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Early View

Original Research Article

Assessment of Meat Oxidative Stability, Nutritional and Fatty Acid Compositions of Early age Acclimated and Genetically Thermoresistant Broilers

Key words

thermotolerance;
meat quality;
oxidative stability;
broiler

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Abstract: This study explores the implication of early-age heat conditioning (EHC) and genetic thermotolerance on fatty acid compositions and oxidative stability in broiler meat quality. We employed an early heat stress strategy, involving a 24-hour exposure to 39±1°C on the fifth-day post-hatch, to acclimatise broiler chicks. Three groups were compared: control (C), acclimated (Ac), and naked neck (NN). The acclimated group exhibited significant changes in fatty acid composition compared to the control. The concentration of oleic acid (C18:1 n9) in the acclimated group was higher by approximately 8.5% compared to the control group. Similarly, linoleic acid (C18:2 n6) increased with a fold change of about 12%. The essential fatty acids like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) also showed notable increases, with a more balanced n-6/n-3 ratio. In terms of meat composition, Ac and NN demonstrated increased levels of ash and minerals, while maintaining comparable protein contents to C. The percentage differences in these fatty acids highlight the impact of EHC on improving the nutritional broilers' meat quality. Furthermore, lipid peroxidation was significantly reduced in the Ac group, with thiobarbituric reactive substances (TBARS) concentrations at 0.100 mg/kg, a 56% reduction compared to that of controls 0.227 mg/kg. This reduction underscores the effectiveness of EHC in enhancing meat's oxidative stability. These results suggest that early-age heat stress acclimatization and genetic thermotolerance strategies can play a key role in enhancing the quality and nutritional value of broiler meat, offering a sustainable method to counter the challenges posed by increasing global temperatures in poultry production.

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Introduction

Innovative adaptive strategies are required to maintain physiological integrity and productive efficiency in the face of rising global temperatures that threaten broiler productivity (1, 2). Heat stress impacts broiler chickens by reducing growth, compromising health, and increasing mortality, leading to economic losses (3, 4). With the growing demand for poultry meat, it's crucial to preserve the quality and quantity of poultry production, particularly as heat stress can alter the nutritional composition of broiler meat, affecting human health (5, 6).

Genetic and early-life physiological interventions, such as exploiting genetic traits for thermotolerance in certain chicken genotypes like the Naked Neck and early heat conditioning (EHC), offer sustainable, cost-effective alternatives to environmental modulation (7-9). EHC, for instance, prepares chicks for heat stress by inducing long-term physiological adjustments (10-12).

The ISA Hubbard and Naked Neck breeds demonstrated significant growth and carcass yield advantages for the Isa breed. The significant disparity in live weights and adipose tissue distribution highlights the Isa breed's superior growth efficiency and proclivity for fat accumulation. In contrast, Naked

Neck chickens, with their leaner physique, are a preferred choice for markets that prioritize lean meat (13).

This study aims to explore the effects of thermal acclimatization and genetic thermotolerance on broiler meat's fatty acid profile oxidative stability and nutritional compositions. By comparing standard commercial, thermally acclimated, and Naked Neck chickens, we aim to understand the metabolic adaptations and fatty acid profiles resulting from both induced and innate survival strategies. This could inform future genetic selection and husbandry practices, enhancing the poultry industry's resilience to global warming.

Material and methods

Birds

The study, which was carried out at the Avian Research Unit of the University of Mostaganem, involved 300 one-day-old male chicks, subdivided into 200 ISA Hubbard commercial varieties and 100 Naked Neck breeds, distributed across three groups with five replicates of 20 birds each. The Control group (C) contained non-acclimated ISA Hubbards, while the Acclimated group (Ac) underwent a 24-hour heat exposure at 39±1°C on the fifth-day post-hatch to induce later-in-age thermotolerance. The Naked Neck group (NN), with natural thermoresistance, offered insight into innate versus induced thermotolerance. All groups were raised in a controlled, seasonally adjusted, humidity-controlled environment (22-34°C; 70-78%).

Diet

Birds in the study had free access to a diet transitioning from a starter to a grower-finisher regimen. These feeding protocols have been carefully formulated to meet the nutritional standards set by the ONAB (2012). The diet composition for chickens is strategically designed to transition from the "Starter" to the "Finisher" phases, addressing the evolving nutritional requirements of birds from initial growth stages to maturity. Initial preliminary analyses of the starter and finisher diets were conducted. This approach not only meets their changing dietary needs but also aids in understanding the alterations in meat composition at the market slaughtering age.

Feedstuff ingredients and chemical composition of the starter and finisher feeds

The diet for the chickens has been adapted to their stage of growth, with a "Starter" phase focusing on energy, protein, and micronutrients essential for early development, and a "Finisher" phase adjusted for a matured metabolic state with higher energy and moderated protein needs. The starter diet consisted mainly of maize (60.5%), soybean meal (29.2%), and wheat bran (6%), while the finisher diet increased corn content (68.7%) and adjusted other ingredients accordingly. Chemical analysis revealed a decrease in Dry Matter, protein, lipids, and cellulose from starter to finisher, aligning with the evolving nutritional demands of growing chickens, as shown in Table 1. The consistent ash content across both phases ensures stable mineral intake.

Table 1: Dietary composition of starter and finisher rations fed to experimental broilers

Feedstuff (%)	Starter	Finisher
Maïze	60.5	68.7
Soybean Meal	29.2	26.8
Fine wheat bran	6	0.85
Dicalcium phosphate	1.7	1.65
Calcium carbonate	0.6	0.6
Vitamin-mineral premix	1	1
Methionine	1	0.4
Chemical composition (%)		
Dry Matter	94.11	92.8
Protein	21.2	19
Lipids	1.91	1.12
Cellulose	3.8	3.2
Ash	5.36	5.25

Table 2: Fatty Acid Profile Changes in Broiler Diets from Starter to Finisher Phases

Fatty acids	Starter	Finisher
C14 :0	0.1	0.24
C14 :1	0.4	0
C16 :0	12.53	12.43
C16 :1(n7)	0.34	0.31
C18 :0	3.14	2.73
C18 :1(n9)	25.15	50.06
C18 :2(n6)	21.01	26.11
C20 :0	0.47	0.45
C18 :3(n3)	3.29	3.12
C20 :1(n9)	0.61	0.64
SFA	16.24	16.85
MUFA	51.05	25.66
PUFA	32.71	57.5
N-6	21.11	26.11
N-3	3.29	3.12
n6/n3	6.41	8.36

Table 3: Composition of breast meat of Control, early age acclimated, and Naked Neck broiler breeds

	Control	Acclimated	Naked Neck
Moisture (%)	22.37 ± 1.29 [c]	24.35 ± 2.54 [b]	27.71 ± 1.96 [a]
Ash (%)	1.21 ± 0.38 [c]	2.05 ± 0.15 [b]	2.71 ± 0.3 [a]
Protein (%)	21.15 ± 0.67 [c]	22.02 ± 1.25 [b]	22.37 ± 0.7 [a]
Lipids (%)	4.22 ± 0.29 [a]	3.89 ± 0.43 [b]	3.59 ± 0.5 [c]

(n=20), Different superscript letters indicated significant differences between groups (p<0.05)

Table 4: Comparison of the fatty acid profiles of breast samples from Control, Acclimatised, and Naked Neck birds

	Groups		
	Control	Acclimated	Naked Neck
C14:0	0.56±0.05 [b]	0.52±0.04 [c]	0.7±0.05[a]
C16:0	23.5±0.7 [a]	9.91±0.89 [c]	13.8±1.15 [b]
C18:0	8.63±0.81 [a]	5.18±1.14 [c]	7.19±0.21 [a]
SFA	32.69±2.23 [a]	15.61±2.1 [c]	21.69±1.81 [b]
C16:1	0.91±0.21 [a]	0.87±0.07 [b]	0.57±0.01 [c]
C18:1 n9	38.02±0.19 [c]	41.26±1.6 [a]	40.47±1.01 [b]
MUFA	38.93±0.76 [c]	42.13±0.71 [a]	41.04±1.13 [b]
C18:2 n-6	12.41±0.84 [c]	14.42±0.35 [b]	15.66±2.21 [a]
C18:3 n-3	2.81±0.17 [b]	3.09±0.38 [a]	2.21±0.91 [c]
C20:4 n-6	2.43±0.92 [c]	3.01±0.57 [a]	2.81±0.19 [b]
C20:5 n-3	1.47±0.78 [c]	3.27±0.16 [a]	3.02±0.71 [b]
C22:5 n-6	0.98±0.36 [c]	1.01±0.22 [b]	1.15±0.19 [a]
C22:5 n-3	1.04±0.01 [c]	1.41±0.01 [a]	1.29±0.1 [b]
C22:5 n-6	1.08±0.08 [a]	1±0.02 [b]	0.98±0.01 [c]
C22:6 n-3	0.41±0.05 [a]	0.32±0.01 [b]	0.36±0.01 [b]
n-3	5.36±1.2 [c]	8.09±0.9 [a]	6.88±1.15 [b]
n-6	16.9±0.85 [b]	19.44±1.14 [a]	19.6±1.2 [a]
n-6/n-3 Ratio	3.15±1.1 [a]	2.4±1.3 [c]	2.84±1.2 [b]
PUFA	22.63±2.84 [c]	27.53±1.19 [a]	27.48±2.01 [a]

(n=20), different superscript letters indicated significant differences between groups (p<0.05), SFA: Saturated Fatty Acids; MUFAs: Monounsaturated Fatty Acids; PUFAs: Polyunsaturated Fatty Acids.

Dietary fatty acid composition of the starter and finisher feeds

The fatty acid composition of broiler diets shifts from the starter to the finisher phase (Table 2), with an increase in saturated fatty acids (SFA) from 16.24% to 16.85% and a rise in polyunsaturated fatty acids (PUFA) from 32.71% to 57.5%, mainly due to more C18:2(n6) fatty acids. Monounsaturated fatty acids (MUFA) decreased from 51.05% to 25.66%, largely from reduced level C18:1(n9). The omega-6 to omega-3 ratio also rose from 6.41 to 8.36, indicating a higher omega-6 content in the finisher phase, impacting the nutritional quality of broiler meat.

Sample collection and slaughter process

Twenty males from each group with an average weight of 1800g were selected for slaughter: Control and Acclimated at 53 days, and Naked Neck at 126 days, aligning with their growth rates and local market standards. Slaughtering followed halal standards by trained staff, and 100 g breast fillets were collected from each for analysis (14, 15), ensuring the study's ethical and scientific integrity.

Fatty acid composition

Diet and meat lipids were extracted using 25 mg samples and a chloroform/methanol mixture, followed by phase separation with sodium phosphate buffer (16, 17). The pooled organic phases were esterified using BF3-methanol with fatty acid analysis conducted via gas chromatography on a CP Sil 88 column (18). The procedure included specific temperature protocols and used helium and nitrogen gases. Fatty acids were identified by retention time comparison against standards, with quantification based on the lipid conversion factor (19).

TBARS (Thiobarbituric Reactive Substances) measuring

The detection of malondialdehyde (MDA) in broiler meat using 3.0 g sample homogenized and alkaline hydrolysed, followed by acetonitrile extraction for HPLC analysis (20). The HPLC utilizes a C18 RP Atlantis T3 column, with specific conditions for accurate MDA quantification, highlighting the method's precision and the importance of addressing ingredient interference (20-23).

Chemical composition of the meat

Samples from the right pectoralis major muscle cranial aspect were ground for analysis at Mostaganem University's Laboratory of Applied Animal Physiology, focusing on dry matter, crude protein, and crude ash. International standards ensured accuracy: moisture by ISO 1442:2023, nitrogen (for crude protein) via the Kjeldahl method (The Kjeldahl Method is a protein determination method that converts free nitrogen to ammonium ions in foods to determine their suitability for the quality standards commonly used for protein identification (24)) according to ISO 937:2023, and crude ash following ISO 936:1998, reflecting strict protocol compliance.

Statistical analysis

The statistical analyses for our data sets were analyzed using a completely randomized design and ANOVA, identifying differences among treatment means (25, 26). Duncan's Multiple Range Test provided detailed group mean comparisons, while single degree of freedom contrasts examined heat stress effects on meat composition, fatty acids, and oxidation status. Significance was determined at $p < 0.05$, with analyses performed in SAS (27, 28).

Results

Table 3 presents the composition of breast meat for C, Ac, and NN broiler breeds, showing significant differences due to genetics and environment. Moisture content increased from C (22.37 ± 1.29%) to Ac (24.35 ± 2.54%) and was highest in NN (27.71 ± 1.96%). Mineral content was higher in Ac (2.05 ± 0.15%) and NN (2.71 ± 0.3%) compared to C (1.21 ± 0.38%). Protein content was consistent: C (21.15 ± 0.67%), Ac (22.02 ± 1.25%), and NN (22.37 ± 0.7%). Lipid percentages decreased from C (4.22 ± 0.29%) to NN (3.59 ± 0.5%).

Fatty acid profile of broiler breast samples

Table 4 reveals differences in fatty acid content among C, Ac, and NN broiler breeds. The Ac group showed a decrease in saturated fatty acids (SFA) and an increase in polyunsaturated fatty acids (PUFA) compared to C and NN, with significant differences in SFA across breeds. SFA levels were highest in C (32.69±2.23), followed by NN (21.69±1.81), and lowest in Ac (15.61±2.1). Monounsaturated fatty acids (MUFA), especially C18:1 n9, were higher in Ac (41.26±1.6) than in NN (40.47±1.01) and C (38.02±0.19). The Ac group also had increased n-3 PUFA and a higher n-6 to n-3 PUFA ratio, indicating the impact of thermal acclimation on fatty acid profiles.

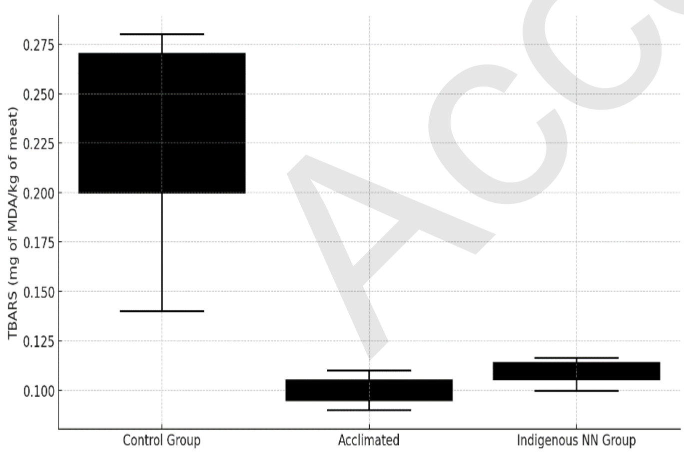

Figure 1: Comparison of the lipid peroxidation of Control, early-age Acclimated, and Naked Neck broilers meat samples

Assessment of oxidative stability of breast meat fatty acids

Oxidative stability in broiler breast meat was evaluated by measuring thiobarbituric acid reactive substances (TBARS) to assess lipid peroxidation, which impacts meat quality and sensory attributes. The analysis across C, Ac, and NN groups revealed distinct oxidative profiles (Figure 1). C meat showed the highest TBARS mean (0.227 mg/kg) with significant variability, suggesting potential quality degradation. In contrast, Ac meat displayed notably lower lipid peroxidation (mean TBARS of 0.100 mg/kg), indicating more consistent quality and the effectiveness of acclimation in reducing oxidative stress.

Discussion

The dietary composition strategically balances nutrients, with the starter diet providing 21.2% protein for early growth, and the finisher diet reducing protein to 19% to align with slower growth and avoid negative effects on health and meat quality. Lipids are decreased from 1.91% to 1.12% to modulate energy metabolism and fat deposition, affecting meat's taste and saleability. Cellulose and ash levels are adjusted minimally, ensuring dietary fiber and mineral needs for optimal health and development. The fatty acid composition of the diets reflects a targeted approach to meeting the nutritional needs of poultry at different stages of growth focusing on optimizing energy supply, supporting physiological functions, and potentially influencing the quality of the meat produced.

Breast meat gradually increased moister content (from C, Ac to NN) intimates a genotype-mediated disparity in water retention capacity, potentially modulated by muscle fiber constitution (2, 29, 30). A pronounced increase in ash content ostensibly reflects an upregulated mineral assimilation, a phenomenon posited to be concomitant with early-age thermal acclimation protocol (30-33). The Indigenous group's augmented mineral content ostensibly corroborates a genetic inclination towards optimized nutrient absorption. Nutritional composition homogeneity in protein concentration suggests a preservation of protein deposition rates irrespective of genotypic and environmental variations, a determinant of paramount importance for meat quality and consumer predilections (34). Thus, thermal stress and inherent genetic factors may exert modulatory effects on lipid metabolism, culminating in a leaner meat phenotype in thermotolerant genotypes (35).

The highest values for C indicate the potential for an increased intake of SFA and/or a metabolic process that favors the synthesis of these fatty acids. This could enhance our insight into the genetic mechanisms behind the regulation of the properties of the fatty acid constitution and the precise regulation of numerous aspects of the involved genes in the FA metabolism in poultry meat (36) which could impact cholesterol levels and the risk of cardiometabolic diseases in consumers (6, 37). In addition, previous studies demonstrated that the ratio of PUFA to SFA in the breast meat was significantly altered, with the Ac and Naked Neck groups showing higher values compared to the control group (38-40). Specifically, breast meat alpha-linolenic acid concentrations were superior in the Ac group, while eicosapentaenoic acid levels were predominant in the Indigenous group when contrasted with other fat categories ($P < 0.05$) (41-43).

Closely linked to its nutritional and health value, the fatty acid profile is an essential quality feature of meat. Therefore, various experiments are being carried out to improve the fatty acid composition of poultry meat, which is known for its high nutritional value. External factors such as thermal conditioning and linseed-supplemented diets have been observed to influence individual fatty acid profiles in chicken breast and thigh muscles, as well as their aggregate quantities (42, 44-47).

Significant variances were observed in carcass characteristics, particularly moisture and ash content, while the crude protein composition remained statistically invariant. The fatty acid profile of poultry meat, an essential determinant of meat quality, is modifiable through dietary interventions and genotypic influences (48, 49). Early age thermal acclimation displayed particular changes to the composition of their adipose tissue influencing essential fatty acids (linoleic and linolenic acids), which must be incorporated into the diet due to their necessity for human and animal health (50, 51). It has been documented that differential deposition of PUFA in breast tissue alters tissue fatty acid profiles, which may be attributed to their distinct roles in these tissues or to their varying phospholipid contents, given that PUFAs are preferentially incorporated into breast muscle phospholipids. As chickens are incapable of synthesizing essential fatty acids endogenously, dietary sources are the sole alternative for their acquisition. Acclimated chickens, possibly due to genetic modifications, showed a higher ability to incorporate PUFA-like stearidonic acid (SDA) and docosahexaenoic acid (DHA) into egg yolks and tissues compared to control groups. This suggests that specific dietary and genetic strategies can significantly enhance PUFA deposition in poultry products (52). Such findings align with the study of (42), who noted that early age acclimation can alleviate stress-induced systemic disorders in broilers, such as increased body temperature, peripheral blood flow, and lipid peroxidation, as also observed by (53, 54).

The indigenous group showed a means TBARS concentration of 0.113 mg/kg, slightly higher than the Acclimated group, but considerably lower than C. The very low standard deviation (0.006) and the tight range (0.11 to 0.12 mg/kg) suggest a remarkably consistent meat quality. Our splitting data depict a significant changes in the antioxidant enzymes of native chicken compared to control groups, which may explain the improved oxidative stability observed in the Indigenous group (55).

Fat oxidation in meat is impacted by natural antioxidants, oxidants, and the degree of polyunsaturation of fatty acids (56). Meats also contain endogenous antioxidants such as enzymes, peptides, and proteins which act as metal ion chelators or free radical scavengers (57). Important antioxidant enzymes, including catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSHPx), are central to the prevention of lipid oxidation (58, 59).

Conclusion

The study demonstrates that early heat conditioning (EHC) and genetic thermotolerance significantly enhance broiler meat's fatty acid profile and nutritional value, offering an economical and effective approach for poultry farming in warm climates. EHC reduces saturated fats while boosting beneficial polyunsaturated fats, including omega-3s. The Naked Neck breed's genetics also play a crucial role in improving meat quality, evidenced by decreased lipid peroxidation in acclimated broilers, hinting at longer shelf life and increased consumer appeal. This research underscores the importance of integrating environmental and genetic strategies to boost poultry's heat stress resilience and meat quality, providing critical insights for the poultry industry amidst climate change challenges. Further, it emphasizes the potential of dietary enrichment with omega-3 fatty acids and the significance of studying genes involved in their synthesis, suggesting avenues for nutritional interventions to improve poultry product healthfulness.

Acknowledgement

Ethics approval. The study complied with the ARRIVE guidelines and followed the U.K. Animals (Scientific Procedures) Act, 1986, and associated guidelines, EU Directive 2010/63/EU for animal experiments.

All methods followed relevant guidelines and regulations with ethical permission from the Ethical Committee for the protection of animals used for scientific purposes and UMAB-Mostaganem Animal Station (*Ref N°22/23; September 2023*).

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Author Contributions. All authors (SD, ZB, DB, SE, and MA) contributed to the conception and design of the experiment. SD, ZB, and DB performed the stress experiment and sampling. SD, ZB, and DB performed chemical and statistical analysis. SD, ZB, DB, SE, and MA and drafted the manuscript. All authors participated in writing, reviewing, and revising the manuscript.

Declaration of Competing Interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ocena oksidativne stabilnosti mesa, hranilne vrednosti in sestave maščobnih kislin zgodaj aklimatiziranih in genetsko termoodpornih brojlerjev

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Izvleček: Raziskava je preučevala vpliv zgodnje toplotne aklimatizacije (EHC) in genetske toplotne odpornosti na sestavo maščobnih kislin in oksidativno stabilnost pri kakovosti mesa brojlerjev. Za aklimatizacijo piščancev brojlerjev smo uporabili strategijo zgodnjega toplotnega stresa, ki je vključevala 24-urno izpostavljenost temperaturi 39 ± 1 °C peti dan po izvalitvi. Primerjali smo tri skupine: kontrolno (C), aklimatizirano (Ac) in skupino z golim vratom (NN). Pri aklimatizirani skupini so se v primerjavi s kontrolno skupino pokazale znatne spremembe v sestavi maščobnih kislin. Koncentracija oleinske kisline (C18:1 n9) v aklimatizirani skupini je bila za približno 8,5 odstotka višja v primerjavi s kontrolno skupino. Podobno se je linolna kislina (C18:2 n6) povečala za približno 12 odstotkov. Tudi esencialne maščobne kisline, kot sta eikozapentaenojska kislina (EPA) in dokozaheksaenojska kislina (DHA), so se občutno zvišale, razmerje n-6/n-3 pa je bilo bolj uravnoteženo. Kar zadeva hranilno vrednost mesa, sta imeli skupini Ac in NN povečano vsebnost pepela in mineralov, pri čemer sta ohranili primerljivo vsebnost beljakovin v primerjavi s skupino C. Odstotne razlike v omenjenih maščobnih kislinah poudarjajo vpliv EHC na izboljšanje prehranske kakovosti mesa brojlerjev. Poleg tega se je lipidna peroksidacija v skupini Ac znatno zmanjšala, pri čemer je bila koncentracija tiobarbituričnih reaktivnih snovi (TBARS) 0,100 mg/kg, kar je 56-odstotno zmanjšanje v primerjavi s kontrolno skupino (0,227 mg/kg). To zmanjšanje poudarja učinkovitost EHC pri izboljšanju oksidativne stabilnosti mesa. Rezultati kažejo, da lahko imajo zgodnja aklimatizacija na vročinski stres in strategije genetske toplotne odpornosti ključno vlogo pri izboljšanju kakovosti in hranilne vrednosti mesa brojlerjev, kar ponuja trajnostno metodo za spopadanje z izzivi, ki jih v proizvodnji perutnine predstavljajo naraščajoče globalne temperature.

Ključne besede: termotoleranca; kakovost mesa; oksidativna stabilnost; brojler