Using of Oil palm (*ELAEIS GUINEENSIS* JACQ.) leaves extract as a supplement in the diet of Boer goats on meat lipid oxidation and color stability.

Wisam Salim Al-Jumaili*

***Alqasim Green University , College of Agriculture , Babylon , Iraq.**

Abstract

Twenty-one male Boer goats were individually penned for 100 days. The oil palm leaves methanolic extract (OPLE) has extracted from oil palm plantation of University Putra Malaysia and used as a supplement in treatment groups. Animals have separated into three groups (7 animal / group), control (Ctrl = 0 mg OPLE), treatment 1 (T1= 300 mg OPLE) and treatment 2 (T2= 600 mg OPLE) / kg DM of animals' diet. In the end of fattening period, the animals have been slaughtered and samples of *longissimus dorsi* (LD) muscles taken from slaughtered animals after 1, 3 and 6 days of aging to use for lipid oxidation and color measurements. Lipid oxidation results which are presented by thiobarbituric acid-reactive substances (TBARS) showed significant ($P < 0.05$) reduction in lipid oxidation in T1 and T2 when compared with Ctrl group. OPLE supplement groups (T1 and T2) showed more stability in color measurements across aging time. The addition of OPLE as a supplement in goats diets induced lipid oxidation in the meat and it could be a beneficial supplement to produce wholesome meat.

Key words : meat production , oil palm leaves extract , lipid oxidation, meat colour, meat quality.

Introduction

Among the major parameters of meat quality, color can be considered a key attribute, due to its direct impact on consumer's impression of product freshness and wholesomeness. Meat discoloration, due to the oxidation of myoglobin to metmyoglobin over time of storage or retail display, leads to significant product discards (31). Myoglobin oxidation is closely linked with lipid oxidation (17). Generally, an enhanced antioxidant status in muscle results in a lower myoglobin oxidation extent and this often corresponds to an improved meat color stability. For example, dietary antioxidants, such as vitamin E, are able to extend meat oxidative stability by improving the overall muscle's antioxidant status, by lowering the formation of some main oxidation markers and by extending, in turn, meat color stability (8). Several compounds are able to exert antioxidant effects in biological substrates and animal tissues. Among the most known natural antioxidants, the interest in phenolic compounds has been growing in recent years. Phenolic compounds form one of the most

numerous and chemically heterogeneous groups of plant secondary compounds, ranging from simple molecules – such as phenolic acids and flavonoids – to the highly polymerized tannins (14). Although the influence of the molecule's complexity on the antioxidant ability of polyphenols is not fully clear, in some instances, the free radical scavenging activity of polyphenols was shown to be positively related to the number of hydroxyl groups in their molecules and to their polymerisation degree, that making polyphenols potentially important antioxidants (14) . Due to their almost ubiquitous distribution in the plant kingdom, the contribution of phenolic compounds to the dietary intake of antioxidants, which estimated to be higher than that of vitamin E, is of great interest for human nutrition (Hollman & Katan, 1998). Besides *in vitro* assays suggesting the effect of plant phenolic extracts to modify rumen biohydrogenation of unsaturated fatty acids and rumen fermentation (34; 4; 24; 6; 16; 13; 20; 21), the direct addiction of purified phenolics and of polyphenol rich plant extracts to muscle model systems was shown to delay metmyoglobin formation (15) and to improve meat color stability for lamb muscle fed polyphenol rich plant extract diet (26). Moreover, tannins, in particular, are present in several feed resources used for livestock feeding and have been shown to affect *in vitro* rumen characteristics of ruminants (1) and products quality (39). The dietary administration of polyphenol-rich plants and plant extracts has been shown to improve the oxidative stability of meat from different farming animals (25; 33). The direct antioxidant activity of a dietary compound would assume its absorption along the gastrointestinal tract and its deposition in the tissues. Monomeric phenolics can be absorbed through the intestine and found in plasma (38).

Considering the effects of plant secondary phenolic metabolites, such as their ability to modify muscle's fatty acid composition in ruminants (27), or to increase the levels of antioxidant enzymes in rat tissues (3), it may be difficult to explain their direct or indirect effects on the resistance of meat to oxidative damages.

The oil palm (*Elaeis* guinensis) in general is a multipurpose crop grown in Malaysia and other tropical countries. In Malaysia, the oil palm planted areas have increased from 96,900 hectares in 1965 to 5.643 million hectares in 2015 (11; 29). The oil palm fronds (OPF) from the tree constitute one of the main byproducts of the oil palm industry in Malaysia (7). Approximately 51 million tons of Oil palm fronds were produced in 2008, accounting for 53% of the total palm biomass (12; 29) and 45 million tons in 2011 (19). In previous studies , goats fed oil palm fronds produced healthier meat (10) without negative effects on growth performance (9). The oil palm leaf extract (OPLE) showed antioxidant properties against lipoproteins oxidation (22; 36). In the present study, phenolic-rich oil palm leaves extract (OPLE) used as a supplement in Boer goats to examine its activity as antioxidant and to improve meat color stability for the *longissimus dorsi* (LD) muscle.

Materials and methods

Extraction of OPLE

Oil palm (*E. guineensis*) leaves (OPL) were harvested from the Universiti Putra Malaysia (UPM) campus. The extraction process was according to (15 and 35). The leaves were chopped

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into 2-3 cm and immediately transferred to plastic 100-liter drums using methanol. The leaves were left for about 72 hour and the mixture was shake every 3 hours before the methanolic oil palm leaf extract was collected, passed through a 10 mm sieve and stored in a 20-litre bottle. The methanol-extract was vacuum evaporated (Heidolph, Germany) and completely dried using a freeze drier to a dark green OPLE powder and stored sealed at -18°C until used.

Diets preparation

The chemical composition of the substrates of the diet shown in Table (1). The goats were fed twice daily with a diet containing a fixed amount of alfalfa hay (AH) and concentrate (50:50, w/w) with no addition of OPLE for control group (Ctrl) , 300 and 600 mg OPLE kg^{-1} DM as a supplement of diets for treatment 1 group (T1) and treatment 2 group (T2) respectively.

Table 1. Chemical composition of substrates used for the fattening diet.

	AH (g/kg DM)	Concentrate (g/kg DM)
Corn	-	255.40
Soybean meal	-	190.0
Palm kernel cake	-	358.7
Rice bran	-	116.9
Palm kernel oil	-	50.00
Ammonium chloride	-	10.00
Vitamin and mineral	-	10.00
ME (Mcal/Kg)		2.51
DM	907.0	915.0
CP	203.0	167.0
NDF	517.0	244.0
ADF	334.0	117.0
EE	34.70	42.20

Alfalfa hay, AH; ; ME, metabolic energy ;DM, dry matter; CP, crude protein; NDF, neutral detergent fiber, ADF, acid detergent fiber; EE, ether extract.

Management of animals

Twenty-one five-month-old male Boer goats weighing 17.38 ± 0.71 Kg (mean initial body weight ± standard error) were randomly assigned to different treatment groups. Goats were housed individually in wooden pens measuring 1.2 m x 1 m each, built inside a shed with slatted floor 1.8 meters above ground. Goats were allocated randomly to three dietary treatment groups where the experimental diets were (Ctrl, T1 and T2) as explained previously. The experimental diets were fed daily at 3.7% of BW DM basis (23) , with adjustments made weekly according to the changing BW. All goats had free access to water and a mineral block. The feeding trial lasted for 100 days with a three weeks adaptation period. At the end of the 100 day trial, all the animals were slaughtered and stored in 4°C chiller (27,30). The post mortem aging samples was taken from carcass of the *longissimus dorsi* (LD) muscle from 12^{th} to 15^{th} rib after 24 hours, 3 and 6 days to use for color measurements (30). At each aging time, about 1g of LD muscle was taken and snap-frozen in liquid nitrogen and stored at -80°C until using for lipid oxidation measurement (27,30).

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Lipid Peroxidation

Lipid oxidation was measured using thiobarbituric acid-reactive substances (TBARS) according to the method of (28), modified by (32). Meat samples (1 g) were homogenized in 4 mL 0.15 M KCl + 0.1mM BHT with Ultraturrax (1 min, medium speed). After homogenization, 200 µL of the sample were mixed with TBRAS solution and then heated in a water bath at 95 °C for 60 min until the development of a pink color. After cooling, one mL of distilled water and three mL of n-butyl alcohol were added to the extracts and vortexed. The mixtures were centrifuged at 5000 rpm for 10 min. Absorbance of supernatant was read against an appropriate blank at 532 NM using a spectrophotometer (Secomam, Domont, France). The TBARS were calculated from a standard curve of 1, 1, 3, 3-tetraethoxypropane and expressed as mg malondialdehyde (MDA) /Kg sample.

Meat Color Determination

Instrumental color was measured using a ColorFlex system (Hunter Associates Laboratory, Reston, USA.) (27,30). The samples were evaluated for lightness (L*), redness (a*), yellowness (b*), hue angle (arctan, b*/a*), which describes the hue or color of the meat and saturation index or chroma calculated as $\sqrt{(a^{*2} + b^{*2})}$ which describes the brightness or vividness of color (18) . All values were determined from the mean of seven measurements of each muscle at 28 ± 2 oC using the 10˚ standard observer. The spectrocolorimeter was standardized using white (L*=100), and black (L*=0) standard tiles, before being used. Samples were allowed to thaw at 4ºC overnight prior to analysis. The samples were placed directly onto the color meter and measured. A total of three readings of the L*, a* and b* values and spectral reflectance (400-700 nm) were collected from different sites of each sample and averaged.

Statistical analysis

All experimental data were analyzed using the SAS (37). The MIXED procedure of the SAS was used to evaluate the treatment effects. Multiple comparison of the means among times and treatment was performed using the Turkey's method (37). Mean differences were considered significant at $P < 0.05$. In all fattening studies, the polynomial effects were used to evaluate the effects of the treatments.

Results and Discussion

Thiobarbituric acid reactive substance (TBARS) values for LD muscles across all diedeletetary treatments (Ctrl, T1 and T2) after a 6 day postmortem aging period were significantly ($P < 0.05$) higher than day 1 (Figure 1). The dietary supplement groups T1 and T2 showed significant reduction in lipid oxidation as the TBARS value belonged to the Ctrl group was significantly ($P < 0.05$) the highest compared to T1 and T2 treatment group at the 1st and 6th day of aging. This result can observe in the changes of color values (Table 2). The relationship between meat color and lipid oxidation has been studied because myoglobin oxidation is closely linked with lipid oxidation (17).

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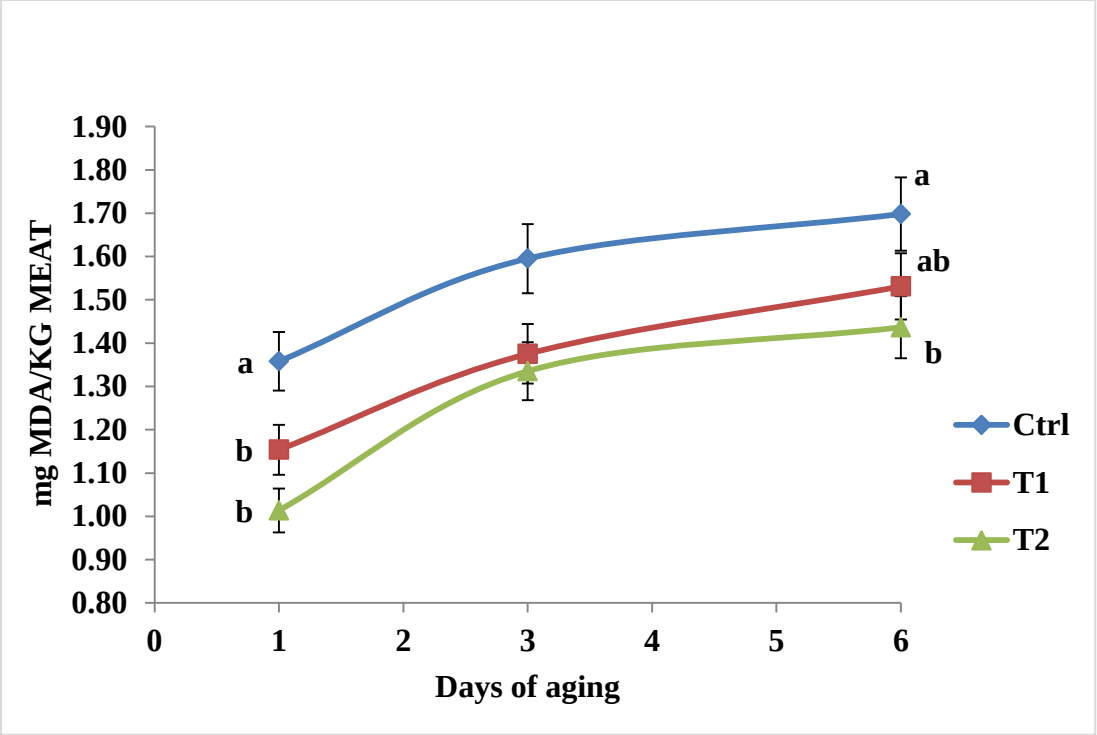

Figure 1: Malondialdehyde (MDA) of the *longissimus dorsi* muscle on different aging times of growing goats fed diets with different levels of OPLE supplementation.
Ctrl = Control (0 OPLE), T1 = 300 mg OPLE\ kg DM diet, T2 = 600 mg OPLE\ kg DM diet. Different letters are significantly different (P<0.05) at same time. Vertical bars are ±1 standard error.

Aldehyde products of lipid oxidation modify myoglobin, causing increased heme oxidation and browning (2). In the present study, the OPLE supplement in post mortem aging periods had an effect on all color parameters obviously in the 6th day of aging. The muscle lightness (L*) significantly (P<0.05) increased in T2 at the 6th day of aging. The values of L* for LD samples belong to T2 was significantly (P<0.05) the lowest in comparison with Ctrl and T1 at the 1st, 3rd and 6th day of aging (Table 2).

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Table 2 . Color of the *longissimus dorsi* muscle of goats fed diets with different levels of OPLE supplementation.

Item	Treatment	Postmortem aging			Pooled SEM
		1day	3day	6day	
L* –Lightness	Ctrl	27.39 (x)	29.37 (x)	29.01 (x)	0.52
	T1	29.41(x)	30.04 (x)	27.96 (xy)	0.49
	T2	24.97 a (y)	25.82 a (y)	27.42b (y)	0.26
a* – Redness	Ctrl	12.49 a	12.97 ab	13.85 b (x)	0.21
	T1	12.42	12.58	12.86(y)	0.24
	T2	12.15	12.24	12.45 (y)	0.16
b*-yellowness	Ctrl	10.48	10.94 (x)	10.93 (x)	0.19
	T1	11.03	11.59 (y)	11.53 (x)	0.18
	T2	10.68	10.58 (x)	10.05 (y)	0.16
Hue angle (HA)	Ctrl	39.94 a	40.34 a	38.24 b (x)	0.44
	T1	41.94	42.73	41.83(y)	0.45
	T2	41.26 a	40.83 a	38.86 b (x)	0.26

Ctrl = Control (0 OPLE), T1 = 300 mg OPLE\ kg DM diet, T2 = 600 mg OPLE\ kg DM diet. SEM= standard error

[x,y] Means within rows with different superscripts are different among treatments ($P < 0.05$).

[a,b,c] Means within columns for each parameters with different superscripts are different among postmortem aging periods ($P < 0.05$).

L*, Measure of darkness to lightness (a greater value indicates a lighter color).

a*, Greater value indicates redder color.

b*, Greater value indicates more yellow color.

HA, Hue angle= tan−1 (b*/a*)*180/π.

The high levels of (L*) observed in Ctrl and T1 groups in connect with lipid oxidation presented in MDA results (Figure 1) could contributed to the high levels of oxymyoglobin which has a lighter color than the myoglobin (30). The results of LD muscle samples from OPLE supplement groups in this study showed more stability in a* value in agreement with (33) and (27). The value of hue angle (HAis useful to indicate shifts in meat color over the storage time, larger values indicate less red, more metmyoglobin (17). There is a significant ($P<0.05$) increasing in the redness (a*) value at the 6th day in Ctrl group and significant ($P<0.05$) reduction in HA at the same day of aging (Table 2). On the surface of stored meat, oxymyoglobin create by the oxidation of myoglobin (17), that explain the appearance of red color in Ctrl group at the 6th day or it could

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contribute to the appearance of yellow spots in the early storage period (1st and 3rd day) which disappeared later (5). The antioxidant activity of oil palm leaves extract (OPLE) has been tasted before by (35) on hyperglycemic/diabetic rats , treated groups with OPLE showed lower levels of TBARS in blood samples than the untreated groups. This antioxidant activity of OPLE might contributed to the stimulation of antioxidant enzymes activities (35) . Furthermore, the antioxidant activity of the polyphenols could explain by the free radical scavenging activity of polyphenols that shown to be positively related to the number of hydroxyl groups in their molecules and to their polymerisation degree (14).
Meat color and MDA results in this research in demonstrate an effect of treatment supplement (OPLE) to reduce lipid oxidation and improved color stability of muscle samples. This results agreed with the results of 26 and 27.

Conclusion

The results of the present study showed that the supplementation of OPLE in the diet of goats improved antioxidant status parameters of longissimus dorsi muscle. Goats fed the control diet showed highest level of lipid oxidation across the aging period. LD muscle samples from the animals fed OPLE supplements was more stable in color measurements. Although the antioxidant effect of OPLE as a polyphenolic supplement in goats' diet is not clear, but it can be concluded that OPLE supplement reduced lipid oxidation in non-frozen stored meat. Further researches need to evaluate the effect of OPLE on meat quality and to produce healthier meat.

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