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RESEARCH ARTICLE

The Effects of Organic Acid and *Origanum onites* Supplementations on Some Physical and Microbial Characteristics of Broiler Meat Obtained from Broilers Kept Under Seasonal Heat Stres

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Summary

This study was conducted to determine the effects of dietary organic acid combination or *Origanum onites* supplementations on the microbiological quality and some meat quality parameters of broiler meat obtained from heat stressed broilers. 540 broiler chicks (Ross 308) were divided into three groups as control, organic acid (OA) supplemented group and *Origanum onites* supplemented group (OO). After the period of 0-3 weeks, chickens were raised under seasonal heat stress. On the 42nd day, 10 birds were randomly chosen from each group and slaughtered then some meat quality parameters, colour, drip and cooking loss were determined on the breast muscles. The microbiological quality and some meat quality parameters of breast muscles and skins were analysed at the 0, 3rd, 8th and 15th days of storage period at 4°C. Coliforms in faces samples taken from gastrointestinal tract were counted. Control group had the lowest pH ultimate value, whereas the values of drip loss (18.28±0.99%) and cooking loss (31.15±0.99%) of this group were the highest (P<0.05). Chickens in control group had the highest L* value (62.23±1.53) whereas the OO supplemented group had the lowest L* value (54.93±1.53) (P<0.05). OA supplementation affected broiler meat microbiological quality significantly (P<0.05). OA supplemented group had significantly lower coliform count (P<0.05). OO supplementation improved the meat quality parameters, such as drip loss, cooking loss and lightness, in broilers under seasonal heat stress. Organic acid supplementations improved microbiological quality of chicken meat during storage.

Keywords: *Broiler, Origanum onites, Organic acid, Microbiologic quality, Meat quality*

Mevsimsel Sıcaklık Stresinde Yetiştirilen Broilerlerin Rasyonlarına İlave Edilen Organik Asit veya *Origanum onites*'in Bazı Fiziksel ve Mikrobiyolojik Et Kalitesi Özellikleri Üzerine Olan Etkileri

Özet

Bu çalışma mevsimsel sıcak stresine maruz kalıp organic asit veya esansiyel yağ asidi ile beslenilmiş olan broilerlerden elde edilen kanatlı etinin bazı et kalitesi özellikleri ve raf ömrünün belirlenmesi amacıyla yapılmıştır. 540 broiler tavuk (Ross 308) kontrol, organik asit ilave edilmiş (OA) veya *Origaum onites* ilave edilmiş (OO) grup olmak üzere 3 ayrı gruba ayrıldı. İlk 3 haftanın takibinde broilerler mevsimsel sıcak stresine maruz bırakıldılar. 42. günde kesilen broilelerden her bir gruptan 10'ar tane rastgele seçilerek göğüs kaslarında renk, pişme ve soğutma kaybı gibi bazı et kalitesi parametreleri incelendi. Göğüs etlerinin raf ömürleri özellikleri soğuk muhafazanın 0, 3., 8. ve 15. günlerinde mikrobiyolojik yöntemlerle incelendi ve bazı et kalitesi özellikleri kontrol edildi. Broilerlerin barsak sisteminden alınan dışkı örnekleri de 0. günde coliform sayısı açısından incelendi. Kontrol grubu en düşük son pH'a ulaşırken fire (%18.28±0.99) ve pişirme kaybı (%31.15±0.99) en yüksek olan gruptu. Kontrol grubunda L*değeri en yüksek bulunurken (62.23±1.53), OO ilave edilen grupta en düşüktü (54.93±1.53). OA ilavesi mikrobiyolojik kaliteyi istatistiksel olarak belirgin bir şekilde (P<0.05) etkiledi. OA ilavesi ile dışkıda coliform sayısında belirgin bir azalma görüldü (P<0.05). Yemlere OO ilavesi ile Lightness, drip ve pişirme kaybı gibi et kalitesi parametrelerinde iyileşme görülürken organic asit ilavesinin raf ömrü süresince mikrobiyel kaliteyi karşılaştırılan diğer gruplara göre arttırdığı gözlemlendi.

Anahtar sözcükler: *Broiler, Origanum onites, Organik asit, Mikrobiyolojik kalite, Et kalitesi*

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INTRODUCTION

Broiler meat quality depends on the inherent characteristics of the animal, long- and short-term environmental influences on the animal and processing variables. Most of these factors are likely to alter muscle post-mortem metabolism which largely determines the processing ability of meat. Environmental conditions existing even shortly before the animal is slaughtered have been known to be stressful for broilers and alter some of their meat characteristics [1].

To the consumer, appearance is the major criterion for purchase selection and initial evaluation of meat quality. Other quality attributes, such as tenderness, juiciness, drip-loss, cook-loss, and shelf-life are important to the consumer after purchasing the product, as well as to the processor when producing value-added meat products [2].

A selective exclusion of the gut pathogens and a consequent promotion of the favourable microbes like the lactobacilli may be the plausible mechanism of action of the organic acids [3]. In addition the beneficial effects of organic acid supplementation in livestock have been attributed to a lowering of pH, mainly in the stomach, and intestines which controls the growth of pathogenic bacteria [4].

Herbs and species have been used for centuries to provide favourable flavours. They also have anti-microbial activity [5,6]. Antibacterial activity of herbs and species is mainly caused by essential oil components such as carvacrol, α-terpineol, terpinen-4-ol, eugenol, (±)-linalool, (-)-thujone, (cis+trans) citral, nerol, geraniol, menthone [7]. The essential oils are hydraphobic and their primary site of toxicity is the membrane. They accumulate in the lipid bilayer according to a partition coefficient that is specific for the compound applied, leading to disruption of the membrane structure and function.

The aim of this study is to determine the effects of organic acid or *Origanum onites* supplementation on some meat quality parameters and microbiological quality of broiler meat of which obtained from of broilers kept under seasonal heat stress.

MATERIAL and METHODS

This study was conducted in summer, during July and August, 2007. 540 broiler chicks (Ross 308), mixed sex kept in deep litter. The temperature was 32°C in housing on day 1 and was decreased weekly by 2.5°C until wk 3. From 3 to 6 wk, groups were kept at ambient temperature which was reaching to 43°C during the day time. From 3 to 6 wk, the average temperature and relative humidity from 10:00 to 18:00 and from 18:00 to 10:00 were 34.2°C, 46% and 26.6°C, 57%, respectively. For each group, broiler chicks were kept in different pens (1.75x2.5 m) based on the diet regimes for 42 days. Birds were randomly divided into 3 groups (180 birds for each group; 60 birds x 3 pens): Control group (basal diet), *Origanum onites* group (the basal diet supplemented with *Origanum onites*: *Origanum onites,* 15 g/kg) and organic acid group (the basal diet supplemented organic acid combination; Lactic acid 200 g/kg; Formic acid 250 g/kg, and Propionic acid, 80 g/kg). Diets were fed ad libitum and birds had free access to drinking water. Ingredients of the diets were shown in *Table 1*.

Birds were slaughtered on 42[nd] day of age. Slaughtering was carried out under commercial conditions in a pilot plant (Poultry Processing plant of ADU, Faculty of Agriculture). The equipment and plant were disinfected after slaughtering for each group. Samples were taken immediately after air chilling.

Table 1. *Composition of experimental basal diets and calculated nutrient content*

Tablo 1. *Deneysel bazal diyet kompozisyonu ve hesaplanmış besin içeriği*

Basal Diets		
Ingredients	**%**	
Corn	52.00	60.00
Soybean Meal	34.50	29.50
Fish Meal	5.00	1.50
Vegetable Oil	5.00	6.00
Di Calcium Phosphate	1.50	1.30
Calcium Carbonate	1.05	0.94
Salt	0.30	0.30
Trace Vitamin Premix [1]	0.25	0.25
Trace Mineral Premix [1]	0.10	0.10
DL-Methionin	0.10	0.10
L-Lysine	0.05	0
Choline	0.05	0.01
Coccidiostat (Cygro) [2]	0.10	0.10
Origanum onites [3]	0	0
Organic Acid Combination [4]	0	0
Calculated chemical analyses, %		
Crude protein	23.11	19.35
Calcium	1.18	0.92
Total Phosphor	0.79	0.66
Methionin	0.51	0.35
Lysine	1.47	1.06

[1]: For each kg of the diet; vitamin A 12.000 IU; vitamin E 35.0 mg; vitamin K_3 5.0 mg; vitamin B_1 3.0 mg; vitamin B_2 7.0 mg; vitamin B_6 5.0 mg; vitamin B_{12} 0.015 mg; Calcium D-Pentotenat 10.0 mg; Folic acid 1.0 mg; D-Biotin 0.045 mg; Choline chloride 125.0 mg; vitamin C 50.0 mg; Mn 80 mg; Fe 60.0 mg; Cu 5 mg; Co 0.2 mg; Se 0.15 mg,
[2]: For each kg of cygro; maduramycin Amonium, 5.000 ppm
[3]: Origanum onites, 15 g/kg
[4]: Lactic acid 200 g/kg; Formic acid 250 g/kg, and Propionic acid, 80 g/kg

Determination of Meat Quality Parameters
pH and Color Measurements

The pH values were determined after 15 min post slaughter (initial pH, pH_{15}) and after chilling for 24 h at 4°C in sealed plastic bags (ultimate pH, pHu), on the left breast muscles of carcasses by using pH meter (Hanna Instrument Model 211) with a penetration electrode (FC-200). Color (L*, a*, and b*) was measured on the left breast muscle at 24 h using a Minolta chromameter (Minolta Corp., Ramsey, NJ).

Drip Loss and Cooking Loss

Drip loss (DL) of the Pectoralis major muscle at 24 h post-mortem was measured as described by Remington et al [8]. The left Pectoralis major muscle was excised and weighed, then placed in a plastic bag and stored at 4°C. The muscle was removed from the bag 24 h post slaughter, wiped, and weighed to evaluate DL which was expressed as a percentage of the initial muscle weight. To obtain cooking loss, samples were cooked individually in heat-and-seal bags immersed in 75°C water to internal temperature of 70°C. Temperature was measured with a penetrative probe (Hanna Instrument 8521) in a meat sample in a bag. Samples were weighed before and after cooking to determine cooking loss. Weight loss was expressed as a percentage of initial weight for drip and cooking losses.

Microbiological Analyses

Ten birds from each group (30 broiler chickens in total) were slaughtered under commercial conditions. After chilling, the left breasts were removed and put in sterile bags. The intestinal truct of the birds were also collected separately and put into individual sterile bags. Breasts (skin on), and intestinal tracts were brought to the laboratory in two cold boxes (4°C). The shelf life and organoleptic features of breast muscles and skins were analysed at the 0, 3rd, 8th and 15th days of storage period at 4°C. 10 gram of skin and flesh samples from each breast were put in a sterile bag and homogenised in 90 ml of pepton water (Merck 1.07228) then serial dilutions were carried out. Plating out was done on PCA (Plate count Agar, Oxoid CM 325) from appropriate serial dilutions and the plates were incubated at 30°C for 3 days. The results were found as log cfu/g of the meat and skin samples analysed.

Organoleptic Analyses

The samples were analyzed by 3 referees on the sampling days based on their odors and appearances. The referees recorded the sensory evaluation results as good and spoiled. The spoiled evaluation was carried out by smelling uncommon flesh odors and observing sticky exudates on the skin and flesh of meat.

Analysis of Faeces

Ten gram of faces samples taken from gastro-intestinal truct were homogenised in 90 ml of pepton water then serial dilutions were carried out. Dilutions were plated out on Violet Bile Lactose Agar (VRBA: Lab M, Lab 31) and plates were incubated at 37°C for 1 day then colonies were counted. The results were found as log cfu/g of the material analysed.

Statistics

All data were subjected to Univariate using the General Linear Models procedure of SPSS. Means differing significantly were separated using the Duncan's test option of SPSS (P<0.05).

RESULTS

Meat Quality Parameters

Meat quality parameters of broiler chickens for different groups are given at *(Table 2)*. Although no statistical differences were observed at the initial pH values measured at 15 min postmortem, *Origanum onites* (OO) supplemented group had the highest pH ultimate value (6.10±0.49) then the others at the pH measurement conducted 24 h post mortem. The drip loss and cooking loss parameters were statistically different between groups. The highest losses for these parameters were in control group. The lowest drip loss (7.40±0.99%) and cooking loss (19.69±0.99%) were observed in the group supplemented with OO *(Table 2)*.

The colour measurements showed that there were significant differences between groups. Chickens kept at

Table 2. *Effect of diets on breast meat quality parameters of broiler chickens under seasonal heat stress (X±Sx)*

Tablo 2. *Mevsimsel sıcaklık stresine maruz kalan broilerlerin et kalitesi üzerine verilen diyetlerin etkisi (X±Sx)*

Parameters	Control	Organic Acid	*Origanum onites*
pH 15	6.01±0.60 [a]	6.13±0.60 [a]	6.19±0.60 [a]
pHu 24	5.84±0.49 [a]	5.96±0.49 [ab]	6.10±0. 49 [b]
DL %	18.28±0.99 [a]	11.86±0.99 [b]	7.40±0.99 [c]
CL %	31.15±0.99 [a]	27.26±0.99 [b]	19.69±0.99 [c]
L*	62.23±1.53 [a]	58.63±1.53 [ab]	54.93±1.53 [b]
a *	3.41±0.41 [a]	2.46±0.41 [a]	2.32±0.41 [a]
b*	5.60±0.54 [a]	2.26±0.54 [b]	3.16±0.54 [ab]

a-c *Means in the same column with no common superscript differ significantly,* ***DL:*** *Drip loss;* ***CL:*** *cooking loss;* ***L**** *= lightness;* ***a**** *= redness;* ***b**** *= yellowness* control group had the highest lightness (L*) values whereas the lowest was the OO group. Although redness (a*) values did not show any significance, mean yellowness (b*) values obtained from the control birds was significantly higher than OA supplemented group *(Table 2)*.

Microbiological Quality

Examinations carried out during the sampling days showed that OA supplementation affected broiler breast meat microbiological quality *(Table 3)*. On day 0, breast muscles from OA group had significantly lower ($P<0.05$) microbial load. On 3rd day of storage, OA group had lowest microbial load (4.98±0.09 log cfu/g) and there was a statistically significant difference ($P<0.05$) between OA supplemented group and the others. No statistical difference was observed between control group and OO group ($P>0.05$). No spoilage signs were observed in any of these groups. The mean microbiological load on the breast muscles from OA supplemented group was 5.51±0.09 log cfu/g and no spoilage signs were observed on the 8th day of storage. However, muscle samples from the other groups showed spoilage signs such as a heavy odour and slight sticky exudates on their surfaces. On the 15th day of trial, all samples were spoiled. Intestinal pH was not affected ($P>0.05$) from the different diets *(Table 4)*. However, OA group had significantly lower coliform count than the other groups.

Table 3. *Numbers of total bacteria grown during the storage on the breast meat of broiler carcasses (log cfu g^{-1}) fed by various feed supplementations under seasonal heat stress*

Tablo 3. *Mevsimsel sıcaklık stresine maruz kalıp çeşitli yem ilaveleriyle beslenilen broilerlerin göğüs etlerinde soğuk muhafaza sırasında gelişen toplam mezofilik bakteri sayıları (log kob g^{-1})*

Storage Time (Day)	Control	Organic Acid Combination	*Origanum onites*
0	4.67±0.10 ^{b}	4.45±0.10 ^{b}	5.30±0.10 ^{a}
3	5.87±0.09 ^{a}	4.98±0.09 ^{b}	5.74±0.09 ^{a}
8	6.40±0.09 ^{a}	5.51±0.09 ^{b}	6.32±0.09 ^{a}
15	8.49±0.15 ^{a}	7.76±0.15 ^{b}	7.88±0.15 ^{b}

$^{a-b}$ Means in the same row with no common superscript differ significantly

Table 4. *Coliform counts in the faecal material taken from broilers (log cfu g^{-1}) and intestinal pH (X±Sx)*

Tabo 4. *Broilerlerden alınan dışkı materyalinde coliform sayısı (log kob g^{-1}) ve barsak içeriğinin pH'ı (X±Sx)*

Characteristic	Control	Organic Acid	*Origanum onites*
Intestinal pH	6.01±0.12 ^{a}	5.82±0.12 ^{a}	5.83±0.12 ^{a}
Coliforms in faeces	6.76±0.58 ^{a}	5.54±0.46 ^{b}	6.67±0.27 ^{a}

$^{a-b}$ Means in the same column with no common superscript differ significantly

DISCUSSION

Although repeated increases in environmental temperature or seasonal temperature changes do not affect broilers [9], acute heat stress does not allow broilers to adopt their physiological and metabolical conditions to increased temperatures causing quality defects and deaths. Especially in the Western and Southern seashore parts of Turkey acute heat stress causes massive deaths and meat quality defects between July and August months in the summer season [10].

The study reported here conducted during the summer season in order to determine the effects of organic acid or *Origanum onites* supplementations on some meat quality parameters and microbiological quality of broiler meat. It was reported that there was a significant correlation between muscle pH and extremes in color variation [11,12]. Cornforth [13] also stated that meat with a high pH has a higher water-holding capacity, hence making it appear darker. Poultry meat with low pH has been associated with low water-holding capacity (WHC), which results in increased cook-loss and drip loss [14,15]. Low pH has also been reported to decrease tenderness [14,16] and increase shelf-life [2]. Heat stress prior to slaughter was reported to cause PSE-like meat in turkeys [17] and broilers [15]. Lee et al [18], who found that birds held at a control temperature of 20°C (68°F), exhibited significantly higher terminal pH values than heat-stressed birds [16]. Mckee and Sams [19] were also showed that lighter meat colour, lower ultimate pH values and higher drip and cooking loss could be observed in turkeys having heat stress prior to slaughter. That is because higher drip losses observed in the control group and organic acid group L* values were higher than *Origanum onites* supplemented group. Based on the information given above meat with high ultimate pH (24 h) with low drip loss had the lowest L* value whereas meat with the low ultimate pH and high drip loss showed high L* value due to the reflectance effect of exudation. From these results, might be predicted that there were some positive effects of OO supplementation on the stress compensation of broilers.

In the food animal industry, organic acids were added to feeds to as fungistats, but in the past 30 yr, formic and propionic acids and various combinations have also been examined for potential bactericidal activity in feeds and feed ingredients contaminated with food-borne pathogens, particularly *Salmonella spp* [20-22]. The supplementation of OA or OO might be considered as beneficial in improving microbiological quality of broiler chickens during storage [23]. Organic acid supplementation of feed or water has also been reported a promising non-antibiotic alternative [24,25] for poultry. *In vitro* studies revealed that the organic acid combinations have anti-microbial activities [25]. At low pH, organic acids are found predominantly in the undissociated (protonated) form. Undissociated organic acids are lipophilic and can diffuse across bacterial cell membranes. Once inside the bacterial cell, the organic acids will dissociate and decrease the cytoplasmic pH, disrupting enzymatic reactions, cellular growth and/or inducing cell death in a variety of bacterial species [4,24-26]. Certain microbes are more or less tolerant to decreases in intracellular pH levels. For example, some enterobacterial species such as *Escherichia coli* are generally more susceptible to organic acid induced toxicity whereas, other species (ie lactobacilli and streptococci) allow their intracellular pH levels to drop, and are often less susceptible [24,26]. In the present study, the total numbers of coliforms in OA supplemented group found in the faeces were significantly lower than the other groups examined, which might also affect the contamination rate of breast skin. As it was predicted, TVC obtained from the breast samples in OA supplemented group was lower than TVC obtained from the other groups. The inhibitory effects of OA combination continued during storage period and significantly lower numbers of total bacteria were observed on 0, 3rd and 8th sampling days when compared with the other groups. The control and OO supplemented groups both had the signs of the spoilage but the OA supplemented group did not show any of the spoilage signs 8th day of cold storage. Therefore, it could be predicted that approximately two days of shelf-life extension could be provided when OA combination supplementation was added to the ration of broilers.

Based on the data gathered from the study presented here it could be concluded that *Origanum onites* supplementation may have some beneficial effects on meat quality parameters of broilers, such as drip loss, cooking loss and meat colour. Organic acid combination supplemented to broiler feed may improve micro-biolgical quality of poultry carcasses during storage.

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