0.02

*Means are statistically different at $p<0.05$

The response of treated birds to infection with *Salmonella*

enteritidis revealed that mortality was recorded between first day and the 12th day post infection (Table 5) in infected non supplemented group; on the other hand, yeast supplementation delayed and decreased mortalities to start from the 4th day till the third week. Dead birds showed severe septicemia. Other birds showed dark brown then turned into white mucoid (foamy) diarrhea, depression, ruffled feather, sunken eyes and decreased feed intake Birds showed severe nephritis, engorged ureters with urates, severe congestion of the liver and spleen, turbidity in abdominal air sacs by the 5th day post infection. At the 10th day, severe pericarditis and cellulites at the site of inoculation (intra-crop) were observed. These findings agree with previous findings of Yang (1992) and Shivaprasad (2000). By the end of observation period, some birds from T2 showed complete blindness. The lesion score showed a trend toward decrease in T4 (1.9) as compared to T2 (2.5).

Intestinal colonization is normally the first step in the infection for orally infected birds leading to the persistent shedding of Salmonella in the feces. In many infected birds, invasion via the gastrointestinal tract results in Salmonella multiplication in reticuloendothelial tissue of liver, spleen and caecum, where caecum is the main colonization site (Barrow et al., 1987 & Hudault et al, 1985). The effect of our studied yeast was adopted for a significant reduction in caecal colonization during the entire period of the experiment as shown in Table 6. Line et al. 1998 and Laegreid and Bauery (2004) stated that, several harmful pathogenic bacteria have been shown to exhibit a binding specific for the sugar mannose in *Saccaromyces cervicaea* wall, which may cause the yeast to act as a decoy for the attachment of pathogens. Because yeast has been demonstrated not to permanently colonize animals, the yeast and any yeast-bound pathogens pass out in the bird excretion and bacterial colonization is diminished. Kabir et al., (2004) reported that probiotic microorganisms, once established in the gut, may produce substances with bactericidal or bacteriostatic properties (bacteriocins) such as lactoferrin, lysozyme, hydrogen peroxide as well as several organic acids. These substances have a detrimental impact on harmful bacteria, which is primarily due to a lowering of the gut pH, which may partially offset the low secretion of hydrochloric acid in the stomach. In addition, competition for energy and nutrients between probiotic and other bacteria may result in a suppression of pathogenic species.

Organ culture for *Salmonella enteritidis* revealed that Adding yeast to the infected birds partially reduced the infection in the liver and intestine. On the other hand, M O was not isolated from the reproductive organs in both infected and treated groups at 7th and 21st days post infection (Table 7). Guard et.al, 2010, suggested that *Salmonella enteritidis* cultures that vary in subpopulation composition have subtle differences in colonization of reproductive tissue, also Withanage et.al, 2003 mentioned that Salmonella-induced changes in T lymphocytes, B lymphocytes and macrophages in the ovaries and statistically significant increases in the numbers of T cells (both CD4+ and CD8+) and macrophages were observed 7 to 14 days after primary inoculation, followed by a peak in B-cell numbers from the 14th day post-primary inoculation onwards in the secretory areas of the oviducts. The peak in lymphocyte numbers immediately preceded a decline in the rate of SE recovery from the reproductive tract beginning at day 14. The correlation of decreased Salmonella recovery with elevated lymphocyte and macrophage numbers strongly suggests that local cell-mediated immunity is involved in controlling SE injection in the ovaries and oviducts.

Erythrogram results (Table 8) showed normocytic normochromic anemia in infected non treated group (T2) which may be due to bacterial endotoxins that suppress to the bone marrow (Feldman et al., 2000). Supplementation of yeast to infected group returned the picture to normal and yeast supplementation didn't significantly differ from controls. This is in agreement with Fleischer et al., (2000) who found no significant changes in packed cell volume and number of erythrocytes in glucan (active principle of yeast) treated groups. Also, agreed with Shareef and Al Dabbagh (2009). On the other hand, our results differ from Banerjee and Pradhan,) 2006) and Huff et al., (2010) who reported increased erythrocyte number, hemoglobin and hematocrit value. The difference may be due to different durations and/or species.

Avian leukocytes serve as the first line of defense against invading microorganisms. Infected non treated group showed leukocytosis at the end of the experimental period, lymphocytosis then lymphopenia, and heterophilia (Table 9), these results were in agreement with Hanan (2002) and Fatma (2005). The infected treated groups T.L.C. showed leukocytosis and heterophilia. While, the treated groups fed *S. cerevisiae* revealed leukocytosis, lymphocytosis and monocytosis at 21 days. These results were partially in agreement with Paryad and Mahmoud (2008). Our results disagree with Woo et al., (2006), who stated that supplementary Safmannan (beta-glucan and mannan oligosaccharide complex) and World-Labs (multiple probiotics) on broiler of chicks, lowered leukocytes and red blood cells (RBCs) than that of control groups. The increased lymphocyte populations at 21days may be indicative of higher activity of humeral immunity in chicks fed yeast supplemented diets Paryad and Mahmoudi (2008).

Table 5. Effect of yeast supplementation on body weights and mortality rate of infected birds

Week	Body weight		Mortality	
	Control	Yeast supplemented	Control	Yeast supplemented
1st PI	33.00±6.42	65.27±1.7	4 (d 1, d 2, d 5)	2 (d 4, d 7)
2nd PI	124.00±4.51	146.67±12.84	2 (d8, d 12)	3 (d 10, 13,)
3rd PI	173.33±6.36	207.00±4.04*		1 (d 14)
Total and %			Total 6 = 30%	Total 5=25%
Lesion score			2.5	1.9

*Means are statistically significant at P<0.05.

Table 6. Effect of yeast supplementation on reducing cecal microbial colonization after experimental infection with *Salmonella enteritidis*

Period		Control	Yeast supplemented
7 days post infection	LF	$4.03x10^7±1.285x10^7$*	$1.701x10^6±70378x10^5$
	NLF	$1.203x10^6±4.495x10^5$*	$1.033x10^5±4.399x10^4$
	TEn	$4.133x10^7±1.328x10^5$*	$2.55x10^6±7.74x10^5$
	TBC	$1.307x10^9±4.01x10^8$*	$2.383x10^6±1.033x10^5$
21 days post infection	LF	$1.29x10^6±3.938x10^5$*	$2.333x10^3±3.94x10^2$
	NLF	$3x10^5±1.078x10^5$*	$7.01x10^3±2.907x10^2$
	TEn	$1.513x10^6±4.578x10^5$*	$1.543x10^3±2.98x10^2$
	TBC	$4.17x10^7±1.84x10^7$*	$1.933x10^5±6.90x10^4$

LF= lactose fermenter NLF= non lactose fermenter Ten= total *Enterobactereacae* TBC= total bacterial count

*. Means are statistically significant between treatments at $p<0.05$

Table 7. Effect of yeast supplementation on Bacteriological examination of different organs after experimental infection with *Salmonella enteritidis*

		Rap	XLD	SS	EMB	TSI
Control infected	Liver	++	++	++	++	++
	Intestine	++	++	++	++	++
	Tests/ovary	-ve	-ve	-ve	-ve	-ve
Yeast supplemented	Liver	+	+	+	+	+
	Intestine	+	+	+	+	+
	Tests/ovary	-ve	-ve	-ve	-ve	-ve

++: all examined samples were positive, +: half of the examined samples were positive, -ve: all the examined samples were negative.

Table 8. Effect of yeast supplementation on the erythrogram of Japanese quails (Mean ± S.E.) at the age of 21 and 42 days.

Parameters & groups	RBC (10^6/μl)		Hb (gm/dl)		PCV (%)	
	21 days	42 days	21 days	42 days	21 days	42 days
Control	2.23± 0.11[a]	2.5±0.04[a]	9.73±0.24[a]	10.37±0.23[a]	26.68±0.23[a]	29.13±0.08[a]
Yeast	2.45±0.6[a]	2.6±0.04[a]	9.9±0.14[a]	10.25±0.14[a]	27.03±0.18[a]	28.83±0.48[a]
Yeast+infection	2.25±0.6[a]	2.45±0.03[a]	9.78±0.08[a]	9.98±0.09[a]	26.58±0.22[a]	28.35±0.48[a]
Control Infected	1.95±0.05[b]	2.22±0.10[b]	8.6±0.23[b]	9.08±0.05[b]	23.75±0.25[b]	25.88±0.13[b]

Means with different or not sharing same superscripts at the same column are statistically different at $p<0.05$

Table 9. Effect of yeast supplementation on leukogram of Japanese quail (Mean ± S.E.) at the age of 21 and 42 days

Parameters & groups	TLC (10^3 / μl)	Hetero (10^3 / μl)	Lymph (10^3 /μl)	Monocyt (10^3 / μl)
Control	22.88±0.43[b]	7.65±0.24[c]	14.58±0.27[bc]	0.40±0.08[c]
Yeast	27.38±0.38[a]	7.10±0.10[c]	18.73±0.33[a]	1.28±0.05[a]
Yeast+infection	27.30±0.61[a]	12.40±0.48[a]	13.63±1.11[c]	0.63±0.03[b]
Control Infected	26.63±0.24[a]	9.75±0.14[b]	15.53±0.37[b]	0.81±0.11[b]

Means with different or not sharing same superscripts at the same column are statistically different at $p<0.05$

CONCLUSION

Our obtained results showed that using yeast is important concept for enhancing quail performance and a good tool for competitive exclusion of pathogenic microorganisms in quail which can greatly assist in control of *Salmonella enteritidis* colonization. Infection of Salmonella is expensive for both industry and society. Exiting a program of such probiotic with the aim of eliminating Salmonella reduces the significant infection source for both man and animal. Eventually yeast can make a valuable contribution to flock health and safety of quail products as food. This may provide a significant tool for quail industry to compete the occurrence of intestinal diseases and in reduction of food borne pathogens.

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