

# Effects of Dietary Momordica Charantia Supplementations on Broiler Performance, Blood Parameters, Meat Quality and Intestinal Morphology

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#### Keywords

Phytoextract, Momordica charantia, Broiler, Performance, Intestinal morphology **Abstract:** Momordica charantia (MC) is one of the most often used medicinal plants, a rich source of vitamins and minerals. This study aimed to assess the effects of a food supplementation of MC extract at various doses (0, 0.15, 0.30, 0.45 g kg<sup>-1</sup>) on the performance, carcass yield, meat quality, intestinal morphology, and some blood parameters in broilers. A total of 288 Ross 308 broiler chicks (1 day old) were used in the experiment. Animals were divided into 4 groups with 6 replicates. Feed intake (FI), daily weight gain (DWG), feed conversion rate (FCR), and carcass yield were unaffected by MC extract supplementations. The group treated with a high dosage of MC extract had significantly lower serum phosphor (P) levels than the other groups (P<0.05). In the small intestine sections of the MC supplemented groups, goblet cell number (GCN), crypt depth (CD), villus length (VL), and epithelial height (EH) had significantly increased (P<0.05). Conversely, MC had a quite limited and variable effect on meat quality parameters. In conclusion, the MC extract doses studied in the study have no significant effect on the performance of the broiler. Furthermore, contrasting several dosages in different housing and health conditions plans can be essential.

# Momordica Charantia Katkısının Broilerde Performans, Biyokimyasal Parametreler, Et Kalitesi ve Bağırsak Morfolojisi Üzerine Etkileri

Kelimeler Bitkisel ekstrakt, Momordica charantia, Broiler, Performans, Bağırsak morfolojisi

Anahtar

Öz: açısından zengin, yaygın olarak kullanılan tıbbi bitkilerden biridir. Sunulan çalışmada, etlik piliçlerde rasyona farklı seviyelerde MC ekstraktı (0, 0.15, 0.30, 0.45 g kg-<sup>1</sup>) takviyesinin performans, karkas verimi, et kalitesi, bağırsak morfolojisi ve bazı kan parametreleri üzerine etkileri değerlendirilmiştir. Çalışmada toplam 288 adet Ross 308 etlik civciv (1 günlük) kullanılmıştır. Hayvanlar altı tekerrürlü dört gruba ayrıldı. MC ekstraktı takviyesi yem tüketimi, günlük canlı ağırlık artışı, yemden yararlanma oranı ve karkas verimini etkilememiştir. Yüksek doz MC ekstraktı ilave edilen grupta serum P seviyeleri, diğer gruplardan önemli ölçüde daha düşüktü (P<0.05). MC ekstraktı ilave edilen gruplarını ince bağırsak bölümlerinde, goblet hücre sayısı, kript derinliği, villus uzunluğu ve epitel yüksekliği önemli ölçüde artış göstermiştir (P<0.05). Bununla birlikte MC'nin et kalite parametreleri üzerinde oldukça sınırlı ve değişken bir etkisi oldu. Sonuç olarak, çalışmada kullanılan dozlarda MC ekstraktının Broiler'de performans üzerine herhangi bir etkisi olmamıştır. Farklı barınma ve sağlık koşullarında farklı doz rejimlerini karşılaştırmak gerekli olabilir.

## **1. INTRODUCTION**

Momordica charantia L. (MC) is a plant used for food and medicine that generally grows in tropical regions. The fruit and leaves of MC are rich in minerals and vitamins, and also contain numerous chemicals such as glycosides, saponins, alkaloids, fixed oils, triterpenes, proteins, and steroids, which are biologically active compounds [1,2]. Anti-diabetic [3,4], antimalarial [5], antiviral [6], anthelmintic [7], antitumor [8,9], antifungal, and antimicrobial [10] properties of MC are reported. Also, it is reported that MC extract has sanitizer properties and can be used as a preservative/sanitizer in the food industry [11]. Shahadat et al. [7] reported that, besides its anthelmintic effect, MC extract (30 g/liter drinking water) provides live weight gain in chickens infected with Ascaridia galli. Likewise, it has been reported that extract of MC improved the body weight of broiler chickens as feed additives, reduced liver function enzymes, and changed the lipid profile [12]. Nevertheless, comprehensive studies on the effect of MC on poultry performance are not enough. Thus, it is not clear whether MC has any growth-promoting effects on broiler chicken. This study was conducted to investigate the effects of adding MC extract to broiler rations at different doses on performance, carcass characteristics, some biochemical parameters, meat quality, and intestinal histopathology.

## 2. MATERIAL AND METHOD

The Atatürk University Animal Experiments Local Ethics Committee authorized the study's experimental procedure in its decision dated 30 June 2016 and numbered 113.

## 2.1. Experimental Design

A total of 288 Ross 308 male broiler chicks (1 day old) were used in the experiment. Animals were divided into 4 groups with 6 replicates and 12 animals in each replicate. The first group was separated as control and MC extract was added to the basal ration in other groups respectively 0.15, 0.30, and 0.45 g kg-1. All groups were fed ad libitum with broiler starter feed (22% crude protein, 12.2 MJ ME kg-1) between 1-21 days and broiler grower-finisher feed (19% crude protein, 13.4 MJ ME kg-1) between 22-42 days. The diet formulation meets the nutritional requirements of the National Research Council (NRC) for broiler chickens [13]. The ambient temperature was kept at 32-35 °C during the first week, then gradually lowered to 22 °C until the end of the experiment. During the trial period, fluorescent lighting was applied 23 hours a day.

#### 2.2. Performance Parameters

The experiment lasted for 42 days, and weekly (7, 14, 21, 28, 35, and 42. days) feed intake (FI), and body weights (BW) were determined by weighing. Daily weight gains (DWG) and feed conversion rates (FCR) were determined by calculation (FCR=Total feed consumption/Total weight gain). At the end of the

experiment, 15 broilers from each group (3 broilers from each subgroup) were slaughtered to determine the carcass yield.

#### 2.3. Histopathological Analysis

At the end of the study, animals were sacrificed then for histological analysis of duodenum, jejunum, and ileum tissues were excised. The tissues were fixed in 10% buffered formol solution for 48 hours. Then, the tissues were passed through alcohol and xylol series with standard histological methods and embedded in paraffin blocks. Sections of 5  $\mu$ m thickness taken from paraffin blocks with a Leica RM2125RT microtome (Leica Microsystems, Wetzlar, Germany) were stained with Crossman's triple staining to evaluate the crypt depth (CD), villus length (VL), and epithelial height (EH). In order to determine the distribution and histochemical structure of goblet cells, Periodic Acid Shift (PAS) staining was applied to transversal serial sections of 5-7  $\mu$ m taken from each block.

For this purpose, goblet cells were counted in the villi and crypt epithelium of the 30000  $\mu$ m (20 lenses) long region in the villi and crypts in serial sections taken from each block. Goblet cell averages falling to 1 mm were measured manually by Cameram SLR 6.1 image analysis program (Mikro Sistem Ltd., Turkey) and their arithmetic averages were calculated.

### 2.4. Meat Quality Analysis

Drumstick and breast meat were removed from broilers that had been slaughtered and brought to the lab under sanitary circumstances, and they were kept for nine days at 4°C. On meat samples taken on the first, third, fifth, seventh, and ninth days of storage chemical analyses were carried out such as water activity (aw), pH, thiobarbituric acid reactive substances (TBARS), and colors [L\* (relative lightness), a\* (relative redness), and b\* (relative yellowness)]. Micrococcus/Staphylococcus, Pseudomonas spp., TMAB (total mesophyll aerobic bacteria), TPAB (total psychrophile aerobic bacteria), and the number of Coliform group bacteria were all measured to assess microbiological characteristics.

The aw value was determined using the Aqualab 4TE (USA) instrument. After homogenization, pH values were assessed using a pH meter (WTW Inolab, Germany) [14].

The findings of the analysis of TBARS, which was done as Lemon [15] specified, are presented in  $\mu$ mol malonaldehyde/kg ( $\mu$ molMDA/kg).

The samples' cross-sectional surface color intensities (L \*, a \*, and b \*) were measured using by Minolta colorimeter (CR-200, Minolta Co, Osaka, Japan).

#### 2.5. Biochemical Analyses

The blood samples collected during the sacrifice process of the animals were centrifuged at 1500 rpm for 5

minutes, and the serum samples obtained were stored at -80 °C until biochemical analyses. In blood serum samples, alkaline phosphatase (ALP), triglyceride (TG), cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), calcium (Ca), phosphor (P), total protein, albumin, glucose, creatinine, and uric acid were analyzed using commercial kits on the Beckman Coulter AU5800 (Beckman Coulter Inc., USA) auto analyzer.

#### 2.1. Statistical Analysis

With the aim of determining the statistical significance between the groups, analysis of variance (ANOVA) was performed using the SPSS 20.00 package program (IBM Inc, NY, USA), and the statistical difference between the groups (p<0.05) was determined according to the Duncan Post Hoc test.

### 3. RESULTS AND DISCUSSION

Most medicinal plants can successfully boost animals' growth performance, which makes them more valuable and popular in the poultry industry. Due to its bioactive components such as sterol and saponin triterpenoids, saponins, polypeptides, flavonoids, alkaloids, and sterols, MC has antidiabetic, antiviral, antimalarial, anthelmintic, antitumor, antioxidant and antimicrobial properties [16]. Nevertheless, comprehensive studies on the effect of MC on poultry performance are not enough. A limited number of previous studies have reported that the addition of 3% MC extract to drinking water improves body weight in broilers [12] and increases body weight in Ascaridia galli-infected chickens [7]. The results obtained in the current study showed that MC supplementation improved FCR depending on the dose and reduced FI. Nevertheless, it has no significant effect (P>0.05) on final BW, FI, DWG, FCR, and carcass yield (Table 1).

Days	Parameters	Control	MC-15	MC-30	MC-45	SEM	P-values
	Initial BW (g)	56.3	55.9	55.9	56.0	0.001	ns
	Final BW (g)	2839.17	2669.53	2597.49	2738.76	33.69	ns
0-21	DWG	0.046	0.045	0.044	0.046	0.001	ns
	FI	0.067	0.065	0.065	0.064	0.001	ns
	FCR	1.468	1.47	1.478	1.399	0.038	ns
21-42	DWG	0.087	0.08	0.077	0.082	0.002	ns
	FI	0.192	0.167	0.173	0.174	0.007	ns
	FCR	2.215	2.088	2.245	2.126	0.048	ns
0-42	DWG (g)	66.3 <sup>a</sup>	62.2 <sup>ab</sup>	60.5 <sup>b</sup>	63.9 <sup>ab</sup>	0.001	*
	FI (g)	129.6	116.2	118.8	119.1	0.004	ns
	FCR	1.957	1.867	1.966	1.865	0.046	ns
	Carcass Yield (%)	68.2	66.8	68.7	70.0	0.855	ns

<sup>a, b, c</sup>: Different superscripted means within the same row differ significantly. \*: (p<0.05). ns: not significant (p>0.05). FI: feed intake, BW: body weight, DWG: daily weight gain, FCR: feed conversion rate. MC-15, MC-30, MC-45: basal diet supplemented with MC at 0.15, 0.30, 0.45 g kg<sup>-1</sup> respectively.

It is observed that the supplementation of MC to the diet did not make a significant difference in the biochemical parameters studied (Table 2).

Table 2. Biochemical parameters.

	Control	MC-15	MC-30	MC-45	SEM	P-values
ALP (U L <sup>-1</sup> )	2392.71ª	1337.29 <sup>b</sup>	1754.14 <sup>ab</sup>	1439.43 <sup>b</sup>	266.262	*
Triglyceride (mg dL <sup>-1</sup> )	32.29 <sup>ab</sup>	41.43 <sup>a</sup>	29.57 <sup>ab</sup>	23.00 <sup>b</sup>	3.935	*
Cholesterol (mg dL <sup>-1</sup> )	117.71	103.43	109.86	114.71	7.749	ns
HDL (mg $dL^{-1}$ )	80.14	69.43	76.14	80.71	4.984	ns
$LDL (mg dL^{-1})$	61.71	52.00	53.00	56.57	4.682	ns
$Ca (mg dL^{-1})$	8.34	8.14	7.74	8.26	0.34	ns
$P(mg dL^{-1})$	6.14 <sup>a</sup>	6.27 <sup>a</sup>	5.99 <sup>a</sup>	5.10 <sup>b</sup>	0.254	*
Total Protein (g dL <sup>-1</sup> )	2.89	2.53	2.74	2.73	0.178	ns
Albumin (g $dL^{-1}$ )	0.99	0.88	0.93	0.93	0.06	ns
Glucose (mg dL <sup>-1</sup> )	218.29	233.29	210.14	214.14	10.201	ns
Creatinine (mg dL <sup>-1</sup> )	0.00	0.01	0.01	0.03	0.014	ns
Uric Acid (mg dL <sup>-1</sup> )	2.66	3.89	2.57	3.54	0.522	ns

<sup>a, b, c</sup>: Different superscripted means within the same row differ significantly. \*: (p<0.05). ns: not significant (p>0.05). ALP: alkaline phosphatase, HDL: high-density lipoprotein, LDL: low-density lipoprotein, Ca: calcium, P: phosphor. MC-15, MC-30, MC-45: basal diet supplemented with MC at 0.15, 0.30, 0.45 g kg<sup>-1</sup> respectively.

There was no difference in blood serum parameters except ALP, TG, and P levels among the groups. Serum P levels in the high dose of MC extract-treated group were significantly lower than the other groups (P<0.05). Also, all the treatment groups showed varying degrees of reduction in serum ALP levels compared to the control group. It has been reported that MC has the potential to reduce hepatic triglyceride and cholesterol concentration [17], and this effect is related to the mechanism of

inhibiting lipid metabolism of the sterile glycoside fraction which is the active ingredients in mainly methanolic extracts [18]. In the study, it was observed that TG, cholesterol, HDL, and LDL levels showed a decreasing tendency in the MC groups, but this decrease was not statistically significant. MC has a hypoglycemic effect due to its content of phyto-constituent called charantin, insulin-like peptides, and alkaloids [2,9,19]. Studies on the hypoglycemic effect of MC have

generally been conducted in rats and humans with diabetes [3,4,20]. However, the current study results show that MC has no effect on the blood glucose level in broiler (Table 2).

There was no previous study on the effects of MC on intestinal morphology. In the current study light microscopically, there was no significant change in the intestinal villi surface epithelium of all groups (Figure 1). Histological staining showed that goblet cells in the villi and crypts of the duodenum, jejunum, and ileum give a positive reaction with PAS staining (Figure 2). It was observed that GCN was higher in the ileum compared to the duodenum and jejunum, and the number of goblet cells increased in parallel with the increase in MC dose (Figure 2).

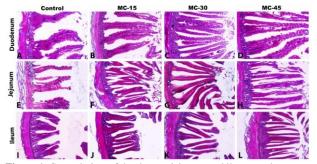


Figure 1. General view of duodenum, jejunum and ileum sections. With Crossman's modified triple stain. MC-15, MC-30, MC-45: basal diet supplemented with MC at 0.15, 0.30, 0.45 g kg-1 respectively.

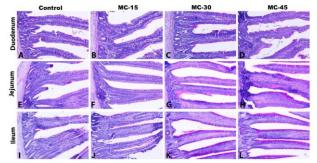


Figure 2. Figure showing PAS reaction and increased staining of duodenum, jejunum, and ileum sections. MC-15, MC-30, MC-45: basal diet supplemented with MC at 0.15, 0.30, 0.45 g kg<sup>-1</sup>respectively.

T

EH (um)

Table 3.	Histometric measurement result	s of intestinal sectior	18.			
		Control	MC-15	MC-30	MC-45	P-values
•	GCN (number 1000µm <sup>-1</sup> )	51.2±2.4 <sup>a</sup>	67.8±1.9 <sup>b</sup>	70.43±2.1 <sup>b</sup>	83.3±3.5°	*
n ga	CD (µm)	31.2±2.7 <sup>a</sup>	33.9±2.3ª	40.2±1.4 <sup>b</sup>	41.5±1.9 <sup>b</sup>	*
Duode	VL (µm)	617.3±80.3ª	728.2±92.1 <sup>b</sup>	$740.5 \pm 95.6^{b}$	999.0±103.3°	*
П	EH (µm)	32.5±1.3ª	$34.5 \pm 0.2^{a}$	$35.8{\pm}1.4^{\mathrm{a}}$	39.6±3.1 <sup>b</sup>	*
-	GCN (number 1000µm <sup>-1</sup> )	68.54±3.4ª	71.1±1.9 <sup>a</sup>	94.8±2.6 <sup>b</sup>	105.9±4.6°	*
Ĩ,	CD (µm)	$32.3 \pm 1.7^{a}$	$34.0\pm2.7^{a}$	35.1±2.5ª	40.5±2.2 <sup>b</sup>	*
Jejunu m	VL (μm)	521.3±63.4ª	582.3±69.5ª	662.4±72.9 <sup>b</sup>	810.3±87.3°	*
ſ	EH (µm)	33.9±2.9ª	$34.8{\pm}2.0^{a}$	$37.4{\pm}1.5^{a}$	$40.8 {\pm} 1.4^{b}$	*
_	GCN (number 1000µm <sup>-1</sup> )	92.4.2±2.5 <sup>a</sup>	99.5±3.1 <sup>b</sup>	118.7±4.2°	136.3±5.7 <sup>d</sup>	*
um Jej	CD (µm)	41.0±2.1ª	38.9±1.7 <sup>b</sup>	37.8±2.2 <sup>b</sup>	42.4±2.3ª	*
llei	VL (µm)	497.3±55.8ª	562.0±69.1 <sup>b</sup>	670.2±76.4°	$706.0 \pm 90.2^{d}$	*

The values are given as mean ± SEM. <sup>a, b, c</sup>: Different superscripted means within the same row differ significantly. \*: (p<0.05). GCN: Number of goblet cells (in 1000 µm area), CD: Crypt depth, VL: Villus length, EH: Epithelial height. MC-15, MC-30, MC-45: basal diet supplemented with MC at 0.15, 0.30, 0.45 g kg-1 respectively.

31.7±2.1<sup>b</sup>

 $29.0\pm2.0^{a}$ 

Physical and chemical properties of meat affect meat quality such as color, odor, flavor, texture, and pH are

28.8±0.6ª

Depending on the dose, it was observed that the GCN, CD, VL, and EH values in the duodenum, jejunum, and ileum tissues increased statistically significantly in the MC-supplemented groups compared to the control group (p<0.05) (Table 3, Figure 3). MC contains flavonoids, phenolic compounds, and saponins compounds such as momordin, momordicoside, momordicin, kuguacin, karavilsodie, and karavilagenin [21,22]. As it is known, saponins and flavonoids have a beneficial effect on intestinal health. Prihambodo et al. [23] reported that flavonoid supplementation has a positive effect on small intestine morphology and villus length increased the in the small intestine of the broiler. According to researchers, due to the antibacterial properties of flavonoids, they reduce pathogenic bacteria that may damage the villi in the intestines, and a low pathogen community stimulates intestinal villi growth and regeneration. Similarly, Youssef et al. [24] reported that the dietary supplements of saponins enhanced intestinal morphology, and increased the villus height in broilers.

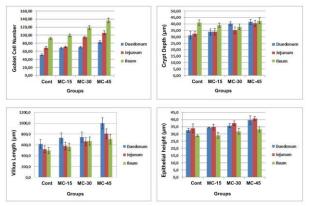


Figure 3. Average epithelial height, goblet cell number, crypt depth, and villus length by intestinal sections and groups. MC-15, MC-30, MC-45: basal diet supplemented with MC at 0.15, 0.30, 0.45 g kg-<sup>1</sup>respectively.

the basic parameters. The microbial load is one of the factors that determine meat quality and shelf life in meat production. The findings of the present study showed

33.2±1.9<sup>b</sup>

that MC has a quite limited and variable effect on these parameters of breast and drumstick meat during the storage period (Table 4-7).

Table 4. Quality parameters in chicken breast meat during	g the storage period.
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Days	Groups	pН	Aw	TBARS	$L^*$	a*	b*
-	_	-		(µmolMDA kg <sup>-1</sup> )			
1	Control	$5.75 \pm 0.01$	0.991±0.001 <sup>b</sup>	4.55±0.20	53.26±0.41ª	3.66±0.39	5.28±0.89
	MC-15	$5.83 \pm 0.03$	$0.990{\pm}0.001^{ab}$	4.59±0.13	55.24±0.75 <sup>b</sup>	$3.48 \pm 0.60$	$7.14 \pm 0.47$
	MC-30	$5.90 \pm 0.05$	0.998±0.001ª	4.56±0.06	49.55±0.78 <sup>a</sup>	$3.98 \pm 0.51$	$5.58 \pm 0.74$
	MC-45	$5.76 \pm 0.07$	$0.988 \pm 0.001^{b}$	4.35±0.04	53.36±1.22 <sup>a</sup>	3.21±0.30	$7.00{\pm}1.03$
	P-values	ns	*	ns	**	ns	ns
3	Control	$5.75 \pm 0.02$	0.993±0.000ª	4.70±0.05	56.20±0.72	3.89±0.73	6.98±0.62
	MC-15	$5.69 \pm 0.12$	$0.987 \pm 0.001^{b}$	$5.05 \pm 0.25$	$57.02 \pm 2.92$	2.20±0.33	6.55±1.42
	MC-30	$5.79 \pm 0.04$	$0.987 \pm 0.000^{b}$	$5.07 \pm 0.07$	57.24±0.79	$2.94{\pm}0.63$	8.13±1.02
	MC-45	$5.78 \pm 0.02$	$0.989 \pm 0.002^{b}$	4.53±0.17	$54.82 \pm 0.46$	4.41±0.30	$7.50{\pm}0.68$
	P-values	ns	*	ns	ns	ns	ns
5	Control	5.95±0.03ª	$0.990 \pm 0.000$	5.30±0.12	50.26±2.12	3.16±0.82	4.78±0.30
	MC-15	5.73±0.02°	$0.993 \pm 0.001$	$4.84{\pm}0.02$	50.94±1.04	3.39±0.64	9.95±0.56
	MC-30	$5.89{\pm}0.00^{ab}$	$0.989 \pm 0.001$	5.24±0.56	50.56±0.25	$3.59 \pm 0.50$	$6.14 \pm 1.70$
	MC-45	5.86±0.02 <sup>b</sup>	0.991±0.002	5.01±0.24	$52.92 \pm 0.50$	$2.90{\pm}0.60$	6.57±1.77
	P-values	**	ns	ns	ns	ns	ns
7	Control	5.77±0.02	0.990±0.001	5.36±0.14	52.89±1.38	$1.84{\pm}0.04$	8.17±0.35ª
	MC-15	$5.59 \pm 0.01$	0.991±0.001	4.88±0.17	53.82±0.70	$3.25 \pm 0.48$	$9.18{\pm}0.39^{a}$
	MC-30	$5.70 \pm 0.04$	0.991±0.002	5.35±0.13	52.48±0.55	2.66±0.61	7.85±1.10 <sup>a</sup>
	MC-45	6.11±0.26	$0.993 \pm 0.002$	5.11±0.21	52.54±0.38	$2.66 \pm 0.59$	4.13±0.36 <sup>b</sup>
	P-values	ns	ns	ns	ns	ns	***
9	Control	$6.04{\pm}0.05$	0.991±0.001	5.39±0.35 <sup>ab</sup>	51.01±1.95 <sup>ab</sup>	3.22±0.91	7.66±0.66ª
	MC-15	$6.08 \pm 0.22$	$0.990 \pm 0.001$	$4.93{\pm}0.04^{b}$	54.29±1.15 <sup>a</sup>	2.39±0.32	$6.20{\pm}0.75^{ab}$
	MC-30	$6.10{\pm}0.07$	$0.995 \pm 0.005$	6.23±0.46 <sup>a</sup>	48.09±0.86 <sup>b</sup>	3.39±0.21	5.09±0.52 <sup>b</sup>
	MC-45	$5.96 \pm 0.02$	0.993±0.002	6.16±0.24 <sup>a</sup>	53.24±1.50 <sup>a</sup>	4.58±1.37	$7.93{\pm}0.50^{a}$
	P-values	ns	ns	*	*	ns	*

The values are shown as mean  $\pm$  SEM. <sup>a, b, c</sup>: Different superscripted means within the same column differ significantly. \*: (p<0.05), \*\*: (p<0.01) \*\*\*: (p<0.001). ns: not significant (p>0.05). aw: water activity, TBARS: thiobarbituric acid reactive substances, L\*: relative lightness, a\*: relative redness, b\*: relative yellowness. MC-15, MC-30, MC-45: basal diet supplemented with MC at 0.15, 0.30, 0.45 g kg-lrespectively.

<b>Table 5.</b> Quality parameters in chicken drumstick meat during the storage period	od.
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Days	Groups	pH	Aw	TBARS	$L^*$	a*	b*
•	-	-		(µmolMDA kg <sup>-1</sup> )			
1	Control	$6.01 \pm 0.01$	$0.994{\pm}0.001$	4.03±0.07 <sup>b</sup>	57.07±2.30	5.34±0.51	$5.46 \pm 0.83$
	MC-15	6.21±0.14	$0.992 \pm 0.001$	4.13±0.07 <sup>b</sup>	56.74±0.71	4.55±0.92	$3.48 \pm 0.57$
	MC-30	$6.12 \pm 0.06$	$0.989 \pm 0.001$	4.50±0.13ª	52.55±0.92	6.81±1.05	6.14±1.36
	MC-45	6.17±0.10	$0.993 {\pm} 0.007$	$4.20 \pm 0.10^{b}$	55.75±1.25	7.30±0.93	$5.07 \pm 0.99$
	P-values	ns	ns	*	ns	ns	ns
3	Control	5.84±0.53	$0.992 \pm 0.002$	4.28±0.13	57.81±0.42	4.59±0.06	1.79±0.51ª
	MC-15	6.25±0.05	$0.992 \pm 0.002$	$4.58 \pm 0.30$	56.36±1.23	$3.24 \pm 0.20$	$3.99 \pm 0.48^{ab}$
	MC-30	$6.19{\pm}0.02$	$0.989 \pm 0.000$	4.56±0.16	54.20±0.84	3.81±0.79	5.92±1.46 <sup>b</sup>
	MC-45	$6.40 \pm 0.04$	$0.994 \pm 0.006$	$4.64 \pm 0.20$	55.55±0.94	5.29±0.77	5.19±0.96 <sup>b</sup>
	P-values	ns	ns	ns	ns	ns	*
5	Control	6.10±0.15	0.991±0.001	4.94±0.13	55.32±2.40	4.04±0.19	6.28±0.83
	MC-15	6.36±0.05	$0.997 \pm 0.010$	4.83±0.03	57.46±3.04	4.62±0.34	3.37±1.59
	MC-30	$6.26 \pm 0.02$	$0.997 \pm 0.002$	4.71±0.05	44.35±0.45	$5.24 \pm 0.56$	$5.40 \pm 0.93$
	MC-45	$6.00 \pm 0.06$	$0.992 \pm 0.001$	$4.70 \pm 0.08$	59.08±0.95	5.81±1.39	4.39±1.17
	P-values	ns	ns	ns	ns	ns	ns
7	Control	5.98±0.03	0.989±0.001	4.84±0.11	52.11±6.15	4.75±1.04	5.152±0.77
	MC-15	$5.76 \pm 0.07$	$0.995 \pm 0.003$	5.13±0.22	$58.83 \pm 0.85$	5.11±0.54	$5.25 \pm 0.28$
	MC-30	6.17±0.02	$0.990 \pm 0.000$	4.81±0.10	55.64±0.45	$3.82 \pm 0.87$	$5.90{\pm}0.58$
	MC-45	6.00±0.23	$0.993 \pm 0.002$	4.76±0.13	55.06±1.17	4.87±0.29	$3.14 \pm 1.14$
	P-values	ns	ns	ns	ns	ns	ns
9	Control	6.46±0.14 <sup>ab</sup>	0.999±0.002	$4.97 \pm 0.07$	50.76±1.58 <sup>b</sup>	5.54±0.35	3.90±0.86
	MC-15	6.15±0.00°	$0.992 \pm 0.000$	5.50±0.40	55.49±1.37ª	4.63±0.37	3.13±1.19
	MC-30	6.19±0.04 <sup>bc</sup>	0.993±0.010	5.40±0.19	$55.78 \pm 0.40^{a}$	4.83±0.35	5.16±0.96
	MC-45	$6.58 \pm 0.05^{a}$	0.993±0.001	5.26±0.29	51.15±1.17 <sup>b</sup>	6.54±1.09	$4.44 \pm 0.47$

The values are shown as mean ± SEM. <sup>a, b, c</sup>: Different superscripted means within the same column differ significantly. \*: (p<0.05). ns: not significant (p>0.05). aw: water activity, TBARS: thiobarbituric acid reactive substances, L\*: relative lightness, a\*: relative redness, b\*: relative yellowness. MC-15, MC-30, MC-45: basal diet supplemented with MC at 0.15, 0.30, 0.45 g kg<sup>-1</sup>respectively.

Days	Groups	TMAB	Coliform	Lactobacillus	Micrococcus-	Pseudomonas	Yeast-Mould	TPAB
-	-		-	spp.	Staphylococcus	spp.		
1	Control	4.94±0.10	3.21±0.07	4.20±0.20°	4.23±0.28	4.27±0.16	2.54±0.06 <sup>b</sup>	3.67±0.29
	MC-15	$5.40\pm0.45$	$3.73 \pm 0.25$	$4.96 \pm 0.15^{ab}$	4.29±0.01	4.37±0.23	3.39±0.09ª	$4.09 \pm 0.09$
	MC-30	$5.82 \pm 0.09$	$3.30 \pm 0.30$	$5.11 \pm 0.05^{a}$	$4.39 \pm 0.09$	$4.18 \pm 0.70$	$3.27 \pm 0.27^{a}$	$4.58 \pm 0.10$
	MC-45	$5.56 \pm 0.30$	$3.39 \pm 0.01$	$4.47 \pm 0.13^{bc}$	4.66±0.17	$4.46 \pm 0.01$	3.467±0.01ª	$3.83 \pm 0.00$
	P-values	ns	ns	*	ns	ns	*	ns
3	Control	$6.03{\pm}0.05^{a}$	3.60±0.00 <sup>b</sup>	$4.68 \pm 0.68$	$4.42 \pm 0.42$	4.30±0.05	3.02±0.02°	4.61±0.02 <sup>b</sup>
	MC-15	5.45±0.15 <sup>b</sup>	4.51±0.13 <sup>a</sup>	$5.26\pm0.30$	$5.28 \pm 0.02$	4.72±0.22	3.63±0.06 <sup>b</sup>	$4.22 \pm 0.08^{\circ}$
	MC-30	$5.94{\pm}0.04^{a}$	3.59±0.21 <sup>b</sup>	5.44±0.13	4.39±0.39	4.32±0.11	$3.80{\pm}0.05^{a}$	$5.25 \pm 0.07^{a}$
	MC-45	6.19±0.11 <sup>a</sup>	$3.87 \pm 0.05^{b}$	4.66±0.10	$4.89 \pm 0.41$	$4.49 \pm 02.4$	3.53±0.02 <sup>b</sup>	4.53±0.02 <sup>b</sup>
	P-values	*	*	ns	ns	ns	***	***
5	Control	6.29±0.69	3.74±0.74	$4.87 \pm 0.04$	4.59±0.11 <sup>b</sup>	5.65±0.69	3.04±0.04	5.39±0.39
	MC-15	$6.99 \pm 0.37$	$4.73 \pm 0.46$	$5.28 \pm 0.28$	5.36±0.11ª	5.21±0.06	3.61±0.17	$5.98 \pm 0.57$
	MC-30	$6.02 \pm 0.32$	$3.66 \pm 0.18$	5.69±0.25	$5.42{\pm}0.08^{a}$	5.87±0.53	$3.83 \pm 0.68$	$5.43 \pm 0.17$
	MC-45	6.21±0.32	3.89±0.11	$4.68 \pm 0.02$	5.59±0.02 <sup>a</sup>	$4.98 \pm 0.28$	$3.55 \pm 0.02$	5.54±0.11
	P-values	ns	ns	ns	**	ns	ns	ns
7	Control	6.34±0.30	3.94±0.34	4.94±0.04 <sup>b</sup>	5.18±0.09	$5.98 {\pm} 0.03^{ab}$	3.30±0.30	6.22±0.27
	MC-15	7.03±0.14	$5.32 \pm 0.00$	$5.60{\pm}0.10^{a}$	5.52±0.31	6.41±0.21ª	4.05±0.10	6.61±0.01
	MC-30	6.71±0.37	3.81±0.09	$5.82{\pm}0.08^{a}$	5.76±0.23	$6.09{\pm}0.04^{a}$	4.91±0.43	$6.24 \pm 0.20$
	MC-45	6.29±0.36	$4.09 \pm 0.61$	5.15±0.11 <sup>b</sup>	$5.93 \pm 0.23$	$5.58 \pm 0.05^{b}$	4.30±0.18	6.45±0.15
	P-values	ns	ns	**	ns	*	ns	ns
9	Control	6.84±0.01 <sup>b</sup>	5.64±0.21ª	5.13±0.25 <sup>b</sup>	6.45±0.42	7.47±0.03 <sup>b</sup>	4.11±0.16 <sup>b</sup>	6.37±0.29
	MC-15	$7.40{\pm}0.10^{a}$	$6.09{\pm}0.09^{a}$	5.73±0.13 <sup>ab</sup>	$5.78 \pm 0.04$	$7.64{\pm}0.03^{ab}$	$4.47{\pm}0.06^{ab}$	7.55±0.29
	MC-30	$7.52{\pm}0.05^{a}$	4.32±0.02 <sup>b</sup>	$6.18{\pm}0.04^{a}$	6.27±0.04	$7.76{\pm}0.08^{a}$	5.31±0.14 <sup>a</sup>	6.32±0.14
	MC-45	6.53±0.07°	$5.00{\pm}0.53^{ab}$	$5.63{\pm}0.16^{ab}$	6.19±0.34	6.14±0.02°	5.19±0.36 <sup>a</sup>	$7.46\pm0.40$
	P-values	***	*	*	ns	***	*	ns

The values are shown as mean  $\pm$  SEM. <sup>a, b, c</sup>: Different superscripted means within the same column differ significantly. \*: (p<0.05), \*\*: (p<0.01), \*\*\*: (p<0.001). ns: not significant (p>0.05). TMAB: total mesophyll aerobic bacteria, TPAB: total psychrophile aerobic bacteria. MC-15, MC-30, MC-45: basal diet supplemented with MC at 0.15, 0.30, 0.45 g kg<sup>-1</sup>respectively.

Table 7. Microbial counts in chicken drumstick meat during the storage period. (log cfu g<sup>-1</sup>).

Days	Groups	TMAB	Coliform	Lactobacillus	Micrococcus/	Pseudomonas	Yeast-Mould	TPAB
			-	spp.	Staphylococcus	spp.		
1	Control	6.39±0.07	3.30±0.30	5.22±0.09	$5.18 \pm 0.18$	4.19±0.11	$2.74{\pm}0.04$	3.71±0.06 <sup>b</sup>
	MC-15	6.03±0.19	$3.52 \pm 0.44$	$4.68 \pm 0.28$	$4.48 \pm 0.48$	4.69±0.25	3.35±0.01	4.31±0.01 <sup>a</sup>
	MC-30	$6.32 \pm 0.43$	$3.18 \pm 0.03$	$4.48 \pm 0.43$	5.55±0.14	4.64±0.21	3.31±0.53	$4.45 \pm 0.20^{a}$
	MC-45	$5.72 \pm 0.21$	$3.24 \pm 0.06$	$4.57 \pm 0.28$	5.29±0.51	4.39±0.24	$2.77 \pm 0.07$	$4.59{\pm}0.19^{a}$
	P-values	ns	ns	ns	ns	ns	ns	*
3	Control	6.63±0.23	$3.59 \pm 0.11$	5.23±0.24	$5.28 \pm 0.04$	4.58±0.08°	$3.04{\pm}0.04^{d}$	4.53±0.35
	MC-15	6.16±0.35	$3.90 \pm 0.30$	$5.09 \pm 0.09$	$5.43 \pm 0.05$	$5.19{\pm}0.07^{ab}$	$3.84{\pm}0.00^{a}$	$5.89 \pm 0.89$
	MC-30	6.53±0.17	3.47±0.23	$4.62 \pm 0.11$	$5.77 \pm 0.05$	5.45±0.22 <sup>a</sup>	3.55±0.01 <sup>b</sup>	$5.22 \pm 0.04$
	MC-45	6.25±0.37	$3.37 \pm 0.05$	$4.84{\pm}0.84$	5.35±0.31	$4.90 \pm 0.06^{bc}$	3.31±0.01°	$4.94 \pm 0.28$
	P-values	ns	ns	ns	ns	*	***	ns
5	Control	6.97±0.01	3.65±0.05°	5.46±0.11	5.79±0.09	5.57±0.16	3.15±0.15	$5.83 \pm 0.08$
	MC-15	6.15±0.15	4.06±0.06 <sup>b</sup>	$5.48 \pm 0.07$	$6.30 \pm 0.04$	6.12±0.23	$4.04{\pm}0.04$	$5.95 \pm 0.15$
	MC-30	$6.62 \pm 0.37$	$4.87 \pm 0.01^{a}$	5.63±0.15	5.62±0.21	$5.68 \pm 0.77$	$3.96 \pm 0.66$	$5.64 \pm 0.19$
	MC-45	$6.27 \pm 0.08$	3.53±0.11°	$5.04 \pm 0.07$	$5.69 \pm 0.20$	$5.84 \pm 0.58$	3.64±0.64	$6.20 \pm 0.08$
	P-values	ns	***	ns	ns	ns	ns	ns
7	Control	$6.78 \pm 0.00$	$4.70\pm0.40$	5.45±0.22 <sup>b</sup>	6.31±0.04 <sup>b</sup>	6.09±0.29	4.00±0.30	6.380.38
	MC-15	$6.24 \pm 0.28$	5.37±0.31	5.64±0.02 <sup>b</sup>	$6.73 \pm 0.10^{a}$	6.69±0.11	4.26±0.22	$6.80 \pm 0.00$
	MC-30	$6.79 \pm 0.30$	$5.31 \pm 0.30$	$6.29{\pm}0.07^{a}$	5.88±0.14 <sup>b</sup>	$5.94 \pm 0.25$	4.51±0.00	$6.37 \pm 0.03$
	MC-45	6.31±0.12	$4.57 \pm 0.27$	5.44±0.01 <sup>b</sup>	6.16±0.12 <sup>b</sup>	$5.88 \pm 0.54$	4.21±0.31	6.41±0.26
	P-values	ns	ns	*	*	ns	ns	ns
9	Control	7.13±0.62	4.72±0.24 <sup>b</sup>	5.49±0.27 <sup>b</sup>	6.33±0.15 <sup>b</sup>	7.44±0.31	4.20±0.15	7.16±0.02
	MC-15	$7.24 \pm 0.04$	$5.66{\pm}0.14^{a}$	$5.84{\pm}0.16^{ab}$	7.23±0.08ª	$7.84 \pm 0.10$	4.43±0.13	$7.03 \pm 0.01$
	MC-30	7.77±0.27	$5.44{\pm}0.10^{a}$	6.41±0.03ª	6.62±0.19b	7.59±0.36	$4.58 \pm 0.20$	$7.09{\pm}0.01$
	MC-45	$6.52 \pm 0.52$	4.75±0.03 <sup>b</sup>	5.58±0.01 <sup>b</sup>	6.33±0.07 <sup>b</sup>	6.59±0.17	$4.80\pm0.50$	7.38±0.33
	P-values	ns	*	*	*	ns	ns	ns

The values are shown as mean  $\pm$  SEM. <sup>a,b,c</sup>: Different superscripted means within the same column differ significantly. \*: (p<0.05), \*\*\*: (p<0.001). ns: not significant (p>0.05). TMAB: total mesophyll aerobic bacteria, TPAB: total psychrophile aerobic bacteria. MC-15, MC-30, MC-45: basal diet supplemented with MC at 0.15, 0.30, 0.45 g kg<sup>-1</sup>respectively.

## 4. CONCLUSION

This study is among the first to look into how MC extract affects the performance, intestinal morphology, and meat quality of broilers. In conclusion, despite the improving effect on intestinal morphology MC extract had quite a limited effect on performance in Broiler in the doses used in the study. Furthermore, contrasting several dosages in different housing and health conditions plans can be essential.

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