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Impact of Prebiotic Supplementation on Productive Performance, Carcass Traits, and Physiological Parameters of Broiler Chickens under High Stocking Density Condition

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ABSTRACT

The present study was performed to investigate the effect of increasing stocking density, prebiotic supplementation, and the interactions on broiler chicken performance and some physiological parameters. A total of 912 one-day-old chickens were used in this study, and they were randomly divided into six groups with 4 replicates each. The experiment included three levels of stocking densities (10, 13, and 15 broiler chicken/m^2) in 6 groups. Groups 1, 3, and 5 were maintained without prebiotic supplementation, while groups 2, 4, and 6 received a diet supplemented with prebiotics in water (1cm/liter). Reducing stocking densities and adding prebiotics improved body weight, feed consumption, feed conversion ratio, hemoglobin, packed cell volume, oxidative stress parameters (total antioxidant capacity), and European production efficiency factor, while decreasing malondialdehyde levels. On the other hand, stocking density and prebiotic supplementation did not affect dressing percentage, the relative weight of giblet parts, hind part, front part, and lymphoid organs (thymus and bursa of Fabricius). In conclusion, adding prebiotics at 1 cm/liter (Mannan-oligo saccharide and B-Glucan) can partially mitigate the negative effects of high stocking density on production performance, physiological and oxidative stress parameters, and European production efficiency factor.

Keywords: Antioxidant biomarkers, Broiler chicken, β-glucan, Mannan oligosaccharide, Oxidant, Prebiotic, Stocking density

INTRODUCTION

Poultry has become an important industry in the economies of countries, and it plays an important role in providing animal protein at reasonable prices compared to meat and fish. The poultry industry has recently produced food with high progress with a decrease in production cost Nasr et al. (2021). Poultry has a fast production cycle and high feed conversion ratio compared to different other types of farm animals except for fish. Broiler chickens should be supplied with the best environmental conditions to fulfill their genetic potential for growth because any flaw in optimal conditions can reduce performance (Feddes et al. 2002). The stocking density is one of the important factors in the poultry industry. Stocking density is an expression of live weight or housed birds per square meter of floor space (Meluzzi and Sirri, 2009). Increasing stocking density had benefits that included increasing income, achieving full use of the available area, and improving productivity Gholami et al. (2020). High environmental temperature is one of the most critical factors that affect broiler chickens' performance. High stocking density may be led to reduced dissipation of body heat to the air due to the reduction of airflow at the level of the bird. Due to high stocking densities, some factors that may decrease performance include poor air quality because of increased ammonia, difficulty access to feed and water, and unsuitable air exchange. Decreasing floor

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space for broiler chickens may reduce feed efficiency, carcass quality, and growth rate and increase mortality (Feddes et al., 2002; Škrbić et al., 2011; Mahrose et al., 2019).

Prebiotics are natural feed supplements that cause many economic advantages by improving broiler chickens' feed efficiency, decreasing mortality rates, and increasing growth rates (Yaqoob et al. 2021). Stress from high stocking densities had a negative effect on microbial population, growth performance, and gut morphology, while the supplementation of prebiotics can reduce the deleterious effect of stress and microbial dysbiosis in the gut of broiler chickens under the condition of high stocking densities (Kridtayopas et al., 2019). A study by Nikpiran et al. (2013b) was conducted to investigate the influence of prebiotics on the performance, blood enzymes, and organ weight of Japanese Quails. They found a diet containing 1 g/kg prebiotic. The pax (yeast cells of *Saccharomyces cerevisiae*) has positive effects on performance. Dietary prebiotics is supposed to be probable important replacements for antibiotic growth promoters in poultry production due to their improvement in productive performance and health status (Froebel et al., 2019). Recently, there was great attention to the use of prebiotics instead the use of antibiotics in the zootechnical sector (Prentza et al. 2022). Using Fermacto® as a prebiotic at a level of 1.6 g/kg in quail's diet improved its growth performance, and it may be due to enhancing digestion, improving intestinal lumen health, and absorption of nutrients by different enzymes (Nikpiran et al. 2014). Different types of oligo and polysaccharides, including mannan-oligosaccharide (MOS), fructo-oligosaccharides (FOS), inulin, galacto-oligosaccharides (GOS), xylo-oligosaccharides (XOS), pyrodextrins, isomalto-oligosaccharides (IMO), lactulose and beta-glucan are generally regarded as prebiotics (Alloui et al., 2013). Adding prebiotics (Aspergillus meal) at a level of 1.6 g/kg to a quail diet has beneficial effects on performance parameters (Babazadeh et al. 2011). This study aimed to determine the effects of different stocking densities and prebiotic supplementation on productive performance, oxidative stress, and physiological parameters.

MATERIAL AND METHODS

Ethical approval

The present study was done and approved by Institutional Animal Care and Use Committee (CU-IACUC) at Cairo University, Cairo, Egypt, under the approval code CU/II/F/42/22.

Study design

A total of 912 unsexed One-day-old Cobb 500 broiler chickens with an average weight of 48 g were used in this study. The trial period lasted from one day of age to slaughter (35 days). The broiler chicks were divided randomly into 6 groups, each group repeated 4 times. The experiment included 3 levels of stocking density (10, 13, and 15 broiler chicken/m^2). The stocking density of groups 1 and 2 was 10 broiler chicken/m^2, groups 3 and 4 were 13 broiler chicken/m^{2,} while groups 5 and 6 were 15 broiler chicken/m^2. Groups 1, 3, and 5 were kept without prebiotic supplementation, while groups 2, 4, and 6 were supplemented with prebiotics in their water 1 cm/liter, Table 1). Feed and water were offered *ad libitum* during the experiment period (35 days). The vaccination program is shown in Table 2. Broiler chickens received starter (1-10 days), grower (11-24 days), and finisher (25-35 days) diets. The compositions of diets are indicated in Table 3.

Broiler chickens were exposed to 23 hours of light, and one hour of dark during the first 3 days, followed by20 hours of light and 4 hours of dark till the end of the experiment, the light intensity was 25 lux for the first 7 days, and then 10 lux after 7 days of age. The brooding temperature was set at 32^{o}C on the first day, and gradually reduced to 24^o C by the end of the third week, then maintained at 24^{o}C until the end of the experiment.

Table 1. Experimental design of the study

Group	Dietary treatment
T1	10 bird/m^2+0 prebiotic
T2	10 bird/m^2+1 cm prebiotic/liter water
T3	13 bird/m^2+0 prebiotic
T4	13 bird/m^2+1 cm prebiotic/liter water
T5	15 bird/m^2+0 prebiotic
T6	15 bird/m^2+1 cm prebiotic/liter water

T: Treatment

Table 2. Vaccination program of broiler chickens (Cobb 500) in the present study

Age (days)	Type	Method	Dose
6	Infectious Bronchities (IB primer) +Newcastle disease (ND-Hitchener B1)	Eye drops	1 dose
10	Newcastle disease (ND- Hitchener B1) + Avian Influenza (H5n3)	Injection under the skin neck	0.5 ml dose
12	Infectious Bursal disease (IBD 78)	Eye drops	1 dose
16	Infectious Bronchities (IBird)	Eye drops	1 dose
18	Newcastle disease (ND-Lasota)	Eye drops	1 dose

Corporation and country made of vaccines: CEVA company, France

Table 3. Composition and calculated analysis of starter, grower, and finisher diets of broiler chickens

Diets	Starter (1-10 days)	Grower (11-24 days)	Finisher (25-35 days)
Ingredients (%)			
Yellow corn	51.2	56.0	61.5
Soya bean meal (46%)	41.3	36.0	30.5
Vegetable oil	3.0	3.5	3.7
Dicalcium phosphate	2.2	2.1	2.1
Limestone	0.8	0.9	0.9
NaCl (salt)	0.4	0.4	0.4
DL-Methionine	0.3	0.3	0.3
Permix*	0.4	0.4	0.3
L-lysine-Hcl	0.2	0.2	0.2
Threonine	0.1	0.2	0.1
Antitoxin	0.1	0	0
Anti-clostridium	0	0	0
Antibiotic	0	0	0
Total	100	100	100
Calculated values			
Crude protein (%)	23.0	21.0	19.0
Crude fat (%)	5.4	6.0	6.4
ME (Kcal/Kg)	2972.7	3056.2	3127.7
Crude fiber (%)	4.1	3.9	3.6
Calcium (%)	0.9	1.0	0.9
Total P (%)	0.8	0.8	0.7
Available P (%)	0.5	0.5	0.5
Lysine (%)	1.4	1.3	1.1
Methionine (%)	0.7	0.6	0.6
Methionine + Cystine (%)	1.0	1.0	0.9
Threonine (%)	1.0	0.9	0.8
Ash (%)	6.3	6.0	5.7

*Each gram premix contained: vitamin A (transretinyl acetate) 9,000 IU; vitamin D3 (cholecalciferol) 2,600 IU; vitamin E (dl-α-tocopheryl acetate) 16 mg; vitamin B1, 1.6 mg; vitamin B2, 6.5 mg; vitamin B6, 2.2 mg; vitamin B12 (cyanocobalamin), 0.015 mg; vitamin K3, 2.5mg; choline (choline chloride) 300 mg; nicotinic acid 30 mg; pantothenic acid (d-calcium pantothenate) 10 mg; folic acid 0.6 mg; biotin 0.07 mg; manganese (MnO) 70 mg; zinc (ZnO) 60 mg; iron ($FeSO_4$ H_2O) 40 mg; copper ($CuSO_4$ $5H_2O$) 7 mg; iodine ($Ca(IO_3)_2$) 0.7 mg; selenium (Na_2SeO_3) 0.3 mg.

Prebiotic

Each liter of prebiotic contains 62.5 g of Mannan-oligo saccharide and 62.5 gm of B-Glucan. Prebiotics were added to the water for 8 hours per day from day 8 until the end of the experiment.

Data collection

Productive performance

The body weight (BW) of the chickens and feed consumption of each group were recorded weekly, and then the Feed Conversation Ratio (FCR) was estimated. European Production Efficiency Factor (EPEF) was calculated at the end of the experiment according to Equation 1:

European Production Efficiency Factor = [Livability % × BW (kg) / Age (d) x FCR] ×100

Carcass characteristics

After 35 days of age, 10 chickens were randomly selected from each batch, weighed, slaughtered, blood filtered, feathered, and then eviscerate. Dressing, front part, hind part, liver, gizzard, heart, spleen, bursa of fabricius, and thymus were weighed, and the relative weight was calculated.

Hematological and oxidative stress parameters

Blood samples were randomly collected at 35 days of age from 10 chickens from each group. The volume of samples was two ml and they were collected from the wing vein, without anticoagulant into a clean centrifuge tube. Three ml of heparinized blood samples were centrifuged at 2500 rpm for 15 minutes (Dacie and Lewis, 1991). Individual plasma samples were stored in a deep freezer at -20^{o}C until the biochemical analysis.

Hemoglobin value and packed cell volume

The hemoglobin concentration (g /100 ml blood) was determined by the spectrophotometer (Jenway, United Kingdom). Packed cell volume was determined by using microhematocrit tubes, blood centrifuged at 4000 rpm/ minute for 10 minutes, and the mean of the reading obtained was recorded (Dacie and Lewis, 1991).

Total antioxidant capacity and malondialdehyde

Plasma samples were assayed for Total antioxidant capacity (T-AOC), and Malondialdehyde (MDA) was determined with a spectrophotometer by Colorimetric Method using commercial detection kits (Diamond Biodiagnostic, Egypt)

Statistical analysis

Enumeration data of the mortality and relative organ weight were tested by the Arcsine transformation method (Roger, 2013). A two-way analysis of variance was used to analyze the data by the least squares procedure of the general linear model (GLM) of SAS program (SAS, 2014). Sources of variation were stocking density and prebiotic supplementation The separation of means was done using Duncan's multiple range test (Duncan, 1955) for comparisons among the significant means ($p < 0.05$).

RESULTS AND DISCUSSION

Productive performance

Live body weight

The results of the present study in Table 4 showed that there was a negative relationship between stocking density and BW throughout the experimental period. The low density had a significantly ($p < 0.05$) higher BW than those of medium and high density. Significant differences ($p < 0.05$) in BW were observed between medium and high density at one, four, and five weeks of age, while at two and three weeks of age, no significant differences were observed ($p < 0.05$). Kridtayopas et al. (2019) consider high stocking density as a stress factor which in turn leads to reduced growth performance, gut bacteria, and intestinal morphology. It was reported that high stocking density could decrease final body weight at 42 days (Sekeroglu et al., 2009). In the same line, Tong et al. (2012) found that increasing stocking density decreased body weight and weight gain. Likewise, Ali (2013) obtained that the highest body weight and daily body weight gain were recorded in the lowest density.

The current results also explain that prebiotic supplementation significantly improved body weight throughout the experimental period ($p < 0.05$). This result agrees with Chae et al. (2006), who reported that weight gain improved due to adding β-glucan supplementation to the broiler chicken diet. In addition, Abdel-Hafeez et al. (2017) reported that birds fed a diet supplemented with prebiotics had a greater body weight. Xu et al. (2003) indicated that feeding broilers diet containing 0.4% fracto-oligosaccharieds (FOS) increased the average daily gain. In another study, Shendare et al. (2008) recorded that the BW of broiler chickens fed a diet with 0.01% mannan-oligosaccharide (MOS) was higher as compared to the control group. Likewise, El-Sheikh et al. (2009) reported that adding 0.2% MOS to the diet of Mandarah hens increased BW and BWG. Tavaniello et al. (2018) found that prebiotics increased broiler's BW irrespective of 3 different routes of delivery (*in ovo*, in water, and *in ovo* + in water) as compared to the control group. In addition, Nikpiran et al. (2014) reported that using Fermacto® as a prebiotic at a level of 1.6 g/kg in quail's diet had increased body weight as compared to the control group. Nikpiran et al. (2013b) found that body weight was higher than the control group when they added 1 g/kg Thepax (prebiotic) to the Japanese Quails diet. Babazadeh et al. (2011) reported that the body weight of quail that fed a diet containing 1.6 g/kg (*Aspergillus* meal) as a prebiotic at a level of was higher than the control group.

Feed consumption

The results of the current study showed that stocking density and the interaction with prebiotics had a significantly higher ($p < 0.05$) weekly and total feed consumption during the experiment period, while prebiotics improved weekly feed consumption for the second, third weeks, and the total feed consumption (Table 5). The Lower stocking density resulted in a significant increase ($p < 0.05$) in weekly feed consumption at one and five weeks of age, while at three and four weeks of age, medium groups consumed the highest amount of feed, on the other hand, during the second week the high-density group consumed significantly more feed as compared with other groups ($p < 0.05$). The interaction effect showed that adding 1 cm β-glucan+MOS /liter drinking water had a contradicting effect on daily feed consumption.

These results agree with previous studies by Dozier et al. (2005); Dozier et al. (2006) reported that feed intake was negatively affected by increasing stocking density (Dozier et al., 2005; Dozier et al., 2006). In the same line, Tong et al. (2012) reported that daily feed intake reduced significantly as density increased. Likewise, Cengiz et al. (2015) indicated that feed intake was significantly decreased at high stocking density (20 birds/m^2) as compared with low stocking density (10 birds/m^2). Heidari et al. (2018) found that feed intake had reduced due to increasing stocking density from 12 to 18 birds/m^2. Recently, Miao et al. (2021) demonstrated that high stocking density (20 birds/m^2) significantly decreased feed intake as compared with low stocking density 14 birds/m^2.

The current results indicated that prebiotic supplementation did not affect feed consumption. These results are in agreement with Benites et al. (2008), who found that the addition of Mannan Oligosaccharide from Bio-Mos (1.0 kg/ton or 0.5 kg/ton) and SAF-Mannanto (0.5 kg/ton) in broiler's diets had no significant effect on feed consumption. Also, Sohail et al. (2013) reported that 0.5% MOS had no significant effect on feed consumption when the broilers were reared under heat stress. Güçlü (2011) fed a Japanese quail diet for 12 weeks with different levels of MOS 0.5 and 1 kg/ton, and they found that prebiotics had no significant effect on feed consumption. Likewise, Iqbal et al. (2017) fed Japanese quail diets supplemented with 0.25%, 0.50%, and 1.0% MOS for 15 weeks, and they found no significant effect on feed intake. And disagree with Piray et al. (2007), who showed that prebiotics increased feed intake during 0-42 days of age. Likewise, it was reported that dietary prebiotic supplementation of 0.5 g mannan oligosaccharide/kg from the first day of age until 42 days of age improved feed consumption (Bozkurt et al., 2009). Abdel-Raheem et al. (2011) reported that cumulative feed intake had increased for birds fed a diet with MOS 2 g/kg in the starter phase and 0.5 g/kg in the grower phase compared to control groups. Also, Rehman et al. (2020) reported that using MOS as a prebiotic in broiler chickens' diet with different levels (0, 1, and 1.5 g/kg) had improved feed intake.

Feed conversion ratio

The current results explained that increasing density resulted in a negative effect on the commutative feed conversion ratio, the best value was recorded when stocking density was 10 birds/m^2 at four and five weeks of age (Table 6). The interaction showed that the low-density group supplemented with prebiotics had the best FCR compared with other groups. The current results agree with the finding of Guardia et al. (2011), who reported that increasing stocking density from 12 to 17 birds/m^2 had a negative effect on FCR and reduced growth performance. Palizdar et al. (2017) found that increasing stocking density had increased FCR, which was the highest value at 21.3 birds/m^2. Abo-Ghanima et al. (2021) indicated that total FCR increased due to increasing stocking density from 25 to 40 kg/m^2.

The current results also explained that cumulative FCR was improved when birds were supplemented with prebiotics. Bozkurt et al. (2009) reported that improving feed conversion was due to stimulated growth of the beneficial microflora in the GIT induced by prebiotic 0.5 g mannan oligosaccharide/kg. Nikpiran et al. (2013a) observed an improvement in broiler performances and FCR due to adding prebiotic Turbo Tox® (1 g/kg) to the diet. In the same line, Waqas et al. (2019) observed an improvement in FCR due to adding MOS 0.2, 0.4, and 0.6 g/Kg. Likewise, Abd-Elsamee et al. (2021) reported that FCR significantly improved when broilers fed diets supplemented with a combination of β-glucan + MOS from yeast or mushroom at rates of 0.01%, 0.02% and 0.03% as compared to control. Nikpiran et al. (2014) reported that FCR had improved due to using Fermacto® as a prebiotic at a level of 1.6 g/kg in the quail's diet as compared to the control group. Nikpiran et al. (2013b) found that FCR had improved due to adding 1 g/kg Thepax to the Japanese Quails diet. Adding prebiotics (*Aspergillus* meal) at a 1.6 g/kg level to a quail diet improved FCR (Babazadeh et al. 2011).

Table 4. Least-square means ± SE of broiler chickens' body weight affected by density, prebiotic, and their interaction at day 35 of age

Items	BW0 (g)	BW1 (g)	BW2 (g)	BW3 (g)	BW4 (g)	BW5 (g)
Density						
10 birds/m^2	49.92 ± 0.27	218.61 ± 1.54^a	518.47 ± 4.44^a	1022.29 ± 8.87^a	1578.21 ± 12.64^a	2190.98 ± 21.69^a
13 birds/m^2	48.80 ± 0.23	209.06 ± 1.24^b	504.94 ± 3.00^b	1004.16 ± 6.09^b	1530.59 ± 9.08^b	2011.90 ± 17.66^b
15 birds/m^2	49.62 ± 0.21	203.02 ± 0.94^c	510.48 ± 2.36^b	1007.85 ± 4.46^b	1483.01 ± 6.22^c	1842.39 ± 13.19^c
Prebiotic						
0 Prebiotic	49.37 ± 0.19	208.36 ± 1.03^b	506.84 ± 2.91^b	1008.60 ± 5.37^b	1514.94 ± 6.21^b	1943.32 ± 14.34^b
1cm/liter water Prebiotic	49.48 ± 0.19	210.02 ± 1.02^a	514.64 ± 2.18^a	1012.29 ± 4.78^a	1533.63 ± 8.62^a	2041.18 ± 15.96^a
Interaction						
10 birds/m^2*0 Prebiotic	49.97 ± 0.36	217.58 ± 2.22^a	511.03 ± 7.70^b	1004.33 ± 14.18^c	1543.46 ± 12.47^b	2188.75 ± 28.28^b
10 birds/m^2*1cm/liter water	49.88 ± 0.40	219.65 ± 2.15^a	525.91 ± 4.36^a	1040.25 ± 10.46^a	1634.96 ± 20.80^a	2203.32 ± 33.00^a
13 birds/m^2*0 Prebiotic	48.70 ± 0.35	206.97 ± 2.00^c	501.36 ± 4.59^c	1005.30 ± 8.48^c	1537.43 ± 11.66^c	1887.24 ± 21.67^d
13 birds/m^2*1cm/liter water	48.91 ± 0.32	211.15 ± 1.47^b	508.51 ± 3.87^b	1003.03 ± 8.77^c	1523.75 ± 13.94^d	2137.38 ± 23.97^c
15 birds/m^2*0 Prebiotic	49.55 ± 0.28	203.38 ± 1.10^d	508.66 ± 3.46^b	1014.22 ± 6.46^b	1491.61 ± 8.52^f	1832.50 ± 17.85^f
15 birds/m^2*1cm/liter water	49.70 ± 0.31	202.66 ± 1.54^d	512.30 ± 3.21^b	1001.47 ± 6.14^c	1474.42 ± 9.04^e	1852.23 ± 19.43^e

$^{a-f}$ Mean, within a column, with different superscripts are significantly different ($p < 0.05$)

Table 5. Least-square means ± SE of broiler chickens' weekly feed intake affected by stocking density, prebiotic, and their interaction at day 35 of age

Items	FW1 (g)	FW2 (g)	FW3 (g)	FW4 (g)	FW5 (g)	TF (g)
Density						
10 birds/m^2	196.37 ± 1.37^a	407.82 ± 4.71^b	738.37 ± 7.95^b	952.63 ± 11.38^b	995.81 ± 27.37^a	3094.68 ± 42.46^a
13 birds/m^2	179.87 ± 1.04^b	408.62 ± 1.64^b	753.08 ± 3.88^a	968.96 ± 11.62^a	949.90 ± 32.14^b	3080.56 ± 41.74^a
15 birds/m^2	177.12 ± 0.39^b	431.35 ± 2.86^a	697.37 ± 7.47^c	931.77 ± 15.24^c	911.05 ± 34.60^c	2971.55 ± 50.60^b
Prebiotic						
0 Prebiotic	184.58 ± 2.90	412.44 ± 5.04^b	726.77 ± 10.67^b	952.19 ± 10.37	946.28 ± 29.02	3037.71 ± 40.81^b
1cm/liter water Prebiotic	184.33 ± 2.45	419.42 ± 2.82^a	732.45 ± 6.52^a	950.05 ± 12.05	958.22 ± 25.17	3060.15 ± 38.10^a
Interaction						
10 birds/m^2*0 Prebiotic	197.50 ± 2.32^a	399.62 ± 6.93^e	737.70 ± 13.19^c	944.77 ± 18.69^c	1002.25 ± 41.64^a	3084.38 ± 62.52^b
10 birds/m^2*1cm/liter water	195.25 ± 1.60^a	416.02 ± 3.28^b	739.05 ± 11.01^c	960.50 ± 14.65^b	989.37 ± 41.66^b	3104.98 ± 66.59^a
13 birds/m^2*0 Prebiotic	178.75 ± 1.93^c	406.02 ± 2.15^d	757.72 ± 1.08^a	971.60 ± 22.25^a	946.67 ± 50.88^c	3082.03 ± 72.51^b
13 birds/m^2*1cm/liter water	181.00 ± 0.70^b	411.22 ± 1.85^c	748.45 ± 7.41^b	966.32 ± 11.45^a	953.12 ± 47.17^c	3079.10 ± 53.58^b
15 birds/m^2*0 Prebiotic	177.50 ± 0.28^c	431.67 ± 5.97^a	684.90 ± 11.65^e	940.20 ± 12.13^c	889.92 ± 53.27^e	2946.73 ± 70.83^d
15 birds/m^2*1cm/liter water	176.75 ± 0.75^d	431.02 ± 1.62^a	709.85 ± 4.62^d	923.35 ± 29.84^d	932.17 ± 49.52^d	2996.38 ± 80.75^c

[a-e]Mean, within a column, with different superscripts are significantly different ($p < 0.05$). FW: Weekly feed intake, TF: Total feed intake.

Table 6. Least-square means ± SE of broiler chickens' weekly cumulative feed conversion ratio affected by stocking density, prebiotic, and their interaction at day 35 day of age

Items	FCR1	FCR2	FCR3	FCR4	FCR5
Density					
10 birds/m^2	0.90 ± 0.01^a	1.15 ± 0.01^b	1.31 ± 0.01^a	1.44 ± 0.02^b	1.50 ± 0.02^c
13 birds/m^2	0.86 ± 0.01^b	1.16 ± 0.01^b	1.33 ± 0.01^a	1.51 ± 0.01^a	1.62 ± 0.04^b
15 birds/m^2	0.87 ± 0.01^b	1.19 ± 0.01^a	1.29 ± 0.01^b	1.50 ± 0.01^a	1.71 ± 0.01^a
Prebiotic					
0 Prebiotic	0.88 ± 0.01	1.17 ± 0.01	1.31 ± 0.01	1.49 ± 0.01	1.64 ± 0.03^a
1cm/liter water Prebiotic	0.87 ± 0.01	1.17 ± 0.01	1.31 ± 0.01	1.48 ± 0.01	1.58 ± 0.03^b
Interaction					
10 birds/m^2*0 Prebiotic	0.91 ± 0.01^a	1.14 ± 0.01^c	1.32 ± 0.02^b	1.48 ± 0.03^b	1.50 ± 0.03^c
10 birds/m^2*1cm/liter water	0.89 ± 0.01^b	1.16 ± 0.01^b	1.30 ± 0.01^b	1.41 ± 0.01^c	1.50 ± 0.03^c
13 birds/m^2*0 Prebiotic	0.86 ± 0.01^d	1.16 ± 0.01^b	1.34 ± 0.01^a	1.50 ± 0.01^b	1.73 ± 0.03^a
13 birds/m^2*1cm/liter water	0.85 ± 0.01^d	1.16 ± 0.01^b	1.33 ± 0.01^a	1.51 ± 0.01^a	1.52 ± 0.01^c
15 birds/m^2*0 Prebiotic	0.87 ± 0.01^c	1.20 ± 0.03^a	1.27 ± 0.01^c	1.49 ± 0.01^b	1.70 ± 0.01^b
15 birds/m^2*1cm/liter water	0.87 ± 0.01^c	1.18 ± 0.01^a	1.31 ± 0.01^b	1.52 ± 0.02^a	1.71 ± 0.04^a

[a-d] Mean, within a column, with different superscripts are significantly different ($p < 0.05$). FCR: feed conversion ratio

Table 7. Least-square means ± SE of broiler chickens' carcass traits affected by stocking density, prebiotic, and their interaction at 35 day of age

Items	Dressing W (%)	Front part (%)	Hind part (%)	Liver W (%)	Heart W (%)	Gizzard W (%)
Density						
10 birds/m^2	71.24 ± 0.57	41.58 ± 0.78	29.70 ± 0.76	2.40 ± 0.07	0.46 ± 0.02	1.99 ± 0.06
13 birds/m^2	70.84 ± 0.53	41.11 ± 0.87	29.79 ± 0.61	2.62 ± 0.07	0.5 ± 0.02	2.01 ± 0.07
15 birds/m^2	71.44 ± 0.61	40.85 ± 0.63	30.59 ± 0.33	2.47 ± 0.11	0.48 ± 0.02	2.15 ± 0.12
Prebiotic						
0 Prebiotic	71.37 ± 0.46	41.31 ± 0.56	29.94 ± 0.52	2.43 ± 0.05	0.48 ± 0.01	2.04 ± 0.05
1cm/liter water Prebiotic	70.98 ± 0.47	41.05 ± 0.67	30.11 ± 0.45	2.57 ± 0.08	0.48 ± 0.01	2.07 ± 0.09
Interaction						
10 birds/m^2*0 Prebiotic	72.14 ± 0.85	42.40 ± 1.35	29.18 ± 1.41	2.43 ± 0.12	0.47 ± 0.04	2.01 ± 0.07
10 birds/m^2*1cm/liter water	70.34 ± 0.70	40.75 ± 0.77	30.22 ± 0.64	2.36 ± 0.07	0.45 ± 0.01	1.98 ± 0.11
13 birds/m^2*0 Prebiotic	70.38 ± 0.52	40.48 ± 0.63	30.16 ± 0.65	2.57 ± 0.07	0.49 ± 0.01	2.14 ± 0.09
13 birds/m^2*1cm/liter water	71.30 ± 0.94	41.75 ± 1.64	29.42 ± 1.07	2.68 ± 0.14	0.51 ± 0.04	1.89 ± 0.10
15 birds/m^2*0 Prebiotic	71.58 ± 0.92	41.05 ± 0.83	30.47 ± 0.37	2.29 ± 0.08	0.49 ± 0.02	1.96 ± 0.12
15 birds/m^2*1cm/liter water	71.29 ± 0.85	40.64 ± 0.99	30.70 ± 0.57	2.66 ± 0.20	0.48 ± 0.03	2.34 ± 0.20

[a-b] Mean, within a column, with different superscripts are significantly different ($p < 0.05$). W: Weight

Table 8. Least-square means ± SE of broiler chickens' lymphoid organs affected by density, prebiotic, and their interaction at day 35 of age

Items	Spleen W (%)	Thymus W (%)	Bursa of fabricius W (%)
Density			
10 birds/m^2	0.11 ± 0.007	0.79 ± 0.02^c	0.08 ± 0.007
13 birds/m^2	0.12 ± 0.008	0.93 ± 0.02^b	0.09 ± 0.005
15 birds/m^2	0.12 ± 0.007	0.99 ± 0.01^a	0.11 ± 0.002
Prebiotic			
0 Prebiotic	0.12 ± 0.005	0.90 ± 0.02	0.09 ± 0.004
1cm/liter water Prebiotic	0.12 ± 0.006	0.91 ± 0.02	0.10 ± 0.005
Interaction			
10 birds/m^2*0 Prebiotic	0.12 ± 0.001	0.78 ± 0.03	0.08 ± 0.001
10 birds/m^2*1cm/liter water	0.11 ± 0.006	0.80 ± 0.03	0.08 ± 0.009
13 birds/m^2*0 Prebiotic	0.12 ± 0.001	0.93 ± 0.03	0.09 ± 0.005
13 birds/m^2*1cm/liter water	0.11 ± 0.001	0.92 ± 0.02	0.10 ± 0.001
15 birds/m^2*0 Prebiotic	0.11 ± 0.004	0.98 ± 0.02	0.10 ± 0.002
15 birds/m^2*1cm/liter water	0.13 ± 0.001	1.01 ± 0.02	0.11 ± 0.004

$^{a-b}$ Mean, within a column, with different superscripts are significantly different ($p < 0.05$). W: Weight.

Carcass traits

The results of carcass traits in Table 7 showed that neither stocking density nor prebiotic supplementation affect the relative weight of all carcass trait parameters in the current experiment. The current results are in agreement with those of Thomas et al. (2004), who reported that stocking density did not affect the carcass traits significantly. In addition, Sekeroglu et al. (2011) indicated that stocking density had no significant effect on the percentage of carcass yield. On the other hand, Dozier et al. (*2006)*; Onbasilar et al. (2008) reported that high stocking densities had a negative effect on final body weight. On the other hand, Yalçınkaya et al. (2012) found that adding 1 g/kg MOS to the broiler diet did not affect the percentage of carcass yield. However, Tavaniello et al. (2018) reported that prebiotics positively affected breast muscle weight and yield and can positively affect carcass traits and meat quality. Piray et al. (2007) revealed a positive effect on carcass quality when broiler chickens were fed prebiotic fermacto at the level of 1.5 and 3 g/kg, compared to the control group. Ahiwe et al. (2019) reported that adding yeast mannan (YM) at 0.15 or 0.20 g/kg to broiler chickens' diet significantly improved the dressing percentage as compared to the control group. The results of Simitzis et al. (2012) demonstrated that the stocking density of 13 birds/m^2 had a lower liver weight than that of 6 birds/m^2. Sekeroglu et al. (2011) reported that stocking density did not significantly affect liver or heart weights. Waqas et al. (2019) reported that the weight of the liver, gizzard, and heart was higher in birds receiving 0.6 g/Kg of MOS than those of the control group. Waqas et al. (2019) found that gizzard, heart, and liver percentages were increased in birds fed 0.6 g/Kg of MOS compared to those fed a control diet. Also, Rehman et al. (2020) found that using three levels of MOS in a broiler's diet had significant effects on liver, heart, and gizzard weights.

Lymphoid organs

The results of lymphoid organs in Table 8 demonstrated that neither stocking density nor prebiotic supplementation affected the relative weight of the spleen and bursa of Fabricius. However, the relative weight of the thymus was significantly increased with the increase in stocking density ($p < 0.05$). Houshmand et al. (2012) reported that the spleen and bursa of Fabricius weights did not significantly affect by stocking density. Likewise, Azzam and El-Gogary (2015) reported that stocking density had no significant effect on the bursa of Fabricius or spleen weights. Thymus weight did not affect by increasing stocking density, and the highest weight was for the medium density 20.35 g, while low and high density was 19.20 and 19.85 g, respectively. The results agree with Tong et al. (2012), who reported that different stocking densities had no significant effect on thymus weight. Thymus weight did not affect by increasing stocking density, and the highest weight was for the medium density 20.35 g, while low and high density was 19.20 and 19.85 g, respectively. Qaid et al. (2016)

reported that the relative weights of the thymus did not affect by stocking density. In addition, Houshmand et al. (2012) reported that adding prebiotics (Bio-Mos) to the starter and finisher diets at 2 and 1 g/kg, respectively, had no significant effect on the spleen or bursa of Fabricius weights. Guo et al. (2003) reported that adding β-Glucan to the diet had increased the relative weights of the spleen, thymus, and bursa as compared to the control. Usama et al. (2018) added β-Glucan + MOS to the diet, had increased the percentage of lymphoid organs. Chand et al. (2019) obtained that the relative weight of the thymus, spleen, and bursa of Fabricius was significantly increased due to adding 100 g/kg MOS to the broiler diet as compared to the control group. On the other hand, Simitzis et al. (2012) reported that increasing stocking density from 6 to 13 birds/m^2 decreased spleen weight and high density had lower weights of spleen and bursa of Fabricius than those in low density. In addition, Ali (2013) reported that high stocking density had reduced the bursa of Fabricius weight and its percentage at day 42 of age.

Hematological parameters

Hemoglobin and packed cell volume

The hemoglobin concentration of chickens at medium density was significantly higher than those of the other densities ($p < 0.05$, Table 9). On the other hand, birds at low density had significantly the lowest value ($p < 0.05$). These results disagreed with Sekeroglu et al. (2011), who found that stocking density had no significant effect on hemoglobin.

The current study showed that supplementation significantly increased the Hg value from 7.05 g/dl to 6.24 g/dl, compared to the control group. The interaction between the density and prebiotic showed that all densities had been affected by adding the prebiotic supplement and had a higher value than those un-supplemented group. The results agreed with those of Mohammed et al. (2016) and Oni et al. (2020) reported that prebiotic supplementation improved hemoglobin value compared to the control group.

There was a significant effect due to stocking density and their interaction on PCV, while prebiotics had no significant effect as compared to low-density and un-supplemented groups. Broiler chickens at high and low stocking density almost had the same PCV value, and it was higher than the medium density value ($p < 0.05$). In the present study, the interaction showed that adding prebiotics to low and high-density birds improved PCV values. AL-Kassie et al. (2008) reported that when the prebiotic was added to the broiler chickens' diet at 10 g/kg increased the percentage of PCV at day 42 of age. Muhammad et al. (2020) and Tarabees et al. (2021) found that adding Isomalto-oligosaccharides 0.5 g/kg to broiler chickens' diet had no significant effect on hemoglobin or PCV.

Oxidative stress parameters

Total antioxidant capacity

Stocking density, prebiotic, and their interaction had a significant effect on TAOC in chickens reared in low density which had the significantly highest level of TOAC followed by those at high and then medium density ($p < 0.05$, Table 9). This result is in agreement with those reported by Zhao et al. (2009) and Cai et al. (2019), that stress caused by high stocking density influenced the antioxidant status of broiler chickens. The results of Wang et al. (2021) demonstrated that adding 200 mg/Kg apple pectic oligosaccharide to breeder chickens' diet increased TAOC values.

Malondialdehyde

The results showed that MDA raised due to the increase in stocking density. Chickens in low density had significantly the lowest value, while MDA in those in medium and high density had almost high concentrations ($p < 0.05$). These results agreed with Simsek et al. (2009), who reported that stocking density of up to 22 birds/m^2 may lead to oxidative stress and MDA increased when the stocking density increased.

On the other hand, the results demonstrated that prebiotic supplementation had no effect on MDA ($p < 0.05$). The interaction between density and prebiotic refers to contradicted results. The results were in agreement with those of Tarabees et al. (2021), who found that prebiotic Isomalto-oligosaccharides had no significant effect on MDA. However, Zhou et al. (2019) noted a decrease in MDA content when they added MOS at levels of 0.5, 1, and 1.5 g/kg to the diet.

European production efficiency factor

The best significant value of EPEF was found at the low density of (415.83) flowed by those in 13 bird/m^2 and then in 15 bird/m^2 ($p < 0.05$, Table 10). Gholami et al. (2020) conducted a study to evaluate the effect of stocking density on broiler chicken performance and economic profit. They found that the stocking density significantly affected economic income when the study included four densities of 10, 15, 17, and 20 chicks/m2, and the highest earnings were a density of 20 chicks/m2. Nasr et al. (2021) studied the impact of stocking density on growth performance to recommend a better density with low production cost and high quality. They found that the medium density of 18 birds/m^2 revealed better performance and the best from the economic point of view than the high density of 20 birds/m^2. Adding prebiotics to the broiler chicken diet positively affected the European production efficiency factor and increased the value to 372.25 compared with the control 341.24. Lowry et al. (2005) reported that using β-glucan as a prebiotic in the diet for broilers challenged with *Salmonella* had decreased mortality rate and economic loss for broiler chickens. Waqas et al. (2019) added different levels of MOS 0.2, 0.4, and 0.6 g/Kg to broiler chicken's diet. They found that the broilers diet with 0.6 g/kg MOS led to the best profit percentage per 1 Kg of meat as compared with the control. In addition, Ibrahim et al. (2021) reported that adding prebiotic AGRIMOS (a high source of mannan oligosaccharides and β glucans) at a level of 0.1% to broiler chicken diets low in protein (95, 90, and 85% of NRC) has a useful effect on economic value.

Table 9. Least-square means ± SE of hematological and oxidative stress parameters affected by broiler chickens' density, prebiotic, and their interaction at day 35 of age

Items	Hg (g/dL)	PCV (%)	TAOC (mM / L)	MDA (nmol/ml)
Density				
10 birds/m^2	5.53 ± 0.24^c	28.05 ± 1.40^a	0.32 ± 0.02^a	16.81 ± 1.81^b
13 birds/m^2	7.47 ± 0.37^a	26.80 ± 0.97^b	0.12 ± 0.01^c	18.51 ± 0.75^a
15 birds/m^2	6.92 ± 0.27^b	28.45 ± 0.84^a	0.14 ± 0.01^b	18.06 ± 1.40^a
Prebiotic				
0 Prebiotic	6.24 ± 0.25^b	27.36 ± 0.96	0.16 ± 0.02^b	17.80 ± 1.22
1cm/liter water Prebiotic	7.05 ± 0.30^a	28.16 ± 0.82	0.23 ± 0.02^a	17.78 ± 1.04
Interaction				
10 birds/m^2*0 Prebiotic	5.01 ± 0.26^c	25.50 ± 2.45^d	0.27 ± 0.02^b	14.57 ± 2.33^c
10 birds/m^2*1cm/liter water	6.06 ± 0.35^b	30.60 ± 0.90^a	0.38 ± 0.03^a	19.05 ± 2.69^a
13 birds/m^2*0 Prebiotic	7.17 ± 0.39^a	27.30 ± 1.21^c	0.09 ± 0.01^d	19.12 ± 0.89^a
13 birds/m^2*1cm/liter water	7.78 ± 0.63^a	26.30 ± 1.57^c	0.15 ± 0.02^c	17.89 ± 1.25^b
15 birds/m^2*0 Prebiotic	6.54 ± 0.35^b	29.30 ± 0.84^b	0.14 ± 0.02^c	19.71 ± 2.49^a
15 birds/m^2*1cm/liter water	7.31 ± 0.41^a	27.60 ± 1.45^c	0.14 ± 0.01^c	16.40 ± 1.19^b

$^{a-b}$ Mean, within a column, with different superscripts are significantly different ($p < 0.05$). TAOC: Total antioxidant capacity, MDA: Malondialdehyde, Hg: Hemoglobin, PCV: Packed cell volume

Table 10. Least-square means ± SE of European production efficiency factor affected by broiler chickens' density, prebiotic, and their interaction at 35th day of age

Items	EPEF (%)
Density	
10 birds/m^2	415.83 ± 15.44^a
13 birds/m^2	351.09 ± 19.30^b
15 birds/m^2	303.32 ± 10.51^c
Significant level	$<.0001^{***}$
Prebiotic	
0 Prebiotic	341.24 ± 17.46^b
1cm/liter water Prebiotic	372.25 ± 18.54^a
Significant level	0.0565^*
Interaction	
10 birds/m^2*0 Prebiotic	414.45 ± 19.06^a
10 birds/m^2*1cm/liter water	417.22 ± 27.36^a
13 birds/m^2*0 Prebiotic	307.23 ± 16.37^c
13 birds/m^2*1cm/liter water	394.96 ± 13.71^b
15 birds/m^2*0 Prebiotic	302.04 ± 6.39^c
15 birds/m^2*1cm/liter water	304.59 ± 21.77^c
Significant level	0.0530^*

$^{a-c}$ Mean, within a column, with different superscripts are significantly different ($p < 0.05$). EPEF: European Production Efficiency Factor

CONCLUSION

It can conclude that adding prebiotics (Mannan-oligo saccharide and B-Glucan) can partially ameliorate the adverse effect of high stocking density on productive performance, physiological and oxidative stress parameters, and European production efficiency factor.

DECLARATION

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Authors' contribution

Essam Mohamed Hassan Karar collected the data, performed the data analysis, and wrote the manuscript draft. Abdel-Rahman Mohamed Mohamed Atta for the study idea, designed the study and revised the manuscript. Mohamed Abdel-Rahman Abdel-Hamed El-Menawey was responsible for the scientific material collection used in the experiment and revised the manuscript. Hassan Bayoumi Ali Gharib was responsible for the laboratory analysis. All authors have read and approved the final data and manuscript.

Availability of data and materials

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author/s.

Competing interests

All research authors agree to publish this research and do not have any conflict of interest.

Ethical consideration

This research was truthful and did not plagiarize or pattern any other papers or ideas. Any fabrication or falsification did not find in this research. This article or any scientific results did not submit to any journals.

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