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EFFECT OF *AZOLLA AND* PROBIOTIC FEEDING ON BROILERS PERFORMANCE, AND BLOOD PARAMETER TRAITS

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ABSTRACT: The present study was conducted to investigate the effect of feeding different levels of Azolla and probiotics on growth performance and some blood biochemical of Indian River (IR) chickens. The study was conducted on 300 IR, which was randomly divided into ten treatment groups each with three replicates of 10 birds. The first treatment (T1) was fed a diet without Azolla while, Azolla 5% (T2), 10% (T3), 15% (T4), and 20% (T5), however birds in group 6 (T6) fed diet without Azolla and in drink water with 10^8 *Enterococcus faecalis*, Azolla 5% with 10^8 *Ent* (T7), 10% with 10^8 *Ent* (T8), 15% with 10^8 *Ent* (T9) and 20% with 10^8 *Ent* (T10). The experiment lasted for 35 days then all animals were slaughtered. data indicated that body weight, body weight gain, feed intake and feed conversion ratio and liver functions (TP, ALT, AST, and ALP), lipids profile on cholesterol, triglycerides, and high density lipoprotein and low density lipoprotein, kidney function (uric acid and creatinine). The obtained results showed that, a treated group with T6 Entero ,(T2) 5%AZ, (T3) 10%AZ, (T7) 5%AZ+Ent and (T8) 10%AZ+Ent improve performance parameters with significantly increase in BW, WG ,DWG and FI and significant decrease of FCR with the treated group with (T2) 5%AZ, (T3) 10%AZ, T6 Entero , (T7) 5%AZ+Ent and (T8) 10%AZ+Ent. As a result, the treated group with T2 (5% azolla), T3 (10% azolla), T6 (Entero), T7 (5%azolla +Entero) and T8(10% azolla +Entero) are the best treatment for growth performance and biochemical parameter.

Keywords: Azolla pinnata, blood biochemical, the haematology, immune competence traits.

INTRODUCTION

Global chicken production has increased dramatically during the past few decades. Due to competition with traditional human food supplies, this increase has caused a shortage and raised the price of traditional feed ingredients. **(**Thirumalaisamy et al., 2016). Therefore, studies on the utilization of unconventional feedstuff as poultry feed ingredient have drawn the attention of scientists throughout the world. FAO programs focus on increasing the feed base production systems to locally available feed resources in developing countries (Sansoucy et al., 1985; and Thirumalaisamy et al., 2016).

Azolla is a rich source of essential amino acids. Minerals including calcium, phosphorus, magnesium, potassium, iron, and zinc are all abundant in Azolla. Animals are fed Azolla because of its nutritious qualities. (Parthasarathy et al., 2003; Reddy, 2011; Chatterjee et al., 2012).

Probiotics are live microorganisms in the development of beneficial intestinal flora. For the purpose of helping to combat harmful organisms such Salmonella or E. coli, beneficial bacteria (such as those from the genera Lactobacillus acidophilus, Bifidbacterium, and Enterococcus) block receptors on the gut wall, generate antimicrobial substances, and enhance the immune system. (Richards *et al.*, 2005). Probiotics' manner of action is undefined. The probiotic's direct effect on the pathogenic microbial species in the intestine or its promotion of the good bacteria's growth may both be factors that contribute. (Parvez *et al.*, 2018). Recently, natural probiotic act as a natural growth promoters or non-antibiotic growth promoters. They are commonly regarded as favorable alternatives to antibiotic growth promoters in livestock production. The main advantage of natural growth promoters is to low risk regarding bacterial resistance or undesired residues in broiler chick's products such as meat, milk or eggs (Männer, 2011).

The current study's goal was to investigate the effect of fed Azolla on growth performance, and some blood parameter of broilers.

MATERIALS AND METHODS

The experimental work of the present study was carried out in the privet farm at Moshtohor

and Laboratories belonging to Regional Center for Food & Feed with the cooperation of Departments of Animal Production, Faculty of Agriculture, Benha University, Egypt.

Experiment birds and treatments:

300 male broilers that are one day old Arbor Acres chicks were weighed equally and randomly divided and distributed in ten dietary treatments groups having three replicate in each. Each dietary treatment group consists of 30 chicks distributed in three replicated pens, with 10 chicks in each. The chicks were maintained on a 24 hours consistent lighting schedule and proper ventilation was ensured. The birds in the control group (T1) were fed diet without Azolla while, Azolla 5% (T2), 10% (T3), 15% (T4), 20% (T5), however birds in group 6 (T6) fed diet without Azolla and drink water with 10^8 *Enterococcus faecalis*, from hatching day to the end of experimental work, Azolla 5% with 10^8 *Enterococcus faecalis* (T7), 10% with10^8 *Enterococcus faecalis* (T8), 15% with10^8 *Enterococcus faecalis* (T9)

and 20% with10^8 *Enterococcus faecalis* (T10). The experiment lasted for 35 days then all animals were slaughtered. The chicks in all treatments were: kept under similar hygienic and environmental conditions, vaccinated against Newcastle and Gumboro diseases, housed in the floor with wire border under continuous fluorescent lighting (10 watt/m^2), and provided on un-medicated corn soybean-based meal diet (containing no added antibiotics, coccidiostats, or growth promoters) and water *ad libitum.*

Experimental diets:

Probiotic strains:

Strains of *Enterococcus Faecalis* used in this study. These strains were isolated, purified, identified, stored and kindly supplied by Food Safety and Biotechnology Laboratory, Regional Center for Food and Feed, A.R.C., Giza, Egypt.

Blood samples:

At the conclusion of the trial, 35 days after slaughter, blood samples were taken. Blood samples from each bird were collected in clear centrifuge tubes and kept at room temperature for 1 1/2 hours; after centrifuging at 3500 rpm for 20 minutes, the clear serum supernatant layer was carefully removed and stored at -20 oC until further analysis (using a Universal-32 centrifuge).

The studied traits:

Productive traits:

Live body weight (LBW):

Data were recorded on individual chicks for live body weight (LBW, g) at hatch, 3 wks. and 5 wks. of age, during the experimental period.

Body weight gain (BWG):

Body weight gain was individually calculated according to the following formula suggested by Broody (1949): Weight gain =W2- W1 Where: W1 and W2 individual body weight at the low successive Period.

Daily weight gain (DWG):

Daily weight gains (BWG, g) were calculated between body weights in different weeks based on the following equation: Daily weight gain (gram/wk) W2- W1/ wks

Where W1= initial body weight at certain age, BW2= final body weight (g). Daily weight gain was calculated during the periods from hatch to 3 wks. and from 3 wks. to 5 wks. and from hatch to 5 wks.

Feeding traits:

Feed intake (FI):

The pre-weighted amount of feed was offered to individual replicates and was accurately recorded at the end of each growth interval, the residual feed as well as that spilled over was accounted. The actual feed intake by chicks from each replicate of the individual dietary treatment was measured , taking due care for loss of feed falling outside the feeders due to scattering by chicks, according to Abdel Azeem (2001). Feed intake values were computed during hatch to 3 wks. and from 3 wks. to 5 wks. and from hatch to 5 wks.

Average feed intake/chick/wks. (g)

$$= \frac{\text{Feed intake (g) / replicate / Period}}{\text{Live chick numbers / replicate / Period}}$$

Feed conversion ratio (FCR):

Feed conversion for each group of chicks was calculated based on the amount of feed consumed (g) per unit of gain (g). It was calculated by using the following equation according to Abdel Azeem (2002).

Feed conversion (FCR) =

$$= \frac{\text{Feed intake (g) / replicate / Period}}{\text{Body weight gain / replicate / Period}}$$

Feed conversion values were computed during hatch to 3 wks and from 3 wks. to 5 wks. and from hatch to 5 wks.

Physiological traits:

Biochemical parameters:

Determination of serum Transaminases (AST and ALT)

Aspartate amino transaminase (AST) and alanine amino transaminase (ALT) were determined calorimetrically according to the method of Reitman and Frankel (1957) using reagent kit of QCA-Spain.

Determination of total protein:

Serum total protein was performed by the Biuret method accotjbb,rding to Csilla *et al*. (2013) using the ready-made kits of Stanbio, Texas.

Alkaline phosphatase (Alk-p)

Alkaline phosphatase level was assayed, by a quantitative method, using a commercial kit from Biomerieux according to Kind and King (1954) method.

Lipids profile:

The determinations of total Cholesterol, triglycerides, high-density lipoprotein (HDL), Low-density lipoprotein (LDL), uric acid and creatinine were applied by spectrophotometer according to the methods of Robert (2003), Ye and Kwiterovich (2000) , Lopez *et al.* (1977) , Nishi *et al.* (2002), Liu *et al*. (2015) and Vickery *et al.*(2006), respectively.

Statistical analysis:

Data were analyzed using SAS, 2004 software (SAS, 2004) by using one way ANOVA strains single factor). Tests of significance for the differences between means were carried out according to Duncan (1955).

Model:

$X_{jk} = \mu + S_j + e_{jk}$ Where: X_{jk} = the k^{th} observation, μ = overall mean, S_j = effect of the j^{th} treatment and e_{jk} = the experimental error.

RESULTS

A-Growth Performance :

1-Body weight :

Data in the Table 3 shows the effect of the inclusion of Azolla supplementation (Azolla pinnata,5% , 10% 15% and 20%), and 108 Ent. group, (Azolla pinnata+ Ent. 5% , 10%, 15% and 20%) on body weight at 3wks and 5wks in IR broilers chicks. Data shows that in 3wks treated groups with T7, T2, T6, T3, T8 , T4 and T9 were significantly ($p \leq 0.05$) increased for LBW in compared to control group. In addition, the treated group with T7 (5% AZ+ Ent.), T2 (5% AZ) and T6 (Ent.) Showed the highest increase in body weight ($P<0.05$) in compared to the T3 (15 % AZ), T8 (10% AZ+ Ent.) and T4 (15% AZ) Treated group with increase in body weight. In 5wks. treated groups with T6, T3, T2, T7 and T8 were significantly increase for live body weight against control group. In addition, the treated group withT6 (Ent.), T3 (10% AZ), and T2 (5%AZ) showed the significant increase in body weight in comparing with the T7 (10% AZ + Ent.) and T8 (15% AZ+ Ent.) treated group with improved in body weight.

2-Weight gain (WG):

Data in the Table 4 shows the effect of the inclusion of Azolla supplementation (Azolla pinnata, 5%, 10% 15% and 20%), and 10^8 Enterococcus faecalis on weight gain (WG) from day hatch to 3 wks., 3 wks. to 5 wks. in IR broilers chicks. data showed that in 0-3wks. and 3-5wks.treated groups with T6 (Ent) ,T3 (10%AZ) ,T2 (5%AZ) and T7(5% AZ+

Ent) were significantly increase ($p \leq 0.05$) for weight gain against control group. In 0-5 wks. Showed that treated groups with T6, T3, T2, T7 and T8 were significantly increase ($p \leq 0.05$) for weight gain compared to control group. In addition, the treated group with T6 (Ent), T3((10%AZ) and T2(5%AZ) Showed the best treatment group in comparing with the T7(5% AZ+ Ent) and T8((10% AZ+ Ent) treated group with improved in weight gain.

3-Daily Weight gain (DWG):

Data in the Table 5 shows the effect of the inclusion of Azolla supplementation (Azolla pinnata,5% , 10% 15% and 20%), and 10^8Enterococcus faecalis in daily weight gain (DWG) from day hatch to 3 wks., 3wks. to 5wks.and from hatch to 5wks. in Indian River (IR) broilers chicks. Data showed that in 0-3 group treated groups withT7, T2, T6 and T3 significantly increase ($p \leq 0.05$) for daily weight gain compared with control group. in 3-5 wks. showed that treated groups with T6, T3 , T2 and T7 and were significantly increase ($p \leq 0.05$) for daily weight gain compared with control group. in 0-5 wks. showed that treated groups with T6, T3, T2, T7 and T8 were significantly increase ($p \leq 0.05$) for daily weight gain compared with control group.

4- Feed Intake (FI):

Data in the Table 6 shows the effect of the inclusion of Azolla supplementation (Azolla pinnata,5% , 10% 15% and 20%), and 10^8Enterococcus faecalis in feed intake (FI) from 0- 3 wks., 3-5 wks. and 0-5wks. in Indian River (IR) broilers chicks. Data showed that in 3-5 group treated groups with T3, T2, T6, T7 significantly increase ($p \leq 0.05$) for Feed intake compared with control group. In 0-5 wks. Showed that treated groups with T6, T3, T2, T7 and T8 were significantly increase ($p \leq 0.05$) for feed intake compared with control group.

5- Feed conversion ratio:

Data in the Table 7 shows the effect of the inclusion of Azolla supplementation (Azolla pinnata,5% , 10% 15% and 20%), and 10^8Enterococcus faecalis in feed conversion ratio (FCR)from 0- 3 wks., 3-5 wks. and 0-5wks. in Indian River (IR) broilers chicks. Obtained data showed that in 0-3 group treated group with T7 and T2 significantly decrease ($p \leq 0.05$) for Feed conversion ratio compared with control group, in 3-5 wks. Showed that treated groups with T6, T2, T3 and T7 were significantly decrease ($p \leq 0.05$) for feed conversion ratio compared with control group. In 0-5 wks. showed that treated groups with T6, T3, T2 and T7 were significantly decrease ($p \leq 0.05$) for feed conversion ratio against control group.

Liver Function:

1-Total protein:

Data in the Table 8 shows the effect of Azolla groups and Enterococci fascism on Total protein (TP mg/dl) in Indian River (IR) broilers chicks. Obtained data in showed that treated groups with T5, T10 and T6 were significantly increase ($p \leq 0.05$) for TP against control group. In addition, the treated group with T5 and T10 showed the best treatment group in comparing with the T6 treated group with increase TP parameter.

2-Alanine amino transaminase (ALT (U/L):

Data in the Table 8 shows the effect of Azolla groups and Enterococci fascism on (ALT (U/L) in (IR) broilers chicks. Obtained data showed that treated groups with, T7 and T8 were significantly

decrease (p≤ 0.05) for ALT compered control group.

Aspartate amino transaminase (AST U/L):

Data shown in the Table 8 shows the effect of Azolla groups and Enterococci fascism on (AST U/L) in (IR) broilers chicks. Obtained data showed that treated groups with T7 significantly decrease (p ≤0.05) for AST against control group.

3-Alkaline phosphatase (ALP nm/min):

Data in the Table 8 shows the effect of Azolla groups and Enterococci fascism on (ALP nm/min) in (IR) broilers chicks. Obtained data in showed that treated groups with T7, and T8 were significantly decrease (p≤ 0.05) for ALP against control group.

Lipid profile

1-Total cholesterol (TC mg/dL):

Data in the Table 9 shows the effect of Azolla groups and Enterococci fascism on Total cholesterol (TC mg/dl) in (IR) broilers chicks. Obtained data showed that treated groups with T7 significantly decrease (p≤ 0.05) for TC against control group.

2- Triglycerides (TG mg/dL):

Data in the Table 9 shows the effect of Azolla groups and Enterococci fascism on (TG mg/dl) in Indian River (IR) broilers chicks. Obtained data in showed that treated groups with T7 were significantly increase (p≤ 0.05) for TG against control group.

3- High density lipoprotein (HDL mg/dl):

Data in the Table 9 shows the effect of Azolla groups and Enterococci fascism on (HDL mg/dl) in Indian River (IR) broilers chicks. Obtained data showed that no significant changes in HDL level of all treated groups and all are in the average range.

4- Low density lipoprotein (LDL mg/dl):

Data presented in Table 9 shows the effect of Azolla groups and Enterococci fascism on (LDL mg/dl) in (IR) broilers chicks. Obtained data showed that treated groups with T7 and T5 were significantly increase (p≤ 0.05) for LDL against control group.

Kidney function

1-Uric acid (mg/DL):

Data in the Table 10 shows the effect of Azolla groups and Enterococci fascism on Uric acid mg/dl in (IR) broilers chicks. Obtained data showed that treated groups with T3, T9, T4, T6 and T2 were significantly increase (p≤ 0.05) for UA against control groups In addition, the treated group with T7 showed the best treatment group in comparing with the other treatment.

2-Creatinine (mg/dL):

Data in the Table 10 shows the effect of Azolla groups and Enterococci fascism on Creatinine (mg/dL) in (IR) broilers chicks. Obtained data showed that treated groups with T2, T3, T4, T9, T6 were significantly increase (p≤ 0.05) for creatinine against control group. In addition, the treated group with T7 showed the best treatment group in comparing with the other treatment.

DISCUSSION

Poultry production aims to produce meat and decrease meat shortage with minimal antibiotics and feeding coasts. (Masud *et al*., 2020). Performance weight parameters of Indian River in presented data revealed that Azolla in feeding (T2 (5% Azolla), and T3 (10% Azolla) decrease feed intake and feed conversion ratio inconsistent with Saini *et al.(*2018) which explains the Azolla (Azolla

pinnata) feeding affects the growth performance and carcass the characteristics of crossbred pigs.. FCR value indicated that how efficiently the feedstuffs are utilised for the production purpose (Parthasarthy *et al.,* 2002).

According to Bacerra *et al.* (1995) the growth performance of make chicken fed with DA (dry azolla) significantly enhanced in the treated group with Azolla. In compared with the control group, all broiler groups fed DA showed lower feed intake and better FCR. The inability of birds to take in much of the substantial Azolla and the high amount of crude fiber in Azolla may be responsible for lower feed intake. The most recent findings support the earlier versions. Joysowal *et al.,* (2018) They documented that all groups fed with Azolla in the diet consumed less feed in compared to the control, with a significant (P 0.01) decrease. Additionally, Chatterjee *et al.* (2020) reported the decrease in feed intake along with an uptick in the amount of Azolla in the diet of chicken birds up to 15%. Similar to our findings that dietary treatment groups with DA had better FCR, Acharya *et al.,* (2015) He suggested that introducing Azolla to the meals could increase the birds' feed efficiency. Wuthijaree *et al.* (2012) also saw an improvement in FCR with the use of 10% and 15% of Azolla to the make ration. On the other hand, Rawat *et al.* (2015) a higher FI was seen in barbecue groups fed diets supplemented with 5% Azolla, according to the study. Added to that (Samad *et al.,* 2020) indicated that the FCR did not differ significantly between the birds fed Azolla. This variation might be due to the used broiler strain, which includes a variable capability for fiber breakdown, as well as other environmental variables. Rout *et al.* (2017). In addition, the final weights and BWG were improved by dietary supplementation with DA. Due to Azolla's high protein content, especially with regard to 5% and 10% Azolla DA showed a significant improvement. Also, having a good supply of vitamins and considerable amounts of minerals like iron, calcium, potassium, magnesium, phosphorus, manganese, and others had a positive impact on the growth performance. Dhumal *et al.* (2009). Moreover, Azolla contains synthetic polymers and carotenoids in acting as natural immune stimulating agents and antioxidants which contribute to a higher level of animal production and health. (Acharya *et al.* (2015). The present study was in accordance with Shambhvi *et al.* (2020) probiotic as feed additives revealed that Azolla had significantly the highest value performance ($p \leq 0.05$) when compared with control group. All obtained values significantly differed from each other. These results are consistent with Parthasarathy *et al.* (2002) who found that the azolla increased growth rate in broilers chickens. These results are consistent with Naghshi *et al.* (2014) prove that the highest in body weight gain that chicken fed 5% Azolla powder had significantly ($p \leq 0.05$) compared with the basal diet.

Anon *et al*.(1980) reported that the treated group with Azolla was similar for blood biochemical parameters, triglycerides (TG), high density lipoprotein (HDL) , low density lipoprotein (LDL), total cholesterol(TC), total protein (TP) , uric acid (UA); creatinine to control group and within the normal values of broiler chicken. In addition, Sharma *et al.* (2018) reported

that the treated group with azolla was similar to aspartate amino transaminase (AST), alanine amino transaminase (ALT) and alkaline phosphatase(ALP) which indicted no harmful effect on liver enzyme to control group and within the normal values of broiler chicken**.** Also these results were similar with those obtained by Brugere-Picoux *et al.* (1987) reported that the treated group with Azolla was similar for blood biochemical parameters, aspartate amino transaminase (AST), alanine amino transaminase (ALT) and alkaline phosphatase (ALP) to control group and within the normal values of broiler chicken.

Probiotics are naturally occurring bacteria that provide health advantages by modifying the commensal microbiota of the host, influencing immunity, improving the function of the intestinal barrier, or affecting how pain is perceived. Enterococci are intestinal commensals found in both humans and animals. They are used as probiotics and in the production of food. Improvements in body weight and other weight parameters may result from natural physiological processes that improve digestion and maintain the integrity of the intestinal mucosal barrier. Cao *et al.* (2013) found that poultry experimentally infected with pathogenic E. coli K88 grew more quickly when given an E. faecium probiotic. According to Zheng *et al.* (2014), dietary E. faecium feeding may alter how nutrients are distributed, which would improve nutrient utilisation. Zheng *et al.*(2014) reported that the E. faecium supplement improved measurements for performance and meat while reduced the amount of abdominal fat. They recommended that changes in the expression of 22 proteins in the pectoral muscle caused variations in meat quality after E. faecium feeding, and that dietary E. faecium probiotics enhanced the meat quality of broilers. This resulted from modifications in the expression of the cytoskeleton, molecular chaperones, and proteins involved in energy and glucose metabolism. These proteins play a key role in regulating the pH and meat's ability to retain water. According to Zheng *et al*. (2014), The pectoral muscle of broiler chickens fed E. faecium supplement had also reduced the cooking loss and drip loss

CONCLUSION

This study concluded that T2 (5% Azolla), T3 (10% Azolla), T6 (Entero), T7 (5% Azolla +Entero) and T8(10% Azolla +Entero) are the best treatment for growth performance and biochemical parameter.

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Table (1): Diet ingredients and calculated chemicals composition of starter diet.

Feed stuff	**Starter**				
	F1	**F2**	**F3**	**F4**	**F5**
Soybean meal %	21.8	19	19	17	16
Maize %	46.5	44.3	46.8	47	46.8
Corn bran %	10	9	5	3	2
Azolla %	0	5	10	15	20
Bone meal %	0	1	0	0.8	0
Salt %	1	1	1	1	1
Oil %	5	5	5	5	5
*Premix %	1	1	1	1	1
Concentrated (52) %	14.5	14.5	12	10	8
Methionine %	0.1	0.1	0.1	0.1	0.1
Lysine %	0.1	0.1	0.1	0.1	0.1
Total	100	100	100	100	100
Ingredient					
Crude protein (%)	22.77	23.10	23.22	23.00	23.07
ME (kcal/kg)	3284	3251	3259	3262	3240

Where: F1: control, F2: 5%Azolla, F3: 10% Azolla, F4: 15% Azolla, F5:20%Azolla.

Table (2): Diet ingredients and calculated chemicals composition of grower diet.

Feed stuff	**Grower**				
	F1	**F2**	**F3**	**F4**	**F5**
Soybean meal %	19.5	18.5	13	10	7
Maize %	47.3	43.3	51	43.3	42
Corn bran %	15	17	9.8	17	17
Azolla %	0	5	10	15	20
Bone meal %	1	2	0	0.5	0.8
Salt %	1	1	1	1	1
Oil %	5	5	5	5	5
*Premix %	1	1	1	1	1
Concentrated (52) %	10	7	9	7	6
Methionine %	0.1	0.1	0.1	0.1	0.1
Lysine %	0.1	0.1	0.1	0.1	0.1
Total	100	100	100	100	100
Ingredient					
Crude protein (%)	20.30	20.15	20.11	20.02	19.94
ME (kcal/kg)	3287	3240	3334	3325	3320

Where: F1: control, F2: 5%Azolla, F3: 10% Azolla, F4: 15% Azolla, F5:20%Azolla.

Table (3): Least–square means and standard error (X±S.E) for body weight (g) at hatch, 3wks. and 5 wks. of broilers chicks of different experimental groups as affected by studied factors**.**

Treatment	Body weight (g) at		
	Hatch	**3wks.**	**5wks.**
T1 Ctrl	42.9± 016	1143±4.71e	2069±5.77 e
T2 (5% AZ)	42.7±0.20	1201.7±7.35b	2568±13.9b
T3 (10%AZ)	42.9± 0.18	1166.4±6.62c	2584.1±14.1b
T4 (15% AZ)	43±0.19	1148.3±4.24d	1968.6±7.43f
T5 (20%AZ)	43±0.19	1139.2±4.35d	1955.4±5.61f
T6 Ent	43±0.22	1172.9±5.82c	2653±21.27 a
T7 (5% AZ + Ent)	42.9±0.20	1219.6±8.14a	2441.8±15.16c
T8 (10%AZ+ Ent)	42.8±0.20	1149.6±4.04d	2198.3±5.89c
T9 (15% AZ+ Ent)	43.2±0.16	1144.6±5.11 d	1898.7±6.26g
T10 (20% AZ + Ent)	43±0.21	1140±4.34d	1893.7±6.011g
PR > F	0.907	<.0001	<.0001

Where: T1: Control group; T2: 5% Azolla; T3: 10% Azolla; T4: 15% Azolla; T5: 20% Azolla; T6: Enterococci; T7; 5% Azolla +Enterococci; T8: 10% Azolla + Enterococci; T9: 15% Azolla + Enterococci; andT10: 20% Azolla + Enterococci. [a,b, c] Means with different superscript in the same column are significantly different at (P<0.05). Data are expressed as Mean ± S.E.M for 4 chicken /group.

Table (4): Least–square means and standard error (X±S.E) for weight gain (g) of broilers chicks of different experimental groups as affected by studied factors

Treatment	Body weight gain(g) at:		
	0-3 wks.	**3-5 wks.**	**0-5wks.**
T1Ctrl	1100±4.68d	926.1±3.48[f]	2026.2±5.74[e]
T2(5% AZ)	1158.9±7.40b	1366.3±14.68[c]	2525.2±13.93[b]
T3(10%AZ)	1123.5±6.63c	1417.6±13.98[b]	2546.2±14[b]
T4(15%AZ)	1105.3±4.27d	820.2±7.39g	1925.5±7.43f
T5(20%AZ)	1096.3±4.33d	816±6.79g	1912.3±5.63e
T6 Ent	1129.9±5.81c	1480±20.9a	2609.9±21.2a
T7(5% AZ+ Ent)	1176.6±8.08a	1222.2±15.45d	2398.9±15.15c
T8 (10%Az+Ent)	1106.8±4[d]	1052.6±2.40f	2155.4 ±5.85d
T9(15%AZ+ Ent)	1101.4±5.12d	754±3.66h	1855.4±6.24g
T10 (20% AZ Ent)	1097±4.31d	753.7±4.19h	1852.7±6.04g
PR > F	<.0001	<.0001	<.0001

Where: T1: Control group; T2: 5% Azolla; T3: 10% Azolla; T4: 15% Azolla; T5: 20% Azolla; T6: Enterococci; T7; 5% Azolla +Enterococci; T8: 10% Azolla + Enterococci; T9: 15% Azolla + Enterococci; andT10: 20% Azolla + Enterococci. [a,b, c] Means with different superscript in the same column are significantly different at (P<0.05). Data are expressed as Mean ± S.E.M for 4 chicken /group.

Table (5): Least–square means and standard error (X±S.E) for daily weight gain (DWG) of broilers chicks of different experimental groups as affected by studied factors

Treatment	Daily weight gain (g) at:		
	0-3 wks.	**3-5 wks.**	**0-5wks.**
T1 Ctrl	52.3±0.22d	66.15±0.24d	57.89±0.16e
T2 (5% AZ)	55.18±0.35b	97.5±1.04a	72.1±0.39b
T3 (10%AZ)	53.5±0.36c	101.2±0.99a	72.60±0.40b
T4 (15% AZ)	52.6±0.20d	58.5±0.52e	55±0.21f
T5 (20% AZ)	52.2±0.20d	58.2±0.48e	54.6±0.16f
T6 Ent	53.8±0.27c	105.7±1.49a	74.5±0.60a
T7 (5% AZ+ Ent)	56 ±0.38a	87.3±1.10b	68.5±0.43c
T8 (10%Az+Ent)	52.7±0.19d	74.9±0.25f	61.5±0.16d
T9 (15% AZ+ Ent)	52.4±0.24d	53.8±0.33f	53±0.17g
T10 (20% AZ +Ent)	52.2±0.20d	53.8±0.30h	52.8±0.17g
PR > f	<.0001	<.0001	<.0001

Where: T1: Control group; T2: 5% Azolla; T3: 10% Azolla; T4: 15% Azolla; T5: 20% Azolla; T6: Enterococci; T7; 5% Azolla +Enterococci; T8: 10% Azolla + Enterococci; T9: 15% Azolla + Enterococci; andT10: 20% Azolla + Enterococci. ^{a,b,c} Means with different superscript in the same column are significantly different at (P<0.05). Data are expressed as Mean ± S.E.M for 4 chicken /group.

Table (6): Least–square means and standard error (X±S.E) for Feed Intake of broilers chicks of different experimental groups as affected by studied factors

Treatment	Feed intake (g/ bird) during		
	0-3 wks.	**3-5wks.**	**0-5wks.**
T1 Ctrl	1915.5±8.82a	1556.6±5.88d	3472.1±10.35b
T2 (5% AZ)	1881.3±7.84b	1779.5±11.9b	3660.9±10.29a
T3 (10%AZ)	1874.1±9.92b	1814.9±13.37a	3689±13.4a
T4 (15% AZ)	1926±7.70a	1373.3±12.38d	3299.3±12.17c
T5 (20% AZ)	19.11±7.40a	1373.1±11.7e	3284.1±9.36c
T6 Ent	1913.5±8.00a	1769.7±13.13b	3683.2±15.32a
T7 (5% AZ+ Ent)	1924.8±8.11a	1576.3±6.06c	3501.2±10.59b
T8 (10%Az+ Ent)	1928.6±7.01a	15713±5.86c	3500.4±9.25b
T9 (15% AZ+Ent)	1922.7±9.97a	1264.8±7.81e	3187.5±12.19d
T10 (20% AZ +Ent)	1912.7±7.77a	1272.9±7.24e	3185.6±10.46d
PR > F	<.0001	<.0001	<.0001

Where: T1: Control group; T2: 5% Azolla; T3: 10% Azolla; T4: 15% Azolla; T5: 20% Azolla; T6: Enterococci; T7; 5% Azolla +Enterococci; T8: 10% Azolla + Enterococci; T9: 15% Azolla + Enterococci; andT10: 20% Azolla + Enterococci. ^{a,b,c} Means with different superscript in the same column are significantly different at (P<0.05). Data are expressed as Mean ± S.E.M for 4 chicken /group.

Table (7): the Least–square means and standard error (X±S.E) for Feed Conversion Ratio (FCR) of broilers chicks of different experimental groups as affected by studied factors

Treatment	Feed conversion ratio (g feed /g gain) during		
	0-3 wks.	3-5wks.	0-5 wks.
T1 Ctrl	1.74±0.002a	1.68±0.002a	1.71±0.001[a]
T2 (5% AZ)	1.62±0.008d	1.30±0.012c	1.45±0.007[c]
T3 (10%AZ)	1.66±0.005c	1.28±0.011c	1.45±0.006[c]
T4 (15% AZ)	1.74±0.002a	1.67±0.004a	1.71±0.002[a]
T5 (20% AZ)	1.74±0.002a	1.68±0.004a	1.71±0.002a
T6 Ent	1.69±0.004b	1.20±0.014d	1.41±0.009[d]
T7 (5% AZ+ Ent)	1.63±0.012d	1.29±0.016c	1.46±0.008[c]
T8 (10%Az+ Ent)	1.74±0.002a	1.49±0.002b	1.62±0.002[b]
T9 (15% AZ+Ent)	1.74±0.002a	1.67±0.007a	1.72±0.002[a]
T10 (20% AZ +Ent)	1.74±0.002a	1.68±0.003a	1.72±0.002[a]
PR > F	<.0001	<.0001	<.0001

Where: T1: Control group; T2: 5% Azolla; T3: 10% Azolla; T4: 15% Azolla; T5: 20% Azolla; T6: Enterococci; T7; 5% Azolla +Enterococci; T8: 10% Azolla + Enterococci; T9: 15% Azolla + Enterococci; andT10: 20% Azolla + Enterococci. [a,b, c] Means with different superscript in the same column are significantly different at (P<0.05). Data are expressed as Mean ± S.E.M for 4 chicken /group.

Table (8): Least–square means and standard error (X±S.E) for Total protein (g/dL), Alanine amino transaminase (ALT, U/L), Aspartate amino transaminase (AST,U/L)) and alkaline phosphatase (ALP nm/min) of broilers of different experimental groups as affected by studied factors

Liver function				
Treatment	**TP g/dL**	**ALT(U/L)**	**AST (U/L)**	**ALP (nm/min)**
T1Ctrl	6.85± 0.240[ab]	43.9± 2.05[bcd]	62.5± 2.957[ab]	40.9± 1.574[ab]
T2 (5% AZ)	6.62± 0.195[ab]	65.5± 4.150[a]	79.7± 7.128[ab]	59.9± 3.149[a]
T3 (10% AZ)	6.43± 0.208[ab]	65.2± 2.334[a]	91.4± 6.039[a]	51.8± 3.431[ab]
T4 (15% AZ)	6.69± 0.302[ab]	60.2± 3.388[ab]	94.3± 9.384[a]	55.9± 2.227[ab]
T5 (20% AZ)	8.05± 0.557[a]	48.6± 4.329[abcd]	67.3± 8.917[ab]	46.3± 1.574[ab]
T6 Ent	7.13± 0.565[ab]	57.4± 3.572[abc]	96.9± 7.998[a]	53.1± 6.047[ab]
T7 (5% AZ+ Ent)	5.92± 0.285ab	29.4± 6.111d	44.6± 10.397b	36.8± 5.622b
T8 (10% AZ+ Ent)	4.81± 1.641b	38.0± 11.344cd	64.6± 21.237ab	36.8± 10.736b
T9 (15% AZ+ Ent)	6.72± 0.167ab	57.4± 3.556abc	87.3± 6.679a	59.9± 3.521a
T10 (20% AZ + Ent)	7.85± 0.447a	47.5± 3.392abcd	61.7± 3.520ab	43.6± 2.227ab
PR > F	0.17	0.009	0.07	0.09

Where: T1: Control group; T2: 5% Azolla; T3: 10% Azolla; T4: 15% Azolla; T5: 20% Azolla; T6: Enterococci; T7; 5% Azolla +Enterococci; T8: 10% Azolla + Enterococci; T9: 15% Azolla + Enterococci; andT10: 20% Azolla + Enterococci. [a,b, c] Means with different superscript in the same column are significantly different at (P<0.05). Data are expressed as Mean ± S.E.M for 4 chicken /group.

Table (9): Least–square means and standard error (X±S.E) for on lipids profile on (total cholesterol (TC mg/dL), triglycerides (TG mg/dL), and high density lipoprotein (HDL mg/dL and low density lipoprotein (LDL mg/dl) of broilers of different experimental groups as affected by studied factors

lipid profile(mg/dL)				
TRT	**TC mg/dL**	**TG mg/dL**	**HDL mg/dL**	**LDL mg/dL**
T1 Ctrl	136.7± 4.306^{ab}	107.9± 3.403^{d}	49.1± 2.099	44.43± 4.930^{d}
T2 (5% AZ)	182.0± 14.510^{ab}	143.6± 11.459^{abc}	46.3± 1.423	78.30± 8.628^{ab}
T3 (10% AZ)	180.1± 5.456^{ab}	142.1± 4.319^{abc}	51.7± 0.772	71.63± 4.198^{abc}
T4 (15% AZ)	178.9± 1.619^{ab}	141.2± 1.275^{abc}	52.0± 0.886	70.53± 1.081^{abc}
T5 (20% AZ)	163.7± 2.918^{ab}	129.2± 2.297^{bcd}	55.7± 2.112	56.43± 3.692^{bcd}
T6 Ent	172.4± 22.845^{ab}	136.1± 18.024^{bcd}	47.1± 3.869	70.88± 17.830^{abc}
T7 (5% AZ + Ent)	111.9± 6.636^{b}	115.8± 5.254^{cd}	40.0± 1.490	48.13± 4.266^{cd}
T8 (10% AZ+ Ent)	137.8± 38.088.^{ab}	146.9± 9.696^{ab}	37.4± 12.087	75.20± 8.922^{ab}
T9 (15% AZ+ Ent)	199.7± 8.079^{a}	166.7± 6.375^{a}	50.4± 0.942	94.47± 5.259^{a}
T10 (20% AZ + Ent)	173.1± 11.619^{ab}	136.6± 9.184^{bcd}	53.8± 4.243	64.65± 4.894^{bcd}
PR > F	0.1	0.01	0.2	0.01

Where: T1: Control group; T2: 5% Azolla; T3: 10% Azolla; T4: 15% Azolla; T5: 20% Azolla; T6: Enterococci; T7; 5% Azolla +Enterococci; T8: 10% Azolla + Enterococci; T9: 15% Azolla + Enterococci; andT10: 20% Azolla + Enterococci. ^{a,b,c} Means with different superscript in the same column are significantly different at (P<0.05). Data are expressed as Mean ± S.E.M for 4 chicken /group.

Table (10): Least–square means and standard error (X±S.E) for on Kidney Function (Uric acid mg/dl) and creatinine (mg/dl) of broilers of different experimental groups as affected by studied factors

Kidney Functions (mg/dl)		
Treatment	**UA (mg/dL)**	**CREA(mg/dL)**
T1 Ctrl	13.3± 0.319^{ab}	0.84± 0.043^{d}
T2 (5% AZ)	17.3± 1.287^{a}	1.23± 0.084^{a}
T3 (10% AZ)	19.3± 1.188^{a}	1.21± 0.036^{a}
T4 (15% AZ)	18.2± 0.906^{a}	1.14± 0.038^{ab}
T5 (20% AZ)	16.4± 0.688^{ab}	0.94± 0.075^{bcd}
T6 Ent	17.6± 1.380^{a}	1.09± 0.062^{abc}
T7 (5% AZ + Ent)	10.3± 1.132^{ab}	0.77± 0.115^{cd}
T8 (10% AZ+ Ent)	13.6^{a}± 3.935^{b}	0.94± 0.051^{bcd}
T9 (15% AZ+ Ent)	18.4± 0.759^{a}	1.10± 0.056^{abc}
T10 (20%AZ+Ent)	15.9± 1.148^{ab}	0.91± 0.059^{cd}
PR > F	0.03	0.0003

Where: T1: Control group; T2: 5% Azolla; T3: 10% Azolla; T4: 15% Azolla; T5: 20% Azolla; T6: Enterococci; T7; 5% Azolla +Enterococci; T8: 10% Azolla + Enterococci; T9: 15% Azolla + Enterococci; andT10: 20% Azolla + Enterococci. ^{a,b,c} Means with different superscript in the same column are significantly different at (P<0.05). Data are expressed as Mean ± S.E.M for 4 chicken /group.

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الملخص العربى
تأثير التغذية بالأزولا علي الأداء وقياسات الدم وصفات المناعة في دجاج التسمين

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ا
أجريت هذه الدراسة لمعرفة تأثير التغذية بمستويات مختلفة من الأزولا والبروبيوتك علي الأداء الإنتاجي والفسيولوجي لدجاج التسمين. وأستخدم في هذه الدراسة 300 كتكوت قسمت عشوائيا إلي عشر مجموعات كل منها ثلاث مكررات من 10 كتكوت . تمت معاملة المجموعة الأولي كمجموعة ضابطة ، ثم معاملة المجموعة الثانية بالأزولا بمعدل 5% ، والمجموعة الثالثة والرابعة والخامسة بمعدل 10- 15- 20% على التوالي ،ثم معاملة المجموعة السادسة بالبروبيوتك *Enterococcus faecails* في مياة الشرب من يوم الفقس حتي نهاية التجربة بمعدل 10^8 CFU/ml ماء شرب . والمجموعة السابعه والثامنة والتاسعة والعاشرة بمعدل 5% من الأزولا + البروبيوتك ، 10% أزولا + بروبيوتك ، 15% أزولا + بروبيوتك ، 20% أزولا + بروبيوتك .واستمرت التجربة لمدة 35 يوما ثم ذبح الطيور وجمع عينات الدم في نهاية التجربة. وأظهرت النتائج وزن الجسم والزيادة الوزنية اليومية ومعدل تحويل الغذاء ووظائف الكبد AST , ALT , ALP ,والدهون الكوليسترول والدهون الثلاثيه والبروتين الدهني منخفض وعالي الكثافة ووظائف الكلي من حامض اليوريك والكرياتينين. وأظهرت النتائج التي تم الحصول عليها أن المجموعة السادسة المعاملة ب البروبيوتك والمجموعة الثانية والثالثه من 5% ازولا – 10 % أزولا والمجموعة السابعة والثامنة المعالجة 5% أزولا + بروبيوتك ، و10% ازولا + بروبيوتك أدت الي تحسن الأداء الأنتاجي والفسيولوجي مع زيادة معنوية كل من BW, WG , DWG , FI وانخفاض في FCR وعلي النقيض الجرعات العالية من الازولا أدت إلي خلل لمعظم الوظائف الفسيولوجية . الخلاصة نستنتج من تلك الدراسة أن المجموعة الثانية 5% أزولا ، والثالثه 10% أزولا، والسادسه البروبيوتك ، والسابعه 5% أزولا+ بروبيوتك والثامنة 10% أزولا + بروبيوتك هي الأفضل في الأداء الإنتاجي والفسيولوجي.