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Protective Effect of Date Pits on Growth Performance, Carcass Traits, Blood Indices, Intestinal Morphology, Nutrient Digestibility, and Hepatic Aflatoxin Residues of Aflatoxin B1-Exposed Broilers

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Citation: Alharthi, A.S.; Al Sulaiman, A.R.; Aljumaah, R.S.; Alabdullatif, A.A.; Elolimy, A.A.; Alqhtani, A.H.; Al-Garadi, M.A.; Abudabos, A.M. Protective Effect of Date Pits on Growth Performance, Carcass Traits, Blood Indices, Intestinal Morphology, Nutrient Digestibility, and Hepatic Aflatoxin Residues of Aflatoxin B1-Exposed Broilers. *Agriculture* **2022**, *12*, 476. https://doi.org/10.3390/ agriculture12040476

Academic Editor: Lin Zhang

Received: 26 February 2022 Accepted: 27 March 2022 Published: 28 March 2022

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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Abstract: This research aimed to assess the influence of date pits (DP) on reducing toxic influences of aflatoxin B1 (AFB1) in broilers by examining the growth performance, carcass characteristics, serum indices, ileum morphology, apparent nutrient digestibility, and liver AFB1 residues. In total, 360 11-day-old straight-run broilers (Ross 308) were randomly allocated into 6 dietary treatments, with 10 replications of 6 birds each, in a 20-day experiment. The treatments were as follows: standard basal diet (negative control, NC); NC + 0.25 mg/kg AFB1 (positive control, PC); NC + 2% DP; NC + 4% DP; PC + 2% DP; PC + 4% DP. Compared to the NC diet, feeding the PC diet decreased the daily feed intake (DFI) during the grower and overall periods (p < 0.01); reduced the daily weight gain (DWG) and production efficiency factor (PEF); increased the feed conversion ratio (FCR) during all experimental periods (p < 0.001); diminished the dressing percentage, breast meat yield, serum concentrations of total protein (TP), albumin (ALB), glucose (GLU), total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD), villus height (VH), villus surface area (VSA), apparent digestibility of crude protein (CP) and ether extract (EE), apparent metabolizable energy (AME), and nitrogen-corrected AME (AMEn) (p < 0.001); and raised the proportional liver weight, serum activities of glutamic oxaloacetic transaminase (GOT) and glutamate pyruvate transaminase (GPT), and residues of AFB1 in the liver (p < 0.001). Compared to the PC diet, feeding the PC + 2% DP or PC + 4% DP diets increased the DWG during finisher and overall periods, PEF during the finisher period, serum levels of TP, GLU, T-AOC, and T-SOD, VH, and apparent digestibility of CP and EE, AME, and AMEn, and decreased FCR during all experimental periods and AFB1 residues in the liver (p < 0.001). Moreover, feeding the PC + 4% DP diet increased the DWG during the grower period, PEF during the overall period, dressing percentage, serum ALB level, and VSA, and decreased the proportional liver weight and serum GOT and GPT activities when compared to the PC diet (p < 0.001). To conclude, our findings demonstrate that a dietary addition of 4% DP can deliver a better safeguard against the adverse influences of AFB1 in broiler chickens.

Keywords: aflatoxin B1; broilers; date pits; digestibility; liver health; performance

1. Introduction

There is a considerable number of biological toxins existent in the natural environment, which would be hazardous for the well-being of farm animals. Mycotoxins, predominantly including aflatoxins, are presently regarded as amongst the most threatening ones in

poultry [1]. Aflatoxins are difuranceoumarin derivatives formed chiefly by strains of Aspergillus flavus—parasiticus, and can infect plenty of different crops, especially corn, groundnut, and wheat [2,3]. Crops contaminated with these toxic metabolites adversely impact the health status of humans and animals, the revenue of producers, and trading opportunities [4,5]. Among the different kinds of aflatoxins, aflatoxin B1 (AFB1) is the most predominant and toxic one for poultry [6,7]. The European Union has specified the maximum allowed concentration of AFB1 to be 0.02 mg/kg in the poultry feedingstuffs to safeguard these chickens from wellbeing dangers and to prohibit the transference of these toxic substances into their meat and egg products [8,9]. AFB1 is converted into secondary toxic metabolites chiefly in the liver, provoking liver injury and alterations in hepatic function [10]. Numerous studies have demonstrated the harmful influences of AFB1 in broiler chickens, involving a decrease in growth responses [11,12], changes in carcass quality [13], liver damage [14], a poor immune response [15], an enhanced susceptibility to infectious diseases [16], and an augmented mortality [17], causing considerable financial losses [18]. Furthermore, AFB1 residue in the edible tissues is a possible risk to human health [19].

During the past years, numerous approaches for the detoxification or inactivation of AFB1 have been employed comprising physical, chemical, and biological techniques [20]. More recently natural phytochemicals were examined for their capability to adsorb aflatoxins [21]. Agricultural wastes, which are wealthy sources of beneficial phytochemicals, have been examined for their ability to neutralize aflatoxin toxicity, and several studies have demonstrated that polyphenols from various sources have the potential to decrease the concentration and toxicity of AFB1 [22,23]. Date pits (DP), which comprise roughly 10% of the date's weight, are a by-product of the date palm (Phoenix dactylifera L.) processing industries [24]. In recent years, some research reports have shown that DP meal can be partly utilized as an alternative feed ingredient in poultry ration [25,26]. The nutritive values for DP have been reviewed recently by Attia et al. [27]. It has also been applied as an effective adsorbent for extracting certain types of mycotoxins from contaminated milk, owing to the existence of oxygenated functional groups in its lignocellulose substrates, such as phenolic compounds [28]. Abdel-Sattar et al. [29] demonstrated that the inclusion of DP into broiler feeds alleviated the detrimental influences of aflatoxicosis on carcass attributes, biochemical variables, and liver histopathological alterations in a dose-related manner. Nevertheless, to our knowledge, there is extremely limited knowledge on the usage of DP as a mycotoxin adsorbent in broiler chickens. Therefore, the current research was performed to evaluate the influence of dietary DP supplementation at different levels on the growth efficiency, carcass yields and visceral organs, serum biochemical parameters and enzyme activities, ileal histomorphology, apparent total tract digestibility of nutrients, and liver residual AFB1 level in broilers fed AFB1-contaminated diets.

2. Materials and Methods

2.1. Birds and Trial Design

This experiment was performed in the poultry farm of the Animal Production Department at King Saud University. A total of 360 one-day-old Ross 308 straight-run broiler chicks of similar initial body weight (45.21 ± 0.36 g) were placed in battery cages (n = 6 chicks/cage; 30 kg BW/m²) in an environmentally controlled room and were fed a basal starter diet until 10 d of age. At 11 d of age, each cage was randomly assigned to 1 of 6 dietary treatments (10 replications each) in a completely randomized design. The treatments were as follows: standard basal diet (negative control, NC); NC + 0.25 mg/kg AFB1 (positive control, PC); NC + 2% DP; NC + 4% DP; PC + 2% DP; PC + 4% DP. The trial lasted for 20 d. The PC diet was prepared using corn naturally contaminated with aflatoxins as previously described by Yang et al. [30]. A high-performance liquid chromatography system (Shimadzu Corp., Kyoto, Japan) was used for the determination of AFB1 concentration in the infected corn and diet as described previously [31]. Fresh DP (*Phoenix dactylifera* L., Khalas variety) was acquired from Riyadh Dates Factory in Al Kharj, KSA. The DP was

ground in a medium-sized mill (Skiold, Saeby, Denmark) to obtain 1 mm sized particles. The starter (1 to 10 d), grower (11 to 20 d), and finisher (21 to 30 d) diets (Table 1) were formulated according to the Aviagen [32] broiler nutrition specifications. All birds were given unrestricted access to mash feed and freshwater through the rearing duration. Birds were raised and monitored according to the *Ross Broiler Management Handbook* [33].

Table 1. Formulation of the starter (1–10 d), grower (11–20 d), and finisher (21–30 d) experimental diets (%, as-fed basis).

In one diam to	<u>.</u>		Grower			Finisher	
Ingredients	Starter	Control	2% DP	4% DP	Control	2% DP	4% DP
Yellow corn	54.2	57.3	55.3	53.3	59.8	58.5	56.0
Soybean meal (46% CP)	31.4	29.3	27.8	27.8	27.0	25.0	25.0
Corn oil	2.20	3.40	3.50	3.50	4.34	4.22	4.56
Corn gluten meal	7.30	6.11	7.40	7.40	5.10	6.40	6.60
Date pits (DP)	0.00	0.00	2.00	4.00	0.00	2.00	4.00
Dicalcium phosphate	1.88	1.82	1.85	1.85	1.68	1.68	1.68
Ground limestone	1.10	0.88	0.87	0.87	0.87	0.87	0.87
Choline chloride	0.50	0.00	0.00	0.00	0.00	0.00	0.00
DL-methionine	0.30	0.23	0.24	0.24	0.25	0.26	0.26
L-lysine	0.40	0.26	0.35	0.35	0.26	0.33	0.33
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Threonine	0.14	0.10	0.10	0.10	0.08	0.10	0.10
Vitamin-mineral premix ¹	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Nutritional levels ²							
ME (kcal/kg)	3000	3100	3100	3100	3200	3200	3200
Crude protein	23.0	21.5	21.5	21.5	20.0	20.0	20.0
Non-phytate phosphorus	0.48	0.44	0.44	0.44	0.41	0.41	0.41
Calcium	0.96	0.87	0.87	0.87	0.81	0.81	0.81
Digestible lysine	1.28	1.15	1.15	1.15	1.06	1.06	1.06
Sulfur amino acids	0.95	0.85	0.85	0.85	0.83	0.83	0.83
Threonine	0.86	0.77	0.77	0.77	0.71	0.71	0.71

¹ Vitamin–mineral premix provided the following per kg of the diets: vitamin A, 12,000,000 IU; vitamin D3, 5,000,000 IU; vitamin E, 80,000 IU; vitamin K3, 3200 mg; vitamin B1, 3200 mg; vitamin B2, 8600 mg; vitamin B3, 65,000 mg; pantothenic acid, 20,000 mg; vitamin B6, 4300 mg; biotin 220 mg; vitamin B9, 2200 mg; vitamin B12, 17 mg; antioxidant (BHA + BHT), 50,000 mg; copper, 16,000 mg; iodine, 1250 mg; iron, 20,000 mg; manganese, 120,000 mg; selenium, 300 mg, and zinc, 110,000 mg. ² Calculated based on AMINODAT 5.0 (Evonik Animal Nutrition, Hanau-Wolfgang, Germany).

2.2. Sampling and Measurements

Feed intake and body weight were recorded weekly on a replicate basis for calculating daily feed intake (DFI), daily weight gain (DWG), mortality-adjusted feed conversion rate (FCR), and production efficiency factor (PEF) of broilers.

At 25 d, 10 birds per treatment (1 bird/replicate) were selected randomly and housed individually in metabolic cages. After acclimatization for 3 d, excreta from each cage were collected for 48 h using the total collection method [34]. Feed intake was recorded during the collection period. Afterward, excreta were oven-dried until constant weight and ground to pass through a 0.5 mm sieve prior to chemical examination. The diets and excreta were analyzed for crude protein (CP) by the Kjeldahl procedure (AOAC method 984.13 [35]) and ether extract (EE) by the Soxhlet extraction procedure (AOAC method 920.39 [35]). The gross energy of feeds and excreta was quantified employing a bomb calorimeter (IKA Works, Wilmington, NC, USA) standardized with benzoic acid. The apparent digestibility of CP and EE and apparent metabolizable energy (AME) and nitrogen-corrected AME (AMEn) of the experimental diets were calculated according to De Marco et al. [36].

At the end of the feeding trial (30 d), 10 birds per treatment (1 bird/replicate) were randomly picked, and blood specimens were gathered from the brachial vein and centrifuged to acquire serum. The serum levels of total protein (TP), albumin (ALB), glucose (GLU), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD), and glutathione reductase (GR) were quantified utilizing commercial ELISA kits (MyBioSource, San Diego, CA, USA) following the manufacturer's guidelines. Serum globulin (GLO) level was calculated by subtracting ALB from TP [37].

After taking blood specimens, the birds were weighed, euthanized, plucked, processed, and eviscerated. The dressing was computed by dividing the hot carcass weight by preslaughter weight and expressed as a percentage. The weights of cut-up parts (breast muscles, leg quarters, and abdominal fat pad) and visceral organs (liver, spleen, bursa of Fabricius, and empty gizzard) were taken and expressed as a percentage of the preslaughter weight.

Approximately 2 cm long segments from the center of the ileum were cut, flushed with phosphate buffer saline, and fixed in 10% neutral buffered formalin. Fixed sections were further dehydrated, cleared, embedded in paraffin, sectioned at 5 µm thickness, placed on glass slides, and stained with hematoxylin and eosin. The slides were photographed under a light microscope fitted with a digital camera (Olympus Corporation, Tokyo, Japan). Villus height (VH) and villus width (VW) based on at least 10 well-oriented villi per sample were measured utilizing ImageJ software (National Institutes of Health, Bethesda, MD, USA). The villus surface area (VSA) was then computed from the VH and the VW at half-height according to Abudabos et al. [38].

Residues of AFB1 in the liver tissues were extracted and purified following the procedure previously illustrated by Magnoli et al. [39]. A Shimadzu high-performance liquid chromatography system with fluorescence detection was used for the detection and mensuration of AFB1 in the final solution, as formerly illustrated [40].

2.3. Statistical Analysis

The experimental unit was the individual animal, except for the performance measures, where the pen of animals was the experimental unit. The data were analyzed by one-way analysis of variance and Tukey test of multiple comparisons utilizing SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA). The statistical model involved treatment as a fixed factor and pen as a random factor. The significance level was specified at p < 0.05. Findings are presented as least square means with pooled standard error of the mean.

3. Results

3.1. Growth Performance

The impacts of dietary treatments on the broiler growth performance for the grower (11–20 d), finisher (21–30 d), and overall (11–30 d) phases are summarized in Table 2. Broilers fed the PC diet had a decreased DFI for the grower and overall phases (p < 0.01) and a reduced DWG and PEF and increased FCR in the grower, finisher, and overall phases (p < 0.001) compared with those fed the NC diet. In contrast, the inclusion of DP in the PC diet either at 2 or 4% increased the DWG to similar levels as in the NC groups for the finisher and overall phases, improved the PEF for the finisher phase, and decreased the PC diet increased the DWG to similar levels as in the NC groups for the PC diet increased the DWG to similar levels as in the NC groups for the PC diet increased the DWG to similar levels as in the NC groups for the PC diet increased the DWG to similar levels as in the NC groups for the grower phase and improved the PEF for the overall phase (p < 0.001). No differences were detected in the DFI for the finisher phase among the treatments (p > 0.05).

		11-2	20 d		21–30 d				11–30 d			
Treatments	DFI ²	DWG	FCR	PEF	DFI	DWG	FCR	PEF	DFI	DWG	FCR	PEF
	g	G	g:g	%	g	g	g:g	%	g	g	g:g	%
NC ¹	67.3 ^a	49.4 ^a	1.36 ^b	282 ^a	119	79.6 ^a	1.49 ^c	349 ^a	92.0 ^a	64.5 ^a	1.44 ^c	360 ^a
NC + 2% DP	67.4 ^a	49.1 ^a	1.37 ^b	278 ^a	119	78.7 ^a	1.52 ^{bc}	344 ^a	93.2 ^a	63.9 ^a	1.46 ^{bc}	348 ^{ab}
NC + 4% DP	68.1 ^a	49.5 ^a	1.38 ^b	282 ^a	122	79.9 ^a	1.53 ^{bc}	344 ^a	95.2 ^a	64.7 ^a	1.47 ^{bc}	357 ^a
PC	59.9 ^b	38.8 ^b	1.55 ^a	200 ^b	112	64.3 ^b	1.74 ^a	257 ^c	85.8 ^b	51.6 ^b	1.67 ^a	287 ^d
PC + 2% DP	64.5 ^b	44.7 ^{ab}	1.45 ^b	233 ^b	119	74.0 ^a	1.58 ^b	300 ^b	91.5 ^{ab}	59.8 ^a	1.53 ^b	310 cd
PC + 4% DP	64.9 ^b	45.4 ^a	1.43 ^b	239 ^{ab}	118	76.5 ^a	1.55 ^{bc}	315 ^{ab}	91.6 ^{ab}	60.9 ^a	1.50 ^{bc}	324 ^{bc}
SEM ³	1.62	1.32	0.02	10.20	2.21	1.86	0.02	8.49	1.49	1.14	0.02	7.47
<i>p</i> -value	0.012	0.001	0.001	0.001	0.060	0.001	0.001	0.001	0.003	0.001	0.001	0.001

Table 2. Effect of adding two levels of date pits (DP) as aflatoxin adsorbent to aflatoxin B1 (AFB1)contaminated diets on growth performance of broilers during grower (11–20 d), finisher (21–30 d), and overall (11–30 d) phases.

^{a-d} Means within the same column with different superscripts differ (p < 0.05). ¹ NC (negative control), standard basal diet; PC (positive control), NC + 0.25 mg/kg AFB1. ² DFI, daily feed intake; DWG, daily weight gain; FCR, feed conversion ratio; PEF, production efficiency factor. ³ SEM, pooled standard error of the mean.

3.2. Carcass Parameters

The impacts of dietary treatments on the carcass yield of broilers are summarized in Table 3. Broilers fed the PC diet had a decreased dressing percentage and breast meat yield and increased proportional liver weight (p < 0.001) compared with those fed the NC diet. In contrast, the inclusion of 4% DP in the PC diet increased the dressing percentage and decreased proportional liver weight (p < 0.001). No differences were detected in the relative weights of the leg meat yield, abdominal fat, bursa, spleen, and empty gizzard among the treatments (p > 0.05).

Table 3. Effect of adding two levels of date pits (DP) as aflatoxin adsorbent to aflatoxin B1 (AFB1)contaminated diets on the relative weights (% of pre-slaughter weight) of carcass yields and internal organs in broilers at 30 d.

Treatments	Dressing	Breast	Leg	Fat	Liver	Bursa	Spleen	Gizzard
NC ¹	71.2 ^a	27.8 ^a	20.4	0.95	2.01 ^c	0.16	0.10	1.74
NC + 2% DP	71.3 ^a	27.3 ^a	20.5	1.16	2.03 ^c	0.15	0.10	1.96
NC + 4% DP	71.2 ^a	27.8 ^a	20.0	0.87	2.00 ^c	0.19	0.19	2.18
PC	69.0 ^c	24.7 ^b	20.1	1.07	2.77 ^a	0.18	0.12	2.37
PC + 2% DP	69.8 ^{bc}	24.0 ^b	21.2	1.04	2.47 ^{ab}	0.17	0.09	2.02
PC + 4% DP	70.5 ^{ab}	26.5 ^{ab}	20.5	0.81	2.27 ^{bc}	0.19	0.12	2.06
SEM ²	0.28	0.54	0.30	0.14	0.08	0.01	0.04	0.19
<i>p</i> -value	0.001	0.001	0.109	0.533	0.001	0.407	0.588	0.306

^{a-c} Means within the same column with different superscripts differ (p < 0.05). ¹ NC (negative control), standard basal diet; PC (positive control), NC + 0.25 mg/kg AFB1. ² SEM, pooled standard error of the mean (n = 10).

3.3. Blood Constituents

The impacts of dietary treatments on serum analyses of broilers, including biochemical indices, liver function enzymes, and the antioxidant status of broilers, are given in Table 4. Broilers fed the PC diet had decreased levels of TP, ALB, GLU, T-AOC, and T-SOD and increased activities of GOT and GPT (p < 0.001) compared with those fed the NC diet. In contrast, the inclusion of DP in the PC diet either at 2 or 4% increased the levels of TP, GLU, T-AOC, and T-SOD (p < 0.001). The increase in TP and T-AOC levels has reached similar levels as in the NC groups. Moreover, adding 4% DP to the PC diet increased the ALB level and decreased GOT and GPT activities (p < 0.001). The decrease in GPT has reached similar levels as in the NC groups.

		Biochemi	cal Indices		Liver H	unction	Antioxidant Capacity				
Treatments	TP ²	ALB	GLO	GLU	GOT	GPT	T-AOC	T-SOD	GR		
	g/dL	g/dL	g/dL	mg/dL	U/L	U/L	U	U/mg of Protein			
NC ¹	5.29 ^a	2.56 ^a	2.73 ^{ab}	207 ^a	45.8 ^c	7.44 ^b	1.86 ^a	185 ^a	3.36 ^{ab}		
NC + 2% DP	5.30 ^a	2.37 ^{ab}	2.94 ^b	208 ^a	46.2 ^c	7.54 ^b	1.85 ^a	186 ^a	3.26 ^{ab}		
NC + 4% DP	5.33 ^a	2.48 ^{ab}	2.85 ^{ab}	208 ^a	45.4 ^c	7.76 ^b	1.84 ^a	186 ^a	2.98 ^{ab}		
PC	4.42 ^b	1.84 ^d	2.58 ^{ab}	165 ^c	57.8 ^a	10.83 ^a	1.41 ^b	171 ^c	2.68 ^b		
PC + 2% DP	5.00 ^a	1.94 ^{cd}	3.06 ^a	188 ^b	55.5 ^{ab}	10.10 ^a	1.69 ^a	179 ^b	3.48 ^a		
PC + 4% DP	5.08 ^a	2.18 ^{bc}	2.90 ^{ab}	189 ^b	52.9 ^b	8.34 ^b	1.81 ^a	181 ^{ab}	3.03 ^{ab}		
SEM ³	0.08	0.08	0.10	2.78	0.79	0.29	0.05	1.31	0.17		
<i>p</i> -value	0.001	0.001	0.027	0.001	0.001	0.001	0.001	0.001	0.039		

Table 4. Effect of adding two levels of date pits (DP) as aflatoxin adsorbent to aflatoxin B1 (AFB1)-contaminated diets on blood serum parameters of broilers at 30 d.

^{a-d} Means within the same column with different superscripts differ (p < 0.05). ¹ NC (negative control), standard basal diet; PC (positive control), NC + 0.25 mg/kg AFB1. ² TP, total protein; ALB, albumin; GLO, globulin; GLU, glucose; GOT, glutamic oxalacetic transaminase; GPT, glutamic pyruvic transaminase; T-AOC, total antioxidant capacity; T-SOD, total superoxide dismutase; GR, glutathione reductase. ³ SEM, pooled standard error of the mean (n = 10).

3.4. Ileal Morphology, Nutrient Digestibility, and Hepatic AFB1 Residues

The impacts of dietary treatments on ileal histomorphometry, nutrient apparent digestibility, and AFB1 concentration in liver tissue of broilers are summarized in Table 5. Broilers fed the PC diet had a decreased VH, VSA, apparent digestibility of CP and EE, AME, and AMEn, and increased AFB1 residues in the liver (p < 0.001) compared with those fed the NC diet. In contrast, the inclusion of DP in the PC diet either at 2 or 4% increased the VH, apparent digestibility of CP and EE, AME, and AMEn, and decreased AFB1 residues in the liver (p < 0.001). The increases in VH and EE digestibility have reached similar levels as in the NC groups. Moreover, adding 4% DP to the PC diet increased the VSA to similar levels as in the NC groups (p < 0.001). No differences were detected in the VW between the treatments (p > 0.05).

Table 5. Effect of adding two levels of date pits (DP) as aflatoxin adsorbent to aflatoxin B1 (AFB1)contaminated diets on ileal histology, apparent nutrient digestibility, and residual AFB1 in the liver of broilers.

Treatments	Ι	leal Histolog	S y					
	VH ²	VW	VSA	СР	EE	AME	AMEn	AFB1
	μm	μm	μm^2	%	%	kcal/kg	kcal/kg	μg/kg
NC ¹	609 ^a	67.1	0.13 ^a	63.4 ^{ab}	77.3 ^a	3284 ^a	3154 ^a	0.00 ^c
NC + 2% DP	616 ^a	72.1	0.13 ^a	63.3 ^{ab}	77.3 ^a	3287 ^a	3151 ^a	0.00 ^c
NC + 4% DP	622 ^a	71.0	0.14 ^a	63.7 ^a	77.6 ^a	3295 ^a	3153 ^a	0.00 ^c
PC	518 ^b	59.5	0.10 ^b	56.6 ^c	74.8 ^b	3209 ^c	3049 ^c	3.32 ^a
PC + 2% DP	608 ^a	66.2	0.12 ^{ab}	61.5 ^{ab}	77.5 ^a	3248 ^b	3099 ^b	1.23 ^b
PC + 4% DP	610 ^a	65.0	0.13 ^a	61.7 ^{ab}	77.1 ^a	3256 ^b	3124 ^{ab}	1.02 ^b
SEM ³	17.94	3.50	0.01	0.49	0.26	5.98	9.87	0.11
<i>p</i> -value	0.001	0.144	0.001	0.001	0.001	0.001	0.001	0.001

^{a-c} Means within the same column with different superscripts differ (p < 0.05). ¹ NC (negative control), standard basal diet; PC (positive control), NC + 0.25 mg/kg AFB1. ² VH, villus height; VW, villus width; VSA, villus surface area; CP, crude protein; EE, ether extract; AME, apparent metabolizable energy; AMEn, nitrogen-corrected AME. ³ SEM, pooled standard error of the mean (n = 10).

4. Discussion

Aflatoxin B1 can lead to enormous financial losses in the broiler industry by lowering the growth rate and feed efficiency and heightening the occurrence of diseases, therefore

increasing mortality [21,41]. The toxic influence of dietary AFB1 (0.25 mg/kg) and the ameliorative efficacy of dietary adsorbent (2 or 4% DP) on the growth performance, carcass characteristics, serum biochemical markers and enzyme activities, ileum morphology, apparent nutrient digestibility, and liver AFB1 residues of broilers were evaluated. Several experiments have demonstrated the detrimental impact of AFB1 on the growth performance [42,43] and carcass characteristics [44,45] of broilers. Our results are in agreement with the aforementioned reports and showed that broilers fed a diet naturally contaminated with AFB1 had a significantly reduced DFI, DWG, and PEF and augmented FCR during the experimental period, along with lowered dressing and breast meat yields compared to those provided with the uncontaminated diet. In addition, previous researchers revealed that AFB1 adversely influenced the intestinal morphology [43] and diminished the digestibility of nutrients [46], bringing about a lowered growth efficiency of broilers. Likewise, feeding the PC diet in this study reduced VH and VSA in the ileal mucosa and apparent digestibility of CP, EE, AME, and AMEn. This decrease might be related to the impairments in epithelial cell proliferation and protein synthesis in the small intestine, which could, in turn, lower the nutrient digestibility [43,47].

In the present study, birds in the PC group had a higher liver relative weight, which is similar to the results of other investigators, who reported that the relative liver weights were significantly augmented in broilers after exposure to AFB1 [48,49]. Furthermore, feeding AFB1 to the PC group significantly reduced the contents of serum TP, ALB, and GLU, which is in agreement with the results of Bagherzadeh Kasmani et al. [50] and Chen et al. [51]. The toxicity of AFB1 has been demonstrated to trigger the suppression of hepatic protein, carbohydrate, and lipid metabolism, and might therefore lead to liver enlargement and serum biochemical changes [37,52]. The present study showed that serum GOT and GPT concentrations and residual levels of AFB1 were significantly elevated in broilers fed with the AFB1-contaminated diet. This finding is in accordance with former studies on broiler chickens regarding transaminases activities [46,53] and AFB1 residues in the liver [54,55], illustrating that the accumulation of AFB1 in the liver could trigger apoptosis and inflammation in broiler hepatocytes. During aflatoxicosis, AFB1 is chiefly metabolized in the liver, and its secondary metabolite of AFB1-8,9-expoxide can attach to cellular macromolecules, such as proteins, lipids, and nucleic acids, bringing about hepatocyte cancerization and liver damage thereafter [56]. When hepatocyte permeability increased following liver injury, transaminases could be liberated from the infected hepatocyte into the bloodstream and elevate the serum activity of transaminases, including GOT and GPT [57]. Similar to the results of Rajput et al. [49], our results also showed that broilers given the AFB1-contaminated diet had significantly lower levels of T-AOC and T-SOD in the serum than those provided with the uncontaminated feed. A reduced protein biosynthesis can be held accountable for lowering enzyme activities [53]. Besides, the metabolites of AFB1 induce cellular oxidative stress through increasing lipid peroxidation reactions, possibly leading to the inhibition of serum antioxidant enzyme activities [58]. Comprehensively, AFB1-induced impairments in both liver function and intestinal integrity are probably responsible for the reduced growth rate and carcass yield of broilers.

Interestingly, the use of DP as a waste by-product adsorbent that is produced utilizing a straightforward, uncomplicated, and cost-efficient process without the usage of chemical treatments is regarded as being green chemistry and sustains the environment by endeavoring to transform undesirable agricultural wastes into advantageous adsorbents [59,60]. Based on our findings, a dietary supplementation of 4% DP was more efficient in mitigating the toxic influences of AFB1 on the growth efficiency by improving the DWG and FCR during the entire period; dressing yield; biochemical metabolites by increasing TP, ALB, and GLU levels; liver health by lowering liver weight; GOT and GPT activities; AFB1 residues; antioxidant capacity by increasing T-AOC and T-SOD; ileal morphometry by improving VH and VSA; and apparent nutrient digestibility by enhancing CP, EE, AME, and AMEn retentions of broilers, showing its high binding capacity to AFB1 molecules. This can be explained by electrostatic attractions and the hydrogen-bonding interaction

for the DP. Besides, hydroxyl and carboxyl derivatives are plentiful on the surface of DP, boosting hydrogen-bonding synthesis. In addition, DP is considered to be an effective adsorbent, ascribable to its granular structure; physical–chemical features, such as poor aqueous solubility; elevated mechanical strength; chemical stabilization; high availability; and cost efficiency [61,62]. These results are in accordance with the former research reports. Abdel-Sattar et al. [29] concluded that the inclusion of DP in broiler's diets ameliorated the hazardous influences of aflatoxins on carcass traits, biochemical parameters, and liver histopathological changes in a dose-dependent pattern. Daneshyar et al. [63] stated that mannan-oligosaccharides in DP could be able to reduce the negative influences of AFB1 on the performance and carcass characteristics of broiler birds. However, there is a lack of data on the usage of DP in broiler feed to reduce the concentrations of aflatoxins, Hence, further research would be necessary for this area of experimentation.

5. Conclusions

In conclusion, our results indicate that dietary AFB1 at a dose of 0.25 mg/kg gave rise to a lowered growth efficiency, deteriorated carcass yield, altered serum biochemical metabolites, liver injury, depressed antioxidant capacity, impaired ileal architecture, and diminished nutrient digestibility of broilers. On the other hand, a dietary supplementation of 4% DP provided better protection against the detrimental influences of AFB1 on the productive and health indicators of the birds. Accordingly, we can recommend using it as a feed additive for assisting in the prevention of aflatoxicosis in broiler flocks.

Author Contributions: Conceptualization and methodology, A.M.A., A.S.A., A.A.E. and R.S.A.; investigation and validation, A.M.A., A.A.A. and A.H.A.; data curation: A.M.A., A.R.A.S. and A.A.E.; formal analysis, M.A.A.-G. and A.A.E.; writing—original draft, review and editing: A.M.A. and A.R.A.S.; funding acquisition, resources and project administration, A.M.A. and R.S.A.; supervision, A.M.A. All authors have read and agreed to the published version of the manuscript.

Funding: This project was funded by the National Plan for Science, Technology and Innovation (MAARIFAH), King Abdulaziz City for Science and Technology, Kingdom of Saudi Arabia, Award Number (15-AGR5339-02).

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of King Saud University, Riyadh, Saudi Arabia (KSU-SE-20-22).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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