CLUJ VETERINARY JOURNAL

Article

Effect of partial substitution of prickly pear seed meal (*Opuntia ficus indica* L.) on serum biochemical parameters and intestinal microbiota in broiler chickens

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Abstract: The aim of the present study was to assess the effect of prickly pear seed (PPS) cake on biochemical parameter and gut microbiota in broiler chickens. One hundred and fifty 1-day-old broiler chicks were allocated into 5 groups with 3 replicates of 10 birds per group. The experimental groups received the diets substituted with 0, 10%, 20%, 30%, and 40% prickly pear seeds meal of OFI during a 6-week. At 45 d of age, five chickens were randomly selected from each treatment group. After 12 h of feed withdrawal, serum and cecal samples were collected use for biochemical parameters assay and PCR Amplification. The results of serum lipid parameters shown that the different PPS dietary treatments significantly ($P<0.05$) reduced the concentration of glucose concentration, total proteins total cholesterol, and lipidemia compared to control group of broiler chickens. It showed significantly decreased ($P < 0.05$) liver enzymatic activity in treated group with PPS cake compared to control group of broiler chickens. Also, ionogram showed that values of Mg, P, and Ca reduced significantly ($P<0.05$) after treatment of chickens with PPS cake concentrations compared to control group. At the phylum level, Firmicutes and Bacteroidetes were the major groups of microorganisms in the gut of broiler chickens received PPS cake diet. In conclusion, this study demonstrated that PPS cake can have a protective effect on the kidney and liver function of chickens. Also, it found that broiler chickens treated with PPS cake meal exhibited a variety of phylum and genus in gut microbiota.

Keywords: Biochemical parameters; gut microbiota; prickly pear; seeds meal; broiler chicken.

1. Introduction

The cactus (*Opuntia ficus-indica L*), commonly known as prickly pear, belongs to the family *Cactacea* [1]. This plant family is reported to contain about 130 genera and nearly 1500 species [2]. Family *Cactaceae* is native to Mexico and is widely distributed in all American countries, Africa and the Mediterranean basin, and some Asian areas [3]. *Opuntia cactus* grows mainly in arid with the annual rainfall is less considerable (< 250 mm and 250-450 mm, respectively) [4]. It is noted that cactus was introduced to North Africa in 17th century in order to combat erosion and desertification.

A program for the promotion of pastoralism based on cactus agriculture has also been implemented by the Algerian High Commission for the promotion of the Steppe (HCDS) in dry and semi-arid regions including Tebessa, Khenchela, and Souk-Ahras (Northern-East Algeria). Cactus pear has taken a large importance in agricultural economics [5]. Also, *Opuntia ficus-indica* (OFI) contributes to sustainable food and feed production for humans and animals in countries with very low rainfall [6]. It produces a highly nutritive fruit and the cladodes which are used fresh green vegetable and salad. In Algeria, the cactus is grown mainly as a fruit crop and is a major consumer product generating huge quantities of seeds. In addition, the cactus could replace common plant species, especially in arid and semi-arid areas where the animal production section frequently suffers from low efficiency and high losses [7].

According to certain reports, cacti are a source of vitamins and minerals that are highly valuable in food and can be used in a variety of pharmaceutical and cosmetic industries [8]. The cactus pear shows a relatively high polyphenols and betalain compounds [9], which are bioactive compounds with positive health effects [10]. Indeed, it has been used in traditional folk medicine because of its role in treating a number of diseases, including anti-inflammatory effects, hypoglycemic effects, inhibition of stomach ulceration and neuro-protective effects [1,11,12]. Moreover, they are used for anti-hyperlipidemic and antiviral activities [13]. In addition, other investigations have demonstrated the potential role of protecting the liver [14] and inhibition of tumor cell growth [15]. Likewise, several studies showed that *Opuntia ficus-indica* juice present an important source antioxidant [13,16].

The animal gastrointestinal tract contains crucial physiological functions [17]. Several factor such as host genetics, nutrition, and treatments with antibiotics can affect the microbiota and its metabolic process [18]. It is important to remind that plant extracts function as prebiotics, altering the commensal microbiome, the composition, and/or the metabolism of the intestinal microbiota, potentially improving the host's health. [19].

It has been demonstrated that phenolic compounds and their metabolites shown a positive impact on maintaining gut health through encouraging the growth of beneficial microbiota [20] and limiting the proliferation of pathogen bacteria such as *Clostridiumperfringens* and *Bacteroides* spp. [21]. The polyphenol compounds may also affect the gut microbiota composition [22-24]. Studies reported that several factors may considerably influence on serum biochemical parameters of chickens including feed additives [25, genotype and environmental temperature [26]. There are numerous locally accessible and nutritionally sufficient plants that can be added to animal diet. Numerous studies have shown that the quantity and characteristics of plant extracts can raise the biochemical indices in broilers [27-30].

In recent years, the use of growth promoters of plant origin as a natural additive in poultry feed has aroused particular interest in poultry farmers. Many studies have been carried out on animal nutrition using the alternative feedstuffs such as co-products due to constraints environmental and food production costs. However, it is very necessary to verify the effect of co-product on the animal health and the quality of food of animal origin. Herb extracts in animal nutrition stimulate feed intake by the secretion of endogenous enzymes, antibacterial effect and antioxidant potential.

The digestive tract's physiological and biochemical processes, particularly the liver's, may be impacted by the active plant compounds. The analysis of biochemical pa-

Received: 03.06.2025
Accepted: 30.06.2025
Published: 17.02.2026

DOI:10.52331/v30i3wr63

rameters is important approach in the management of animal health; it is a tool common to assess nutritional status and monitor health and disease in farm animal [31]. Moreover, the biochemical blood indices are information helpful in revealing health disorders already in the preclinical stage. Additionally, numerous researchers have already demonstrated the effect of plant extracts in the diet on the biochemical parameters in treatment [32-35]. However, to the author's knowledge, little research has focused on the impact of prickly pear seed meal; a plant is widely present in the Algerian rural, in broiler chicken's livestock. Therefore, the aim of the present study was to assess the effect of prickly pear (*Opuntia ficus-indica* L.) seed cake on biochemical parameter (lipid, protein, carbohydrate, mineral metabolism and liver enzymes) and gut microbiota in broiler chickens.

2. Materials and Methods

The experimental protocol was approved by the Scientific Faculty Council of the University of El-Tarf (Report of Faculty Scientific Council #06 dated January 23, 2024, Algeria) and the authors followed the regulations applied in University of Liege (Belgium).

2.1. Animals, Experimental Design, and Management

A total of 150 Arbor Acres broiler chicks male and female were purchased from a commercial hatchery at one day of age. Their average weight was 45±19.54 g. Chicks were randomly allocated to three groups [3 replicates (pens of 2 m²) per group and 10 broilers per replicate] for a 44-day study. Broilers of each group were kept with optimum natural ventilation and room temperature maintained at 35°C during the first week of age, and gradually decreased to 25°C until the end of the study. Lighting regime was used throughout the experimental period (24 hours of lighting for the first five days; then 18 h light/ 6 h dark every day until the end study). Birds were vaccinated against Gumboro (IBA-VAC®), and Newcastle (BIO-VAC® B1) diseases. In order to prevent coccidiosis, the chicks were treated by anticoccidial (HIPRAVIAR®) at 15 and 28 days during 3 days. Chicks were managed according to the guidelines suggested by Cobb Broiler Commercial Management Guide (Arbos Acres Plus).

The animals were fed for 44 d, including starter (Day 1 - Day 12), growth (Day 13 - Day 32), finisher (Day 33 - Day 39) and withdrawal (Day 40 - Day 44) phases. NRC nutrient requirements from 1994 were satisfied by the diets. The chicks were divided into four groups: one control group, which was provided basal diets, and three experimental groups, which were fed meals supplemented with 10%, 20%, 30%, and 40% OFI prickly pear seed (PPS) meal. Every chick had *ad libitum* access to feed and water.

2.2. Blood and intestinal Sample Collection

Five chickens were chosen randomly from each treatment group at 45 days of age. After 12 h of feed withdrawal (except the water offered *ad libitum*), blood samples (n = 5) were collected after slaughter from the jugular vein using in EDTA tube. Serum was rapidly separated after centrifugation at 3,000 rpm for 10 min and stored at -20 °C until use for biochemical parameters assay. To determine the cecal microbiota, five cecal contents were collected aseptically in sterile plastic tubes and kept at 4°C. The cecal digesta was processed within 24h at the Pasteur Institute of Algeria (Algiers, Algeria).

2.3. DNA Extraction and PCR Amplification

Cecal samples were used for DNA extraction using the InnuPREP Stool DNA Kit (PREPStool DNA Kit, AJ Innuscreen GmbH, 13125 Berlin, Germany) following the manufacturer's instructions. The Microbial DNA was transferred to the microbiology laboratory of Department of Food Sciences (Faculty of Veterinary Medicine, University of Liège, Belgium). The total quantity of viable bacteria in each sample was estimated using qPCR after PMA treatment, as previously indicated (Reference). The V2-V3 region of the 16S rRNA genes were PCR-amplified from the microbial genomic DNA with primers (forward (50-GAGAGTTTGATYMTGGCTCAG-30) and reverse (50-ACCGCGGCTGCTGGCAC30)) using a real-time qPCR system (PRISM 7900HT) [36]. Clusters of sequences have been categorized as operational taxonomic units (OTUs), and sample OTU sequences (with a cutoff threshold of 0.03) have been categorized as taxa using the VSEARCH algorithm [37]. Sixteen reference alignments and taxonomic assignments from phylum to genus were conducted using MOTHUR based on the SILVA database (v1.38) of full-length 16S rRNA sequences (ribosomal Silva).

2.4. Serum Biochemical Parameters

A multi-parameters automatic biochemical analyzer (ARCHITECT ci4100®, Abbott, US) was utilized for all blood parameter test procedures. The photometric method was used to measure the serum levels of blood glucose (Glu), total protein (TP), triglycerides (TGs), total cholesterol, HDL, LDL, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), urea (Ur), creatinine (Cre), blood uric acid (BUA), and alpha fetoprotein (AFP). Using the potentiometric approach, ionic parameters like calcium, magnesium, and phosphorus are measured. Each parameter was determined in duplicate simultaneously at 37°C.

2.5. Statistical analysis

The data showed the descriptive mean ± standard deviation. One-way ANOVA analysis was performed using the SAS® software (version 9.4, Institute Inc, Cary, NC, USA). The graphs were performed with the GraphPad Prism 8.00 software package (GraphPad Software, San Diego, CA, USA). Fisher's PLSD post hoc analysis was performed to make statistical comparisons to analyze the differences between groups. P<0.05 was considered to be significant.

3. Results

The effect of the substitution rate of prickly pear cake (*Opuntia ficus indica* L.) on blood glucose and total protein in broiler chickens are presented in Table 1. Blood glucose concentration and total proteins values were significantly low in the group treated with 40% PPS (1.28±0.37 and 12.83±5.52 g/l, respectively) (P<0.05). The results of serum lipid parameters shown that the 10%, 20%, 30% and 40% PPS dietary treatments significantly (P<0.05) reduced the concentration of total cholesterol, HDL, LDL and triglyceride compared to control group of broiler chickens (Table 1).

Table 1. Serum biochemical profile of broiler chickens fed diets with different inclusion levels of prickly pear (*Opuntia ficus-indica* L.) seed cake at 45 days of age.

Parameters	PPS 0%	PPS %	PPS 20%	PPS 30%	PPS 40%	SEM	P >F	Feed effect	R^2
Glu (g/L)	2.36^a±0.52	1.87^b±0.54	1.68^c±0.37	1.63^c±0.33	1.28^d±0.37	0.10	<.0001	***	0.57
TP (g/L)	28.22^a±3.23	26.58^b±10.37	19.49^c±6.69	17.42^d±7.57	12.83^e±5.52	1.63	<.0001	***	0.59
TCH (g/L)	1.12^a±0.22	0.95^b±0.27	0.79^c±0.18	0.8^c±0.12	0.71^c±0.26	0.05	<.0001	***	0.53
HDL (g/L)	0.72^a±0.12	0.59^b±0.19	0.56^b±0.12	0.55^b±0.08	0.50^b±0.18	0.03	0.0001	*	0.53
LDL (g/L)	0.21^a±0.07	0.21^a±0.07	0.14^b±0.07	0.12^b±0.04	0.12^b±0.07	0.01	<.0001	**	0.42
TG (g/L)	0.87^a±0.33	0.71^a±0.31	0.60^b±0.22	0.58^b±0.37	0.43^c±0.29	0.07	0.0016	**	0.43

[a,b,c,d,e] A significant difference in carcass part characteristics between the control group (0% PPS) and the treated groups (10%, 20%, 30% and 40% PPS) is indicated by letters (P< 0.05). SEM = Standard error of the mean.

Glu: glucose, TP: total protein, TCH: total cholesterol, HDL: high-density lipoprotein cholesterol, LDL: low-density lipoprotein cholesterol, TG: triglycerides

According to our findings, the broiler chickens' serum activity of CGT, ASAT, and ALAT was considerably (*P*<0.05) lower in the PPS cake-treated group than in the control group. However, in experimental broiler chickens, PPS cake supplementation had no effect on the blood activity of urea and creatinine (*P*>0.05) (Table 2).

Table 2. Serum biochemical profile of broiler chickens fed diets with different inclusion levels of prickly pear (*Opuntia ficus-indica* L.) seed cake at 45 days of age.

Parameters	PPS 0%	PPS %	PPS 20%	PPS 30%	PPS 40%	SEM	P >F	Feed effect	R^2
GGT (U/L)	22[a]±3.25	20.6[b]±5.65	16.6[c]±4.89	16.9[c]±4.40	15.7[d]±7.37	1.24	0.0016	***	0.42
ALAT (U/L)	7.8[a]±1.89	6.86[b]±1.24	6.4[c]±0.63	6.68[b]±1.10	7.53[a]±1.68	0.34	0.0295	***	0.30
ASAT (U/L)	208.06[b]±35.92	224.2[a]±62.51	193.13[c]±89.72	152.91[d]±38.62	155.46[d]±56.28	16.07	0.0071	***	0.25
Ur (g/L)	0.4[a]±0.0	0.4[a]±0.0	0.4[a]±0.0	0.4[a]±0.0	0.4[a]±0.0	0.00	.	NS	0.00
Cre (mg/L)	2.13[a]±0.35	2.06[b]±0.25	2[b]±0.0	2[b]±0.0	2[b]±0.0	0.05	0.26	NS	0.16
BUA (mg/L)	48.38[a]±15.83	39.48[c]±16.34	41.47[b]±15.66	40.31[b]±15.44	32.42[b]±13.43	3.95	0.09	***	0.23

[a,b,c,d,e] A significant difference in carcass part characteristics between the control group (0% PPS) and the treated groups (10%, 20%, 30% and 40% PPS) is indicated by letters (*P*< 0.05). SEM = Standard error of the mean.

GGT: gamma-glutamyltransferas, ASAT: aspartate aminotransferase, ALAT: alanine aminotransferase, Ur: Urea, Cre: Creatinine, BUA: blood uric acid

The Table 3 illustrates the Calcium (Ca), Phosphorus (P), Magnesium (Mg) concentrations in broiler chickens treated with 10%, 20%, 30% and 40% PPS cake concentrations. In general, blood ionogram showed that values of Mg, P, and Ca reduced significantly after period 44 days of treatment with 10%, 20%, 30% and 40% PPS cake concentrations compared to control group (*P*<0.05). Except, K+ values significantly increased treated group with 10% of PPS (*P*<0.05), and it did not vary in treated group with 20% of PPS in relation to the control group (*P*>0.05).

Table 3. Ionic parameters profil of broiler chickens fed diets with different inclusion levels of prickly pear (*Opuntia ficus-indica* L.) seed cake at 45 days of age.

Parameters	PPS 0%	PPS %	PPS 20%	PPS 30%	PPS 40%	SEM	P >F	Feed effect	R^2
Mg (mg/L)	21.62[a]±3.03	16.8[c]±4.15	18.38[b]±6.09	16.96[c]±3.69	16.69[c]±5.62	1.06	0.0073	***	0.43
P (mg/L)	40.70[b]±13.13	49.83[a]±13.55	40.02[b]±8.57	33.02[d]±10.71	38.21[c]±13.00	2.94	0.0041	***	0.36
Ca (mg/L)	77.3[a]±11.59	62.01[b]±21.36	54.28[c]±12.54	54.72[c]±11.91	45.43[d]±11.83	3.52	<.0001	***	0.51
AFP (ng/ml)	0.01[a]±0.01	0.01[a]±0.00	0.01[a]±0.00	0[a]±0.00	0.01[a]±0.01	0.002	0.0002	NS	0.45

[a,b,c,d,e] A significant difference in carcass part characteristics between the control group (0% PPS) and the treated groups (10%, 20%, 30% and 40% PPS) is indicated by letters (*P*< 0.05). SEM = Standard error of the mean.

Mg: magnesium, P: phosphorus, Ca: calcium, AFP: alpha-fetoprotein.

A principal coordinate analysis (PCoA) plot illustrating the variations in the intestinal microbial composition of broiler chicks fed different cake diet groups (0%, 10%, 20%, 30%, and 40% PPS) is depicted in Figure 1. Samples from the 10% and 20% PPS cake groups clustered closer together than samples from the other groups.

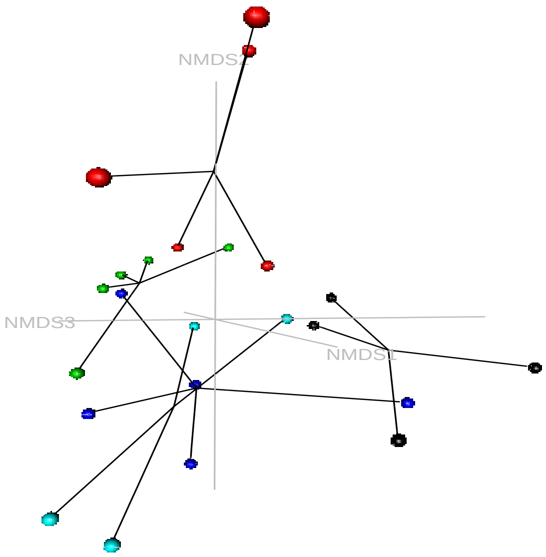

Figure 1. A principal coordinate analysis (PCoA) plot showing dissimilarities among different diet groups. Each dot represents a single sample. Black, red, green, blue and cyan blue color denote 0%, 10%, 20%, 30% and 40% PPS cake diet, respectively.

Permutational multivariate analysis of variance (ANOVA) was also used to determine the significant differences between groups. The significant difference between the five groups was observed (*P*-value < 0.05; Table 4). In the present study, bacterial analysis revealed that broiler chickens treated with *Opuntia ficus-indica* seed meal exhibited a variety of phylum in gut microbiota (Figure 2).

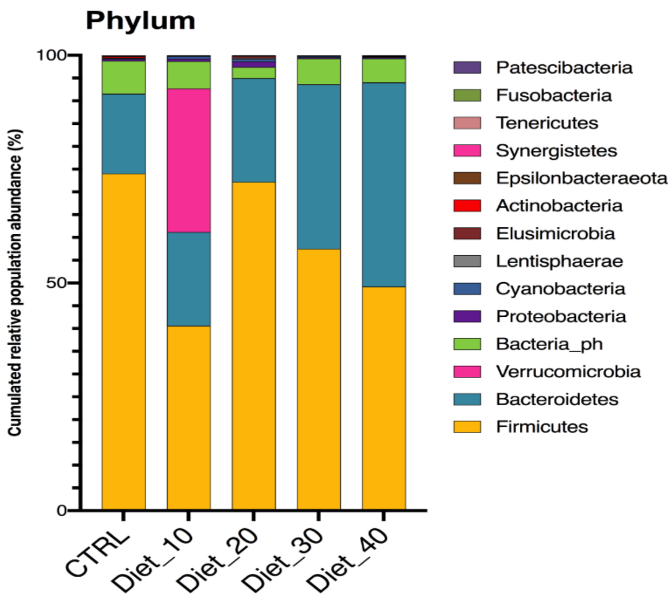

Figure 2. The intestinal microbial composition and species diversity of chickens fed diets with different concentration of PPS cake at the phylum level. Bar plots represent the percentage (%) of average abundance for groups.

At the phylum level, Firmicutes and Bacteroidetes were the major groups of microorganisms in the gut of broiler chickens received PPS cake diet, ranged 40-72% and 20-44%, respectively. On the other hand, the 10% PPS diet contains also 31% of *Verrucomicrobia* compared to other diet groups (*P*< 0.05). As shown in Figure 3, there are some changes in the structure of gut flora after PPS cake treatment compared to control group, according to taxonomic research. *Lachnospiraceae, Bacteroide, Akkermansia, Bacteroidales* were the most prevalent genera.

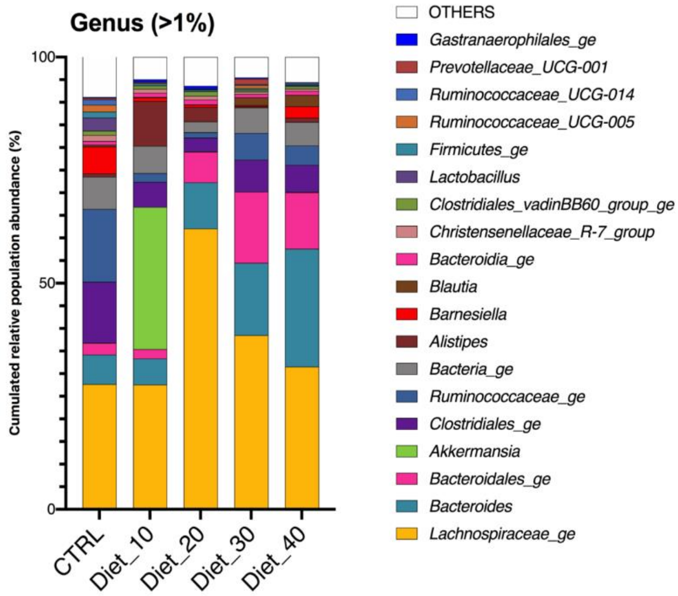

Figure 3. The intestinal microbial composition and species diversity of chickens fed diets with different concentration of PPS cake at the genus level. Bar plots represent the percentage (%) of average abundance for groups.

4. Discussion

Blood biochemical profiles are mainly used as indicators of an animal's physiological and metabolic condition [38]. Many researchers reported on positive effects of herbs inclusion diet on performance and health status of broiler chickens [39-41], and also can have a positive effect on lipid metabolism and antioxidant status [42]. Furthermore, plant extracts have been shown to improve the activity of digestive enzymes [42] and control the kidney and liver functions in chickens [43]. In addition, the use of plant as dietary supplement can improve hematological indices and serum biochemistry profiles of broiler chickens [44]. Plant extracts were continually experimented, as growth additive, in animal feed due to their biological benefits in improving digestion efficiency and animal health status [45-47]. Phytoadditive used on livestock and poultry are excellent alternatives to antibiotic-based growth promoters due to their proven positive effects on animal growth and health [48]. Essential oils are increasingly appreciated in animal feed due to their high biological activity. To our knowledge, the current study is the first on the impact of different feed diet level of OFI seed cake on serum biochemical indices and gut microbiota in Arbor Acres broiler chicken.

According to the current results, the PPS dietary treatments considerably (P<0.05) decreased the concentration of lipid parameters in the broiler chickens compared to the control group, of serum lipid parameters. There were significant differences (*P*<0.05) between the treated groups for blood glucose concentration and total proteins values, with higher values in broiler chickens supplemented with different PPS concentrations compared to the control group. These results are in agreement with results obtained previously, where [49], where *Opuntia ficus-indica* cladode, fruit reduce glycemia and cholesterol and triglycerides level in diabetic experimental animals. Moreover, Moula et al. (2019) [50] reported that the addition of 10% prickly pear cladodes powders decreased significantly (*P*<0.05) the concentrations of glucose, triglycerides and cholesterol in broiler chickens. In another study, the biochemical analysis showed that white male rabbits given an aqueous extract of *Opuntia ficus indica* had considerably lower blood plasma levels of glucose, cholesterol, and triglycerides [1]. This hypoglycemic effect due to the fact that the cactus is very rich in dietary fiber which increases the enzymatic activity of certain microbes, resulting in an increase in sugar production microbial enzymatic activity, which leads to the inhibition of hydrolysis [51].

Likewise, Jibril et al. (2018) [52] demonstrated that supplementation with powdered balsam apple (*Momordica balsamina*), contain biologically active phytochemicals such as flavonoids, leaf had significantly decrease on blood glucose level Japanese quails. Recently, Gazwi et al. (2022) [53] reported that the glucose, cholesterol, LDL and triglyceride concentrations was significantly decreased upon the supplementation of *C. sativum* and *C. intybus* extracts, riche in flavonoids, in the diet of broiler chicks at six weeks of age. The presence of secondary chemicals in cacti may be the cause of the decrease in glucose concentration since they increase insulin secretion and improve muscle absorption and metabolism of glucose. In addition, flavonoids could be linked to antidiabetic properties by binding to starch, boosting hepatic glycolysis and the glycogen level, and reducing hepatic gluconeogenesis [54,55] Moreover, Padilla-Camberos et al. (2015) [56] concluded that *Opuntia ficus-indica* cladode extract is able to prevent hypercholesterolemia by pancreatic lipase inhibition in mice, in part due to its polyphenolic compounds. Similarly, it has been found that plant extracts containing flavonoids can improve the lipid metabolism of broiler chicks [57].

The liver and kidney are of the most vital organs of living organisms. They has a crucial role in detoxification and metabolism, that to be susceptible to damage from metabolic disorders. The status of liver and kidney function can be measured via the increase or decrease in serum levels of biochemical parameters such as CGT, ASAT, ALAT, urea and creatinine, which are considered diagnostic tools to assess toxicity [58,59]. The decreased activity of ALAT and ASAT indicated the hepatoprotective nature of the PPS cake contained bioactive compounds due to its antioxidant properties.

It is important to underline that prickly pear seeds (PPS) meal of OFI possesses a significant concentration of flavonoids and secondary metabolites, including gluconic acid, pscidic acid, ferulic acid diglucoside and quercetin-3-O-galactoside [60]. This is consistent with the findings of Bouazza et al. (2016) [61] studied the effect of OFI in liver steatosis created by feeding rats a high-fat diet, who discovered that incorporation of the vinegar obtained from prickly pear have hepatoprotective effects in rats. Indeed, numerous studies have shown that *O. ficus-indica* has hepatoprotective properties [62-64]. Likewise, the results of the present study showed that serum creatinine, urea and blood uric acid levels were unchanged in the groups treated with PPS cake and the control group. In the other hand, blood uric acid level was significantly low in PPS cake compared to the control group (P<0.05). These results indicated that PPS cake had no deleterious effects on renal function. In addition, the extract of OFI fruits contains antioxidant molecules such as quercetin, myricetin, and luteolin which can synergistically offer a renoprotective effect, as noted by Okur et al. (2020) [65].

A number of factors, such as the manner of supply and the presence of other nutrients, influence the percentage absorbed and the efficiency of macronutrient absorption. It is most likely that the absorption Ca, P and Mg was low in intestine tract during digestion the prickly pear seeds meal of OFI, resulting in reduce in serum concentration. However, it should be noted that *Opuntia ficus indica* seeds contains a large variety of minerals with a predominance of potassium, phosphorus magnesium and calcium [40].

It is very important to remember that cacti (*Opuntia ficus-indica*) possess the antinutritional factor calcium oxalate, which binds to calcium and possibly other minerals in a nutritionally unavailable form, thus interfering with the bioavailability of minerals for animal absorption [66]. Phytate occur in grains and seeds of crops, which this compound forms insoluble complexes with divalent ions of metals (Ca, Mg, Zn, Fe) and with phosphorus, reducing their bioavailability and thus increasing the requirement of such components [67]. This reduce may be due to the effect of phytate and oxalic acid present in the cactus pear seeds because it is an organic compound that binds to calcium or other minerals in an unavailable nutritional form, affecting the availability for absorption by the broiler chickens [68-70]. On the other hand, no significant differences were observed between in alpha-fetoprotein (AFP) values of treated group with PPS cake and control group of broiler chickens (P> 0.05). The results obtained of the present investigation indicate clearly the absence of malignant transformation in broilers chickens. Results of the anticancer activity of *Opuntia ficus-indica* were reported previously by Ali et al. (2022) [71], that flavonoids of OFI possessed many notable biological activities; such as anti-oxidant and anti-carcinogenic activities. Moreover, Chavez-Santoscoy et al. (2009) [72] reported that *Opuntia violaceae* (Spiny, purple peel and purple red pulp) contained the highest flavonoids and diminished both prostate and colon cancer cell viability.

Animal health and growth performance depend on maintaining a healthy degree of intestinal development. The digestive tract of chickens contains to a wide range of microorganisms coexist in symbiotic interactions that impact immunity, metabolism, and nutrition [73]. Several investigations reported that have reported a wide variety of plant extracts improve intestinal health indices in poultry [74-76]. To elucidate the differences in microbiota exposed to different amounts of PPS cake, a principal coordinate analysis (PCoA) based on measurements the intestinal microbial composition was performed, which it makes possible to visualize variation between samples and possibly identify groups by projecting observations into a space of reduced dimensions (Fig. 1).

The results of the current research are consistent with previous scientific report where almost 90% of bacteria in the gut belong to two major phyla Firmicutes and Bacteroidetes [77]. As shown in Fig. 3, there are some changes in the structure of gut flora after PPS cake treatment compared to control group, according to taxonomic research. *Lachnospiraceae, Bacteroide, Akkermansia, Bacteroidales* were most prevalent genus. After the oral administration of 10% PPS cake, compared with the control group, the proportion of *Akkermansia* was significantly increased (P<0.001). In contrast, the 20%, 30%, and 40% PPS cake diet groups had significantly higher (P<0.05) proportions of *Lachnospiraceae*. Additionally, the groups who had 30% and 40% PPS cake diets exhibited significantly larger relative abundances of Bacteroidales than the control group. Over 900 different kinds of bacteria, as well as some protozoa, fungus, yeast, and viruses are housed in the gastrointestinal tract (GIT) of chickens. These microorganisms are collectively referred to as the microbiome or microbiota and help the host break down and utilize the feeds that are consumed [78].

In fact, a number of probiotic strains are added to poultry feed in addition to their natural gut microbiome in order to increase the number of known beneficial microbes, which to prevent dysbiosis or to reduce the load of pathogenic microbes through to the use of antibiotic growth promoters [79,80]. As reported by numerous authors, the essential oils of certain plants fortified the gut microflora by reducing harmful bacteria numbers and increasing beneficial bacteria populations [81]. Our investigation revealed that the PPS cake diet group and the control group differed somewhat in the relative abundance of specific gut bacteria.

It is also important to underline that a dietary intake of PPS seeds cake was able to reduce the levels of Firmicutes while increasing the relative abundance of Bacteroidetes and *Verrucomicrobia*. Phyla Bacteroidetes are gram-negative bacteria that ferment polysaccharides and other indigestible carbohydrates producing short-chain fatty acids that are gut-friendly [82]. The remarkable effect of PPS cake on microbiome could be attributed to the constituent bioactivity of the prickly pear (*Opuntia ficus-indica* L.) seed cake and the nature of the compounds in the functional group. Likewise, Viveros et al. (2011) [83] reported that dietary polyphenol-rich grape products modify the intestinal microflora and increase the biodiversity degree of intestinal bacteria in broiler chickens. It should be noted that prickly pear seeds are an important source of natural fiber and, given its high linoleic acid content, its oil can be used as a nutraceutical agent [84]. Thus, the dietary fiber can modulate the gut microbiome and promote the growth of beneficial bacteria that would be required to improve broiler performance [85]. Moreover, anthocyanins can control intestinal flora because of the diverse range of bioactive compounds found in *Opuntia ficus-indica*[87].

5. Conclusions

Overall, the results obtained in this study demonstrated that broiler chickens' biochemical parameters were lowered when prickly pear seed meal (*Opuntia ficus indica* L.) According to this study, broiler chickens' renal and liver function may be protected by PPS cake. Also, it found that broiler chickens treated with *Opuntia ficus-indica* seed meal exhibited a variety of phylum and genus in gut microbiota. There are increasing the abundance of Firmicutes, Bacteroidetes and *Verrucomicrobia* in broiler chickens received the PPS cake diet. However, further studies are necessary enhance our understanding of the possible mechanism by which bioactive compounds of PPS affects the gut microflora of broilers.

Author contributions: MB: methodology, investigation, writing original draft; JLH: supervision, writing-review & editing; ID: formal analysis, investigation; AB. and LT: data curation, software; OB; E-HB and NBD: visualization, validation, formal analysis, data curation; KI; MI; UA and DAA: writing-review & editing; AA: supervision, conceptualiza-

tion, validation, writing original draft, writing-review & editing, supervision. All authors read and approved the final manuscript.

Funding: Not applicable.

Institutional Review Board Statement: The experimental protocol was approved by the Scientific Faculty Council of the University of El-Tarf (Report of Faculty Scientific Council #06 dated January 23, 2024, Algeria) and the authors followed the regulations applied in University of Liege (Belgium).

Data Availability Statement: All data generated or analyzed during this study are included in this article.

Acknowledgments: The authors thank the General Directorate of Scientific Research and Technological Development (DGRSDT/MESRS-Algeria) for supporting the project. The authors thank the staff of the Medical Biology Laboratory, Institut Pasteur (El-Hamma, Algeria) for their technical assistance.

Conflicts of Interest: The authors declare no competing interests**.**

Consent for publication: Not applicable.

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