



The Impact of Adding Olive Pomace (OP) on Broilers Productivity, Welfare, Stress Levels and Hematological Responses in Battery Brooders with Varying Stocking Densities

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ABSTRACT

Background: Olive byproducts are valuable feed resources for livestock and poultry. The current study aimed to assess the impact of adding olive pomace (OP) to the diet of broilers raised in battery cages with varying stocking densities on their growth performance, carcass characteristics and blood biochemical parameters.

Methods: Sixty-two-day-old unsexed broiler chicks were divided into two treatment groups, each with 31 birds. The first group of 31 chicks were raised in electrically heated battery brooders with varying stocking densities of 8, 16, or 25 birds per m² and fed with a standard diet. The second group of 31 chicks was raised with the same stocking densities and fed with supplementing 5% OP.

Result: Increasing stocking density from 8 to 25 birds/m² reduced growth performance, whereas recorded the highest body weight gain with stocking density of 16 birds/m² in commercial broilers. The eviscerated carcass weight, abdominal adipose tissue (AAT) and breast weight decreased by 8.7% with higher stocking densities. The levels of serum creatine kinase (CK) and alanine amino transferase (ALT) increased in contrast to the decrease in glucose. Plasma ALT and AST levels were considerably reduced when OP was added to the broiler diet.

Key words: Blood parameters, Broilers, Growth, Olive pomace, Stocking density.

INTRODUCTION

Stocking density is expressed as the number of birds per unit of space (m²) or body mass (kg). Globally, poultry farmers strive to optimize their financial return in order to boost chicken output per square meter and prevent production losses resulting from overcrowding. Higher stocking density increases output of chicken meat per area, which boosts profitability (Puron *et al.*, 1995). However, high temperatures and restricted airflow at bird level can decrease performance (Feddes *et al.*, 2002).

In 2007, the European Commission established basic guidelines for broiler welfare, suggesting a maximum stocking density of 30 kg/m² (0.073 m²/bird) for the entire EU. Additionally, the National Chicken Council (2005) developed a voluntary welfare examination system for broiler enterprises. Depending on final body weight, the program recommends densities ranging from 31.8 kg/m² for light broilers to 41.6 kg/m² for roosters.

Stocking density is crucial in poultry farming, directly influencing broiler welfare (Gupta *et al.*, 2017). Studies on stocking density (20 to 40 kg/m²) have shown inconsistent results regarding broiler productivity and performance (Cengiz *et al.*, 2015; Pekel *et al.*, 2020). Welfare-related worries, like stress, have increased. Kang *et al.*, (2016) reported significant differences in corticosterone levels at 10 birds/m² compared to 5, 6 and 7 birds/m². Shakeri *et al.*, (2014) reported similar results at 35 kg/m².

Agricultural by-products are valuable feed resources for livestock, particularly domestic animals (Al-Harhi

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et al., 2009). Olives, rich in natural bio-phenols with antibacterial properties, offer further potential applications in the pharmaceutical, food and agriculture industries (Bensehaila *et al.*, 2022). However, olive mill wastes, including the solid residue known as olive pomace (Rahil *et al.*, 2021), remain largely unused in Algeria and pose a significant environmental hazard due to their abundance, resulting from the extensive agricultural areas dedicated to olive tree production (El Hachemi *et al.*, 2007).

These byproducts contain 6.8% residual oil and can serve as additional energy sources. The unsaturated fatty acids (oleic acid, linoleic acid and linolenic acid) in olive pulp may also influence the fatty acid profile in animal tissue (Molina-Alcaide and Yáñez-Ruiz, 2008). Additionally, there has been renewed interest in extracting bioactive substances, such as polyphenols, oleuropeosides, flavonoids and simple phenolics, from olive byproducts to enhance animal well-being and performance (Liehr *et al.*, 2017; Leskovec *et al.*, 2018). The chemical composition and antioxidant properties of olive byproducts can also improve animal feed, especially under extreme climatic conditions.

There was uncertainty regarding the optimal amount of OP in broiler chicken diets. Thus, this study aims to evaluate the effects of varying stocking densities on growth performance, carcass weight and blood biochemical parameters. It also investigates whether incorporating OP can mitigate the negative impacts of high stocking densities.

MATERIALS AND METHODS

The study was conducted at the Higher School of Agronomy of Mostaganem, Algeria, from March to June 2021, in an 18 m² experimental greenhouse that was cleaned, disinfected and left vacant for two weeks. Two battery cages were used for rearing broilers. Olive pomace was collected in January 2021 from olive oil extraction factories in western Algeria (Sig, Mascara) and was then dried and stored away from light and humidity.

Experimental design and diets

The experiment was conducted using electrically heated battery cages with a completely randomized design, featuring a 2×3 factorial arrangement of feed and three varying stocking densities. A total of 62 day-old unsexed Arbor Acres broiler chicks were weighed and fed a standard starter diet (3035 kcal/kg). The chicks had an average initial body weight of 508.33±3.91 g at 16 days old. They were divided into 2 groups and further distributed into six batches based on diet and rearing stocking density until the 50th day. The two groups consisted of 31 chicks each and were reared in battery cages throughout the experimental period with the following densities:

-Low density: 8 birds/m² (5 birds/cage)

-Medium density: 16 birds/m² (10 birds/cage)

-High density: 25 birds/m² (16 birds/cage)

In the first group, the birds were fed with a standard diet, while in the second group, the birds were offered an experimental diet (standard diet + 5% OP). The ingredients used for diet preparation and nutrient composition of the diets are presented in Table 1.

Extra chicks were used to maintain the desired stocking density. If any birds died, replacements with equivalent body weight were added to keep the density constant until the 50th day of the experiment. Throughout the experimental

period, each battery cage was placed in a climate-controlled environment.

Parameters

Growth performance

Body weight, weight gain and feed intake were recorded and the feed conversion ratio was calculated on 16th, 21st, 26th, 31st, 36th, 41st, 46th and 50th days.

Measurement at slaughter

On the 50th day, 10 chicks from each group were selected randomly, slaughtered by severing the jugular vein and eviscerated at a local commercial slaughterhouse. Measurements were recorded for full and eviscerated carcass weight and weights of the breast, thigh, giblets (liver, heart and gizzard) and abdominal fat.

Blood sampling and serum analysis

Blood samples were collected randomly from five chickens per batch using a syringe from the brachial vein and placed in dry tubes. The blood was then centrifuged at 3000 rpm for 10 minutes to separate the serum, which was immediately frozen at -20°C. Blood tests were conducted using diagnostic kits from BIOLABO SAS, France. The tests included measurements of glucose and creatine kinase, indicators of stress response and serum concentrations of alanine amino transferase, aspartate amino transferase, alkaline phosphatase and gamma-glutamyl transferase.

Statistical analysis

The results for the different parameters are presented as mean values with the standard error of the mean (SEM). Statistical analysis was conducted using SPSS software

Table 1: Calculated and determined compositions of the diets.

Ingredients	Grower and finisher (%) (15 to 50 days of age)	
	Control diet	OP5%
Corn	67.00	32.00
SBM	27.00	15.00
OP	0	5.00
Wheat bran	4.00	4.00
Vitamin-mineral premix*	1.00	1.00
Calcium	0.50	0.50
Phosphorus	0.50	0.50
Analyzed composition (%)		
Crude protein	21.00	20.00
Crude fat	1.12	1.19
Crude fiber	3.85	4.17
Moisture	12.02	9.82
Energy (kcal)		
Metabolisable energy	3035	3085.5

*Vitamin premix: Vitamins (E, K, B1, B2, B6, B12) and minerals (Copper, Manganese, Iode). CD: control diet; GO5%: Diet containing 5% of olive pomace.

employing two-factor analysis of variance (ANOVA) with a significance difference of 0.05. Tukey's test was used for statistical comparison at the 95% confidence level. Differences were considered significant when the calculated F-value exceeded the critical F-value at $P < 0.05$, $P < 0.01$ and $P < 0.001$.

RESULTS AND DISCUSSION

Growth performance

The results of the present findings on BW, WG and FCR are presented in Table 2-3-4. Significant differences were observed in weekly body weight gain ($P < 0.001$) among treatment groups by supplementation of OP and due to stocking density from the 2nd to the 5th week of rearing broilers in battery cages ($P < 0.05$). Broilers in high-density control groups weighed 10.06% less than those in low-density groups, while the better weights were recorded in chicks fed a diet with added OP, regardless of density. However, weight gain increased with rearing density from days 32 to 47 ($P < 0.05$), but at the end of the rearing period (47-50 days), body weight gain decreased as stocking density increased ($P < 0.05$). However, the FCR significantly

decreased with higher stocking density ($P < 0.01$) during the first week of rearing broilers in battery cages. Addition of OP to the broiler diet improved FCR during the grower phase (16-36 days) but had no significant effect during the finisher phase (36-50 days).

The findings are consistent with research showing that higher stocking density reduces broiler growth compared to lower stocking density (Simitzis *et al.*, 2012; Chegini *et al.*, 2018; Shakeri *et al.*, 2014) and reveal FCR at high densities, which aligns with the results of Rambau *et al.*, (2016) and Astaneh *et al.*, (2018). High stocking density reduces growth for several reasons, including stress, which raises corticosterone levels and limits glucose supply. Additionally, the body uses most of its energy to support the immune response, produce antibodies and increase heterophil levels. As lymphocytes decrease, energy use increases, leading to lower food efficiency (Carsia, 2015). However, Dozier *et al.*, (2006) observed that high stocking density reduces body weight gain by decreasing feed consumption. It can also raise temperatures, limit airflow and restrict bird movement, making it difficult for them to reach feeders and drinkers. This leads to nutritional deficiencies, higher energy use and increased stress (Feddes *et al.*, 2002; Cengiz *et al.*, 2015).

Table 2: The effects of stocking density and diet on BW of broiler chickens.

Days	BW (g)	16-21 th	22-26 th	27-31 th	32-36 th	37-41 th	42-46 th	47-51 th
Densities	Diet							
Low	CD	820.00	1092.50	1628.00	1856.00	2380.50	2786.50	3225.50
density	OP5%	754.00	1035.00	1397.00	1829.00	2303.00	2731.50	3047.40
Medium	CD	778.00	1129.00	1544.50	1977.00	2399.50	2831.50	3094.00
density	OP5%	778.00	1128.00	1560.50	1969.00	2502.60	2919.00	3133.00
High	CD	742.50	1068.50	1513.00	1915.00	2391.00	2750.00	2900.50
density	OP5%	728.00	1089.00	1448.50	1849.00	2303.00	2770.00	2958.00
SEM		6.28	9.43	13.69	17.51	20.75	25.10	30.04
Density effect		$P < 0.01$	$P < 0.01$	$P < 0.05$	$P < 0.05$	$P < 0.05$	NS	NS
Diet effect		$P < 0.05$	NS	$P < 0.01$	NS	NS	NS	NS

For each group $n = 10$, results expressed as Mean and Standard Error of the mean (SEM), BW: Body weight, CD: control diet; OP 5%: Diet containing 5% of olive pomace. $P < 0.05$: Significant effect; $P < 0.01$: Highly significant effect. NS: No significant.

Table 3: The effects of stocking density and diet on weight gain of broiler.

Days	WG (g)	16-21 th	22-26 th	27-31 th	32-36 th	37-41 th	42-46 th	47-51 th
Densities	Diet							
Low	CD	311.66	272.50	535.50	228.00	524.50	406.00	338.50
density	OP5%	245.66	281.00	362.00	432.00	474.50	428.00	415.90
Medium	CD	269.66	351.00	415.50	432.50	422.50	432.00	262.50
density	OP5%	269.66	350.00	432.50	408.50	533.50	416.00	214.00
High	CD	234.16	326.00	444.50	402.50	376.00	459	26.00
density	OP5%	219.66	361.00	359.50	400.50	454.00	467	41.50
SEM		6.28	10.54	14.93	13.84	12.56	10.21	19.69
Density effect		$P < 0.01$	$P < 0.01$	NS	NS	NS	NS	$P < 0.05$
Diet effect		$P < 0.05$	NS	$P < 0.01$	NS	NS	NS	NS

For each group $n = 10$, results expressed as mean and standard error of the mean (SEM), WG: Weight gain. CD control diet OP5%: Diet containing 5% of olive pomace. $P < 0.05$: Significant effect. $P < 0.01$: Highly significant effect. NS: No significant.

Table 4: The effect of stocking density and diet on the (FCR) of broiler chickens.

Days FCR		16-21 th	22-26 th	27-31 th	32-36 th	37-41 th	42-46 th	47-51 th
Densities	Diet							
Low	CD	1.63	2.66	1.45	4.57	1.84	2.45	0.62
density	OP5%	2.03	20.16	2.80	1.79	1.89	2.24	2.00
Medium	CD	1.89	1.75	1.78	1.97	3.81	3.46	3.91
density	OP5%	1.86	1.78	1.79	2.31	1.82	2.97	1.69
High	CD	2.15	2.00	1.70	2.10	2.07	2.39	2.37
density	OP5%	2.27	1.66	2.19	2.22	2.05	2.38	1.31
SEM		0.04	0.07	0.08	0.14	0.07	0.09	0.14
Density effect		P<0.01	NS	NS	NS	NS	NS	NS
Diet effect		P<0.05	NS	P<0.01	NS	NS	NS	NS

For each group n = 10, results expressed as mean and standard error of the mean (SEM), FCR: Feed conversion ratio; CD control diet; OP5%: Diet containing 5% of olive pomace. P<0.05: Significant effect. P<0.01: Highly significant effect. NS: No significant.

Table 5: The effect of the stocking density and diet on the carcass parameters and inner body organ weight.

Densities	Diet	Eviscerated weight (g)	*Thigh weight (g)	*Breast weight (g)	AAT (g)	Liver weight (g)	Gizzard weight (g)
Low	CD	2329.4	787.53	335.58	28.64	47.28	59.82
density	OP5%	2318.8	806.23	311.86	33.03	48.59	58.4
Medium	CD	2334.5	798.84	369.58	26.84	48.37	56.28
density	OP5%	2343	858.09	373.98	32.18	48.26	62.5
High	CD	2109	730.29	293.57	23.25	45.43	55.06
density	OP5%	2118.3	801.71	345.05	17.38	54.2	73.54
SEM		29.76	13.93	8.67	1.08	1.03	1.36
Density effect		P<0.05	NS	P<0.05	P<0.01	NS	NS
Diet effect		NS	NS	NS	NS	NS	P<0.01

For each group n = 10,*: For each group n =5, results expressed as Mean and Standard Error of the mean (SEM); AAT: Abdominal adipose tissue; CD: Control diet; OP5%: Diet containing 5% of olive pomace. P<0.05: Significant effect. P<0.01: Highly significant effect. NS: No significant.

Table 6: The effect of stocking density and diet on blood serum components in broilers.

Densities	Diet	Glucose (mg/dl)	Stress indicators			Hepatic profile		
			LDH (UI/L)	CK (UI/L)	ALT (UI/L)	AST (UI/L)	PAL (UI/L)	GGT (UI/L)
Low density	CD	180.67	1027.14	911.33	272.78	510.23	1302.39	125.16
	OP5%	160.89	844.95	1015.74	150.35	158.25	2059.04	115.19
Medium density	CD	211.84	846.25	830.36	521.06	464.33	1508.96	227.08
	OP5%	196.94	619.69	810.71	162.07	196.18	1371.48	61.52
High density	CD	194.83	1375.99	402.47	641.62	466.85	1776.81	79.26
	OP5%	202.72	844.95	376.92	597.09	149.15	1592.46	98.98
SEM		3.84	119.71	62.7	39.39	30.79	221.17	19.78
Density effect		P<0.01	NS	P<0.01	P<0.01	NS	NS	NS
Diet effect		NS	NS	NS	P<0.01	P<0.01	NS	NS

For each group n = 5, results expressed as mean and standard error of the mean (SEM), CK: Creatine kinase; LDH: Lactate deshydrogenase; ALT: Alanine-Amino-Transferase, AST: aspartate aminotransferases, PAL: Phosphatase alkaline, GGT: Gamma glutamyl-transpeptidase, CD: Control diet; OP5%: Diet containing 5% of olive pomace. P<0.01: Highly significant effect. NS: No significant.

On the other hand, inclusion of 5% olive pomace in broiler diets did not negatively influence growth performance. This was described by Al-Harathi (2017), who found that iso-energetic and iso-protein diets containing olive pomace

provide sufficient nutrients for growth performance. Similar findings by El Hachemi *et al.*, (2007) reported that up to 15% OP can be included in broiler diets without negatively affecting feed intake or utilization.

Interestingly, neither the addition of OP to the diet nor a stocking density of up to 25 birds per m² had a detrimental effect on the broilers overall growth performance.

Carcass traits

The analysis on carcass traits revealed that stocking density significantly affected carcass characteristics, including eviscerated weight, breast weight and abdominal fat weight ($P < 0.05$) (Table 5). Low and medium stocking densities recorded higher eviscerated weights, while high stocking densities resulted in the lowest weights and reduced abdominal fat weight ($P < 0.01$). Chicks raised with medium stocking density and fed with inclusion of 5% OP recorded the better breast weight.

However, inclusion of 5% OP did not affect liver weight but significantly increased gizzard weight ($P < 0.01$). Thigh weight was unaffected by increasing stocking density or the addition of OP to the diet.

The present study revealed that the medium stocking density group recorded the highest eviscerated carcass weight high and breast weights, compared to the other groups. Other research corroborated present findings that higher stocking density affects carcass characteristics and reduces carcass quality (Skomorucha *et al.*, 2009; Sekeroglu *et al.*, 2011). While some studies (Hassanein, 2011; Simitzis *et al.*, 2012) observed that increased stocking density reduces carcass weight.

The present study observed a decrease in abdominal fat weight as stocking density increased, due to the fact that higher densities limit feed access, reducing feed consumption and, in turn, abdominal fat weight.

The inclusion of 5% or 10% OP in broiler diets with or without enzymes does not negatively influence carcass yield or internal organs (Al-Harathi *et al.*, 2017), which is corroborated with present research findings. However, inclusion of OP in broiler diet significantly increased gizzard weight, probably due to improved mechanical digestion and muscle growth needed to process the fiber and kernels present in OP (Al-Harathi and Attia, 2015).

Biochemical blood parameters

The inclusion of 5% OP in the broiler diet had no significant impact on glucose, creatine kinase, or LDH levels (Table 6). However, stocking density significantly affected glucose and creatine kinase levels in the blood serum ($P < 0.01$); as stocking density increased, both creatine kinase and glucose levels decreased. While the stocking density had no significant impact on LDH levels. ALT activity increased significantly ($P < 0.01$) in birds raised at the higher stocking density levels. In contrast, adding OP to the broiler diet significantly reduced ALT and AST levels ($P < 0.01$). PAL and GGT activities were unaffected by the addition of OP or by stocking density.

The results of the present study revealed that glucose levels were higher in birds reared at medium and high stocking densities ($P < 0.01$). This is due to stress

stimulating hepatic glycogenolysis in broilers, which increases energy demand and glucose use (Asma, 2018). Additionally, increased CK activity can indicate muscle damage or stress in chickens, sometimes related to heat stress (Terlouw and Rybarczyk, 2008; Sandercock *et al.*, 2001). However, results of the study recorded higher CK levels in the low-density groups compared to other stocking densities. In contrast, no significant impact was observed with supplementing OP on glucose, CK and LDH levels. However, chickens consuming OP recorded lower levels of these parameters, which may be due to the presence of polyphenols and flavonoids in the OP.

Additionally, ALT and AST are frequently employed biomarkers to detect tissue damage, particularly in the liver. Elevated levels of these enzymes can indicate liver disease (Drotman and Lawhan, 1978). In the present investigation, high levels of these enzymes were observed in chickens raised at higher stocking densities. These findings are consistent with the results of Gholami *et al.*, (2020). Broilers raised with high stocking densities in fierce competition for feed and water, which can lead to muscle damage and increased levels of liver enzymes in the blood (Nobakht and Fard, 2016).

As well, olive products are known for their antioxidant properties (Visioli and Galli, 2002). The results of the present study revealed a significant decrease in serum ALT and AST levels in broilers fed with OP supplemented diet. This reduction can be attributed to oleuropein, a bioactive compound present in olive products (Gonzalez *et al.*, 1992), which can reduce hepatic enzyme levels. Similar findings were observed by Nakbi *et al.* (2010), who observed that olive oil reduced elevated ALT and AST levels in rats exposed to 2, 4-dichlorophenoxyacetic acid.

CONCLUSION

The findings of the present study revealed that higher stocking density per m² area affects the growth performance and increases stress. Rearing of broilers with higher stocking density per m² area altered the hematological responses and blood biochemical profiles. The present study observed that raising the density to 25 birds/m² compromises broiler chicken welfare. However, supplementing 5% OP in the broiler diet is beneficial in order to improve growth performance with higher stocking density rearing and decreased blood, liver enzymes and other stress indicators.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not

accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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