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In vitro biological activity of *Opuntia ficus indica cladode* powder and the effect of its incorporation on broiler chick growth performance

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Abstract

This study aims in part to valorize the cladodes of *Opuntia ficus-indica* (OFI), commonly known as prickly pear, widely spread in the Algerian rural landscape, as a feed additive for broiler chickens. The objective of this study was to measure the effects of incorporating OFI powder into the diet on the growth performance of broiler chickens. The experiment was conducted at the animal house of Hassiba Ben Bouali University of Chef, on sixty-four (64) day-old, unsexed Cobb 500 chicks. The chicks were divided into four groups (n=16) according to the diet. The animals received daily 4 rations T1, T2, T3 and T4 containing respectively 0%, 1%, 2% and 3% of the prickly pear pad powder. Analyses revealed a rich nutritional composition and good antioxidant activity of the powder. The incorporation of OFI powder improved the feed conversion ratio (FCR) of the animals ($p<0.0001$), with the lowest FCR recorded in batch 2, while the control batch had the highest values. A non-significant improvement ($p=0.353$) was observed in growth performance. The 2% batch showed the highest slaughter weight (2437g), while the chickens in the control batch appeared the lightest (2320, 78g), compared to the experimental batches. An interesting result is the increase in featherweight in the batch receiving 3% powder.

Keywords: *Opuntia ficus-indica*, broiler chicken, Algeria, growth performance, antioxidant activity.

Introduction

In Algeria, chicken meat consumption is constantly increasing due to its nutritional value and lower production cost compared to red meat (Metidja and Laoumir, 2023). However, food quality and safety are becoming growing concerns for consumers, as is the environmental impact of intensive farming. Indeed, the use of feed additives such as nitrogen, vitamin, and mineral concentrates, as well as coccidiostats and anti-infective, can compromise the health of poultry and, consequently, that of the final consumer (Mbanza et al., 2023). Furthermore, the dependence on imports for animal feed affects prices and raises questions about the quality of poultry feed (Mbanza et al., 2023). Indeed, the majority of components used in animal feed are imported from abroad, which puts pressure on the price of chicken meat on the domestic market and on production costs (Moula et al., 2019). Faced with these challenges, the use of natural additives, such as prickly pear (*Opuntia ficus indica*, OFI), could offer a healthy and promising alternative (Hernández-Becerra et al., 2022). Maiuolo et al., 2024 indicate that OFI cladodes have a very varied composition with the main constituents being fiber, polysaccharides, proteins, fatty acids, vitamins, sterols, minerals and polyphenols, which are responsible for many beneficial activities for human health, such as antioxidant, anti-inflammatory, anticancer and nutritional properties. The objective of this work is to evaluate the effect of adding prickly pear (*Opuntia ficus-indica*, OFI) cladode powder to the diet of broiler chickens on their growth performance.

II. Materials and Methods

II.1. Material

The experimental study was conducted at the animal house of Hassiba Ben Bouali University, Ouled Fares, Chlef, from April 24th to June 9th, 2024, for a total duration of 47 days. The animal house facilities include partitions that create spacious pens for each group of animals, thus ensuring a clean and hygienic environment. Feeders and drinkers were installed to guarantee permanent access to feed and fresh water. Lighting, temperature and ventilation were carefully controlled to create ideal conditions for the growth and comfort of the animals. We ensured that the litter was dry and clean, which reduces stress and thus promotes natural behavior in the chicks

II.2. Plant Material

The prickly pear pads were harvested in the wilaya of Chlef, carefully cleaned and cut, then dried at a temperature not exceeding 50°C, and finally ground into a fine powder ready for analysis and incorporation into the chicks' feed.

II.2. Animal material

Sixty-four (64) day-old, unsexed Cobb 500 chicks were used in this study. They were divided into 4 homogeneous groups (n=16), with 1 control group (T) receiving the standard feed and 3 experimental groups receiving the same standard feed supplemented with 1%, 2% or 3% of prickly pear pad powder for groups 1, 2 and 3 respectively. During the entire rearing period, from starter (1-15 days), grower (16-30 days) to finisher (31-46 days), the animals received a commercial standard feed, supplied by the feed mill NUTRIFOR, located in the commune of Boukadir, wilaya of Chlef. This feed is composed of corn, soybean meal, wheat by-product, phosphorus, limestone, salt and a vitamin-mineral premix. The chemical composition of the feed is given in Table 1."

Table 1: Standard feed composition

	Moisture (%)	**Fat content (%)**	**Protein content (%)**	**Fiber content (%)**	**Ash content (%)**	**Starch content (%)**
Start	11,83	4,27	22,63	2,48	4,39	40,40
Growth	12,82	3,84	20,99	2,59	3,77	41,33
Finishing	12,72	4, 31	18,38	2,71	3,05	45,10

II.2. Methods

II.2.1. Chemical analyses

The analyses of the feed distributed to the animals were carried out by infrared spectrophotometry at the Nutrifor laboratory.. The analyses concerned the moisture, ash, fat, fiber, protein and starch content. The chemical analyses of the powder were carried out in Győr, Hungary, at the MEZOLABOR laboratory and at the analysis laboratory belonging to the feed mill AGROFEED. The analyses focused on the dry matter, protein, fat, fiber, ash, starch, ADF, NDF, ADL, Sodium (Na), Calcium (Ca), Phosphorus (P), vitamin E, cellulose and amino acid content (Lysine,

Methionine, Cystine, Alanine, Arginine, Aspartic acid, Glutamic acid, Glycine, Histidine, Leucine, Isoleucine, Phenylalanine, Proline, Serine, Threonine, Tyrosine, Valine).

II.2.2. Determination of antioxidant activity of *OFI* snowshoe powder

The antioxidant activity of the powder was evaluated at the plant bioresources research laboratory of the University of Hassiba Benbouali of Chlef. Methanolic extracts were prepared to evaluate the antioxidant activity by the DPPH (2,2-diphenyl-1-picrylhydrazyl) test, which measures the ability to scavenge free radicals. The radical scavenging potential was expressed in terms of the median inhibitory concentration (IC50), representing the concentration of the extract necessary to inhibit 50% of the DPPH radicals.

II.2.3. Determination of zootechnical parameters

The zootechnical parameters studied include feed consumption, where the quantities of food ingested were estimated daily by weighing the quantities of feed distributed and refused. The feed conversion ratio (FCR) was measured to evaluate feed efficiency, by relating the quantities of feed consumed to weight gain. The weight performance of the animals was determined by weekly weighing, allowing the calculation of average weight and average daily gain. Carcass weight and yield were also evaluated after slaughter. Mortalities were recorded throughout the study and autopsies were performed to determine the causes. The mortality rate was calculated by the ratio of the number of deaths recorded during the rearing period to the total number.

II.2.4. Statistical analysis

Statistical analyses were performed using XLstat 2016. Means and standard deviations were calculated, and analysis of variance was used to compare the different treatment groups.

III. Results and discussion

III.1. Results of chemical analyses

The water content of our fresh cladodes is 93%, slightly higher than the value of 88.46 to 92% recorded by El Kharrassi et al. (2015). This slight variation can be attributed to differences in growth and harvest conditions. Indeed, the moisture content of fresh pads can vary depending on various factors, such as climate and the growth environment of plants (Stintzing and Carle, 2005).

The protein content of our samples (Table 2) falls within the 4-10% DM range recorded by Stintzing and Carles (2005). The same authors report a lipid content (1-4%) similar to that found for our cladodes. The ash content is within the range of 12.97 - 25.65% reported by El Kharrassi et al. (2015), but it is slightly higher than that of Stintzing and Carle (2005), varying from 19% to 23%. Our samples have a significantly lower fiber content than that of Stintzing and Carle (2005), which is 18%. Our cladodes contain a significant amount of vitamin E, which is a key antioxidant for combating oxidative stress. Cuvelier et al., (2003) indicate that the vitamin E content in vegetables is generally higher in mature green leaves.

Table 2: Main components of prickly pear cladodes

Component	**Test value**
Crude protein	8,767 ± 0,18%
Crude fat 'A	1,77 ± 0,5%
Crude fiber	10,52 ± 0,4%
Crude ash	234 ± 5,9 g/kg
Cellulose	73 g/kg
ADF	93 ± 5 g/kg
NDF	177 ± 9 g/kg
ADL	20 ± 5 g/kg
Starch	11 ± 5 g/kg
Vitamin E	42± 8 mg/kg

The mineral composition of our cladodes (Table 03) reveals the predominance of calcium followed by phosphorus and sodium. The calcium content is lower than the value of 9.04±1.21% DM reported by Moussaoui (2020), but much higher than that of Hernandez-Urbiola et al. (2010) ranging between 17.52 and 34.4 mg/g, depending on the maturity stage of the cladodes. Compared to the cladodes of Hernandez-Urbiola et al. (2010), ours contain more phosphorus but a comparable amount of sodium, i.e. 0.236 to 0.411 mg/g and 0.2-0.55 mg/g, respectively. These variations in the mineral composition of cladodes depend, according to Hernández-Becerra et al. (2022), on various factors such as plant variety, growing region, water quality, soil and cultural practices.

Table 3: Mineral composition of prickly pear cladodes

Component	**Test value (g/kg)**
Sodium (Na)	0,32 ± 0,03
Calcium (Ca)	61,10 ± 4,28
Phosphor total (P)	1,51 ± 1

The amino acid content of our cladodes reveals a predominance of glutamic acid, followed by aspartic acid, leucine, alanine, and valine. Methionine content is the lowest. El-Mostafa et al. (2014) indicate that the main amino acids in cladodes are glutamine, followed by leucine, lysine, valine, arginine, phenylalanine, and isoleucine.

Table 4: Amino acid profile of OFI cladodes

Component	Test value %(m/m)
Méthionine	0,11 ± 0,02
Cystine	0,14 ± 0,02
Alanine	0,42 ± 0,06
Arginine	0,40 ± 0,06
Acide aspartique	0,67 ± 0,1
Acide glutamique	1,62 ± 0,24
Glycine	0,36 ± 0,05
Histidine	0,25 ± 0,04
Leucine	0,52 ± 0,08
Isoleucine	0,33 ± 0,05
Phénylalanine	0,41 ± 0,06
Proline	0,36 ± 0,05
Sérine	0,38 ± 0,06
Thréonine	0,33 ± 0,05
Tyrosine	0,26 ± 0,04
Valine	0,42 ± 0,06

he pH of the OFI powder was measured to be 4.04, which is consistent with the range of 3.54-4.56 reported by Belbahloul et al. (2020). These authors attribute this variation primarily to differences in harvest location.

II.2. Antioxidant activity

The study revealed that both the cladode extract and the control (ascorbic acid) have the ability to neutralize free radicals, and that this activity increases with concentration. The polyphenols present in our extracts are probably responsible for this antioxidant property. Indeed, studies have shown

a strong correlation between IC50 values and the content of polyphenols and flavonoids (Athamena et al., 2010; Mariod et al., 2010). It has been established that antioxidant molecules such as ascorbic acid, flavonoids, and tannins can reduce and decolorize DPPH due to their ability to donate hydrogen atoms (Bougandoura and Bendimerad, 2013). The cladode extract has an IC50 value of 11.59 mg/ml, which is higher than that of ascorbic acid at 0.06 mg/ml. The lower this value, the more powerful the extract is considered as an antioxidant. The radical scavenging activity recorded for our cladodes is lower than that described by Msaddak (2018) (1.45 mg/ml IC50), where his extract is considered a powerful antioxidant compared to our extract for cladodes of the same species.

III.3. Effect of OFI powder on the zootechnical performance of chicks

III.3.1. Effect on feed consumption

The four groups consumed slightly different amounts of feed, however, these variations are not significant (p=0.088). The incorporation of OFI powder had a significant effect (p<0.0001) on the feed conversion ratio of the animals, with group 2 recording the lowest ratio (Table 5).

Table 5: Effect of OFI powder on feed consumption

	T	LOT 1%	LOT 2%	LOT 3%	P-Value
CAM (g)	1969,54±532,03 (n=35)	1966,66±479,68 (n=35)	1959,69 ± 436,68 (n=35)	1959,6±529,01 (n=35)	0,088
IC (g)	2,347±0,68 (b,c) (n=35)	2,188±0,49 (a,b) (n=35)	2,079±0,41 a (n=35)	2,251±0,65 c (n=35)	<0,0001

CAM: Average food consumption, **CI**: Consumption index,

P: statistical significance * p<0.05. On each line, numbers marked with different letters (*a,b,c*) are significantly different at the 5% threshold.

Regarding the evolution of feed conversion ratio (FCR) over the four weeks of experimentation (Figure 1), we observed that significant sources of variation were recorded during the 3rd, 4th, and 6th weeks (p= 0.0001, 0.0001, 0.019 respectively). Indeed, group 2 recorded the lowest FCR, while group T recorded the highest. On the other hand, the incorporation of OFI cladode powder into the chicken feed did not generate any significant difference (p=0.297) in FCR among the four groups

during the 5th week. Our results showed a feed conversion ratio during the last week (day 35 to day 46) ranging from 2.12 to 2.23. These values are slightly higher than those reported at day 42 by Moula et al., (2019) (1.95 to 2.05) and the recommendations for standard broilers (1.90) presented by Sauveur (1997)

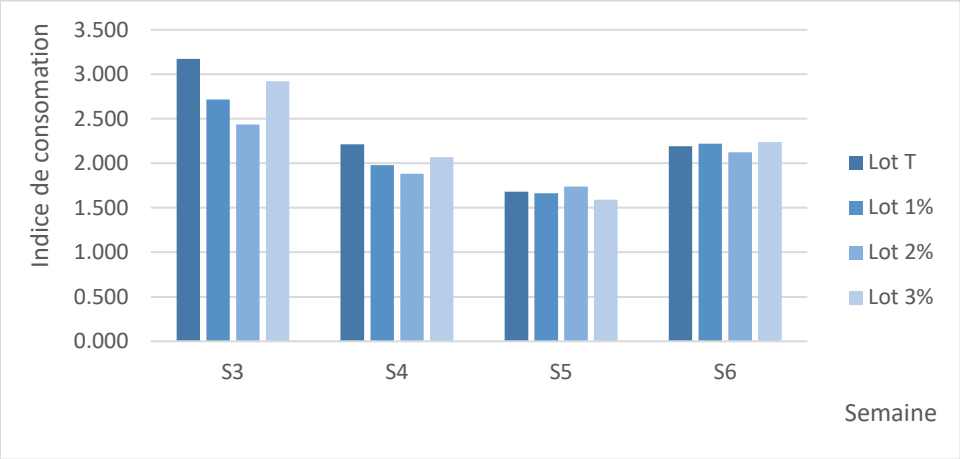

Figure 1: *Evolution of OFI's effect on consumption index over the four weeks of experimentation*

III.3.2. Effect on weight performance

The chicks in all four groups had a comparable initial live weight. Although not significant (p=0.353), the animals in the 2% group had the highest slaughter weight (2437g). In contrast, compared to the experimental groups, the chickens in the control group appeared the lightest (2320.78g). The evolution of the animals' weight (Figure 2) shows a very significant weight difference during the 3rd week (p=0.04) and the 4th week (p=0.01). During these two weeks, the animals in the 2% group were the heaviest while those in the 3% group were the lightest.

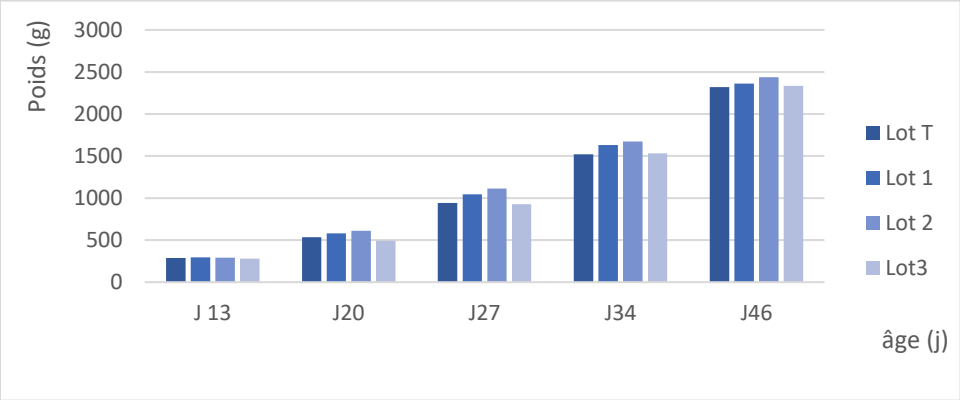

Figure 2: *Effect ofOFI powder on weight change during experimentation*

Concerning average daily gain (ADG), highly significant differences (p= 0.009) were revealed between groups during the 4th week. The 2% group had the highest ADG (71.527g) and the control group the lowest (58.125 g). The absence of significant variation between groups during the 6th week suggests that the effect observed in week 4 did not persist, the gain may have decreased due to the increase in temperature observed during this week. These results indicate that OFI powder could have an impact on average weekly gain under certain conditions or growth phases. However, the lack of significance of the differences between the groups during the following weeks suggests that the effect is not constant and could be influenced by other environmental or physiological factors. A comparable result was indicated by Moula et al. (2019) who showed that ADG was significantly different during the first 2 weeks, in favor of 5% OFI. However, no statistical difference ($p > 0.05$) was observed for the other rearing periods. The work of Lidetewold et al. (2016) shows a significant increase in weight gain with the addition of cactus fruit flour. Similarly, according to Battaa et al. (2019), final body weight, total weight gain and ADG values were significantly higher for supplementation levels of 20 and 30% dried OFI peel than for the control.

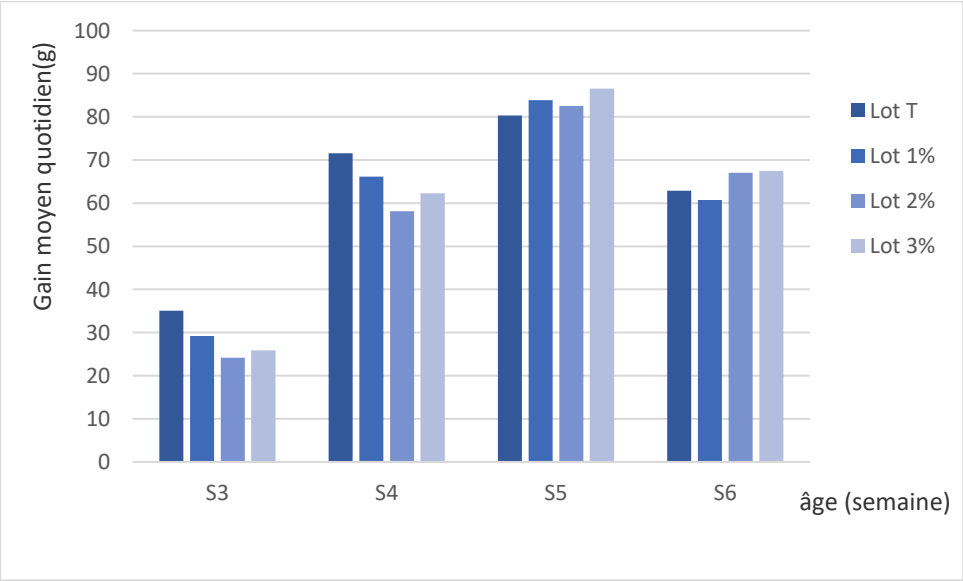

Figure 4: *Effect of OFI powder on the evolution of average daily earnings*

Regarding carcass and organ weights, apart from feather weight ($p=0.045$), no significant differences were found between the four groups. Indeed, other parameters, such as the weight of the carcass, head, legs, intestines, heart, liver, and gizzard (Table 6), did not show significant differences ($p>0.05$). These results are similar to those of Moula et al. (2019), who stated that carcass weight and characteristics were not different between the feed groups. According to the results of lesion severity in chickens presented by Badr et al. (2019), birds fed with 5% and 10% of prickly pear peels showed slightly hypertrophied and congested livers, while the 15% group showed moderate hypertrophy and pallor. The gizzards of all birds in the experimental groups did not undergo any changes.

Table 6: Effect of OFI paddle powder on organ and carcass weight

Poids des organes(g)	**Lot T**	**Lot 1%**	**Lot 2%**	**Lot 3%**	**P-value**
Carcasses **M ± ET**	1889,9±113,54 (n=10)	1919,2±109,02 (n=10)	1980,5±172,24 (n=10)	1882,5±179,18 (n=10)	0,615
Plumes **M ± ET**	93,7 ± 13,62 **a** (n=10)	82,1 ± 15,45 **ab** (n=10)	91,9±11,742**ab** (n=10)	101,4±8,592**ab** (n=10)	**0,045**
Têtes **M ± ET**	52,2 ± 5,22 (n=10)	52,6 ± 4,33 (n=10)	52,7 ± 5,03 (n=10)	50,3 ± 5,17 (n=10)	0,762
Pattes **M ± ET**	94,9 ± 17,62 (n=10)	97,8 ± 10,73 (n=10)	94,1 ± 13,7 (n=10)	95,1 ± 12,02 (n=10)	0,996
Intestins **M ± ET**	98,70 ± 16,62 (n=10)	101,60 ± 13,09 (n=10)	99,90 ± 9,85 (n=10)	116,40 ± 19,55 (n=10)	0,106
Viscères **M ± ET**	237,2 ± 32,58 (n=10)	245,7 ± 27,38 (n=10)	241,3 ± 21,12 (n=10)	255,5 ± 26,79 (n=10)	0,430
Cœurs **M ± ET**	8,60 ± 1,90 (n=10)	8,70 ± 1,34 (n=10)	8,70 ± 0,95 (n=10)	8 ± 0,82 (n=10)	0,233
Foies **M ± ET**	59,60 ± 10,54 (n=10)	55,1 ± 9,98 (n=10)	56,80 ± 9,78 (n=10)	51,20 ± 5,69 (n=10)	0,427
Gésiers **M ± ET**	70,30 ± 12,17 (n=10)	66,10 ± 7,25 (n=10)	68,60 ± 10,09 (n=10)	69,70 ± 11,11 (n=10)	0,516

($p<0.05$).

III.3.3. Effect of OFI powder on mortality rate

All mortalities occurred on the 45th and 46th days and were recorded in the control group and group 3 (one mortality), with a rate of 6.25%, and in group 2 (two mortalities) with a rate of 12.5%. These mortalities can be explained by a sudden increase in temperature (41 and 42°C) during these two days. The mortality rate observed in group 2 is not very far from that recorded by Moula et al. (2019) who reported a mortality rate of 10% in broilers fed 10% cladodes.

Conclusion

This study evaluated the effect of incorporating prickly pear pad powder into the diet of broiler chickens. It showed that this powder has a composition rich in nutrients and a good antioxidant activity. Although feed consumption, carcass yield, and offal weight did not show significant differences between groups, significant variations were observed in weight evolution on the 20th and 27th days, as well as in feed conversion ratio in weeks 3, 4, and 6. The absence of negative effects suggests that the incorporation of this powder, which comes from a low-cost local resource, can reduce feed costs in the Algerian poultry sector. An interesting result was observed in the group of chickens receiving 3% of cladode powder where an increase in feather weight was noted. This preliminary study opens up perspectives for further research. We recommend, first, testing higher rates of OFI cladode powder with a better balanced feed and with a larger number of chicks, or using the cladodes directly in drinking water to preserve all their beneficial molecules. Then, partially or totally substituting some expensive or unavailable constituents in Algeria such as corn or soybean meal which increase the cost of production. Finally, it would be interesting to carry out an analysis of meat quality to evaluate the impact of OFI powder on organoleptic and nutritional qualities.

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