UNIVERSITI PUTRA MALAYSIA

WELFARE, PHYSIOLOGICAL STRESS RESPONSES, AND CARCASS AND MEAT QUALITY IN GOATS SUBJECTED TO DIFFERENT SLAUGHTER METHODS

AZAD BEHNAN SABOW

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By

AZAD BEHNAN SABOW

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

June 2016

DEDICATION

THIS THESIS IS DEDICATED TO MY FATHER AND IN MEMORY OF MY LATE MOTHER AND MY FAMILY WITH LOVE AND RESPECT

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

WELFARE, PHYSIOLOGICAL STRESS RESPONSES, AND CARCASS AND MEAT QUALITY IN GOATS SUBJECTED TO DIFFERENT SLAUGHTER METHODS

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AZAD BEHNAN SABOW

June 2016

Chairman : Awis Qurni Sazili, PhD
Faculty : Agriculture

There is increasing global demand for halal meat. However, halal slaughter is controversial with respect to animal welfare. Therefore, harmonizing spiritual views, animal welfare, slaughter methods and meat quality is a continued research effort. The current study aims to describe two experiments that link physiological stress responses following different methods of slaughter to carcass and meat quality using goats as a model.

In the first experiment, blood biochemistry, electroencephalographic changes and meat quality characteristics associated with possible noxious stimuli following neck cut of goats subjected to conscious halal slaughter and slaughter following minimal anesthesia were assessed in order to validate the minimal anesthesia technique. Ten male Boer crossbred goats were divided into two groups of 5 animals each and subjected to either halal slaughter without stunning (HS) or slaughter following minimal anesthesia (AS) using 5mg/kg propofol administered through cephalic vein and maintained with halothane in 100% oxygen. Blood analysis revealed that the intensity of response for most biochemical blood parameters, except glucose and lactate was not affected by slaughter method. Based on the EEG results, the changes in brain electrical activities were not significantly different between anesthetized and non-anesthetized animals. The HS goats had earlier ($p<0.05$) cessation of heart beats compared with AS goats. Blood loss at exsanguination, residual hemoglobin and myoglobin in meat were not different between HS and AS. At pre-rigor, HS had lower ($p<0.05$) muscle pH and glycogen compared to AS. HS meat had lower ($p<0.05$) drip loss than AS throughout ageing. After 24 h postmortem, AS meat had higher population of lactic acid bacteria than HS group. The present results demonstrate that the noxious stimulus from neck cut is present in both conscious and minimally anaesthetized goats. Slaughtering goats

following minimal anesthesia did not affect bleeding efficiency and meat quality in comparison with slaughtering goats fully conscious.

The second experiment compared different methods of pre-slaughter electrical stunning and slaughter without stunning on physiological stress responses, carcass and meat quality in goats. Thirty-two crossbred Boer bucks were divided into four groups of 8 animals and subjected to halal slaughter without stunning (SWS), low frequency head-only electrical stunning (LFHO; 1 A for 3 s at 50 Hz), low frequency head-to-back electrical stunning (LFHB; 1 A for 3 s at 50 Hz) and high frequency head-to-back electrical stunning (HFHB; 1 A for 3 s at 850 Hz). Slaughtering of animals with or without stunning was performed under minimal anesthesia. Analysis of the sticking blood revealed that all variables were significantly ($p<0.05$) higher than their values in blood samples taken in the farm, lairage, after the point of anesthesia induction and prior to stunning/slaughter. Following slaughter, the SWS animals had higher changes of electrical activity of the brain (EEG) than that of pre-slaughter while electrical stunning maximized the possibility of post stunning insensibility. Based on EEG data, the LFHO animals had the highest changes of electrical activity of the brain at stunning point compared to other stunned animals. LFHB induced a cardiac arrest in all the goats contrary to LFHO and HFHB. The duration between the point of cut and producing cardiac fibrillation for the HFHB goats was similar to that of LFHO and SWS groups. At 12 h postmortem, LFHO, LFHB and HFHB exhibited lower ($p<0.05$) glycogen and pH and higher lactate and glycolytic potential values and faster pH decline than SWS. The pH, glycogen, lactate, glycolytic potential, drip loss, color, shear force, sarcomere length and myofibrillar fragmentation index at 1, 7 and 14 d postmortem did not differ between treatments. The LFHO, LFHB and HFHB exhibited higher ($p<0.05$) cooking loss than SWS at 7 and 14 d postmortem. Incidences of speckles and blood splash were higher ($p<0.05$) in electrical stunned goats compared with SWS goats. Nonetheless, HFHB had lower ($p<0.05$) speckles and blood splash than LFHB and LFHO. The SWS, LFHO and HFHB goats had higher ($p<0.05$) blood loss and lower residual hemoglobin in muscle compared to LFHB. The LFHB meat had higher ($p<0.05$) TBARS value and bacterial counts at 7 and 14 d postmortem than other treatments. Immunoblot analysis, carbonyl and thiol content determination revealed that protein oxidation increased with aging time, but was not affected by slaughter methods. The expression of myosin heavy chain and troponin-T reduced while actin remained stable over storage. The present findings indicate that all slaughter methods caused substantial physiological stress responses which may not necessarily translate into compromising of animal welfare and meat quality, as all blood biochemical parameters level observed fell within the normal physiological range for goats. Moreover, HFHB can be used as a substitute for conventional electrical stunning techniques since it meets both the Islamic requirement of the animal being alive at the moment of slaughter and animal welfare requirement of insensibility during exsanguination.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KEBAJIKAN, TINDAKBALAS TEKANAN FISIOLOGI DAN CIRI-CIRI KUALITI KARKAS DAN DAGING DALAM KAMBING YANG DIPERUNTUKAN PADA KAEDAH RENJATAN DAN SEMBELIHAN YANG BERBEZA

Oleh

AZAD BEHNAN SABOW

Jun 2016

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Terdapat peningkatan terhadap permintaan daging halal di peringkat global. Penyembelihan halal bagaimanapun adalah amat kontroversi apabila dilihat dari sudut kebajikan haiwan. Oleh itu, keselarian perspektif kerohananian, kebajikan haiwan, kaedah penyembelihan dan kualiti daging adalah merupakan satu usaha penyelidikan yang berterusan. Kajian ini menerangkan dua eksperimen yang mengaitkan tindak balas tekanan fisiologi mengikut kaedah pemotongan leher dan salur darah yang berbeza kepada kualiti karkas dan daging menggunakan kambing sebagai model.

Dalam eksperimen pertama, biokimia darah, perubahan elektroensefalografik dan ciri-ciri kualiti daging berkaitan kemungkinan rangsangan berbahaya yang disebabkan oleh pemotongan leher kambing melalui kaedah penyembelihan halal sedar dan penyembelihan selepas pembiusan minimum telah dinilai bagi mengesahkan teknik bius yang minimum. Sepuluh ekor kambing jantan Boer kacukan telah dibahagikan kepada dua kumpulan, dengan jumlah sebanyak 5 ekor kambing bagi setiap kumpulan, yang kemudiannya tertakluk kepada penyembelihan halal tanpa renjatan (HS) atau penyembelihan selepas pembiusan minimum (AS) menggunakan 5mg/kg propofol yang diberi melalui vena sefalik dan dikekalkan dengan halothane dalam 100% oksigen. Analisis darah menunjukkan kebanyakan parameter biokimia darah, kecuali glukosa dan laktat, tidak terjejas oleh penyembelihan kambing samada di dalam keadaan bius minimum atau sedar sepenuhnya. Berdasarkan kepada keputusan EEG, perubahan dalam aktiviti elektrik otak di antara haiwan yang dibius dan tidak dibius didapati tidak berbeza dengan ketara. Kambing-kambing HS mengalami pemberhentian denyutan jantung yang lebih awal ($p<0.05$) berbanding dengan kambing AS. Kehilangan darah semasa pemotongan, sisa hemoglobin dan myoglobin dalam daging di antara HS dan AS adalah tidak berbeza. Pada peringkat pra rigor, HS mempunyai pH otot dan glikogen yang lebih rendah ($p<0.05$) berbanding AS.

Daging HS menunjukkan ($p<0.05$) kehilangan titisan lebih rendah daripada AS di sepanjang proses penuaan. Selepas jam ke 24 postmortem, daging AS merekodkan populasi bakteria asid laktik yang lebih tinggi berbanding dengan kumpulan HS. Keputusan semasa menunjukkan bahawa rangsangan berbahaya kesan pemotongan leher ada di dalam kedua-dua kambing sedar sepenuhnya dan yang telah dibius secara minimum. Penyembelihan kambing diikuti bius minima tidak memberi kesan terhadap keberkesanan pendarahan dan kualiti daging.

Eksperimen kedua membandingkan kesan kaedah penyembelihan dengan renjatan pra sembelihan yang berbeza dan penyembelihan halal ke atas tindak balas tekanan fisiologi, kualiti karkas dan daging kambing. Sebanyak 32 ekor kambing jantan Boer kacukan telah dibahagikan kepada empat kumpulan yang terdiri daripada 8 haiwan dan diagihkan kepada penyembelihan halal tanpa renjatan (SWS), renjatan kepala berfrekuensi rendah (LFHO; 1 A untuk 3 s pada 50 Hz), renjatan kepala hingga ke badan berfrekuensi rendah (LFHB; 1 A untuk 3 s pada 50 Hz) dan renjatan kepala hingga ke badan berfrekuensi tinggi (HFHB; 1 A untuk 3 s pada 850 Hz). Penyembelihan haiwan dengan atau tanpa renjatan telah dilakukan di keadaan bius minimum. Analisis darah menunjukkan bahawa semua pembolehubah adalah lebih tinggi ($p<0.05$) daripada nilai dalam sampel darah yang diambil di ladang, ruang menunggu, selepas titik induksi bius dan sebelum renjatan/penyembelihan dan semua prosedur penyembelihan menyebabkan peningkatan paras katekolamin, hiperkalsemia, hiperglisemia, asidemia laktik dan aktiviti enzim. Berikutan penyembelihan, haiwan SWS mempunyai perubahan aktiviti elektrik otak (EEG) yang lebih tinggi berbanding semasa pra penyembelihan, manakala renjatana elektrik pula memaksimumkan kemungkinan pengsan semasa pasca renjatan. Berdasarkan data EEG, haiwan LFHO mempunyai perubahan aktiviti elektrik otak yang tertinggi ketika renjatan berbanding dengan haiwan dari kumpulan renjatan yang lain, menunjukkan adanya tindak balas tekanan fisiologi. LFHB mendorong serangan jantung dalam semua kambing yang terlibat, sedangkan perkara ini tidak berlaku pada kambing yang direnjat secara LFHO dan HFHB. Tempoh antara titik mula pemotongan dan penghasilan fibrilasi jantung bagi kambing HFHB adalah sama sepertimana yang diperhatikan di dalam kumpulan LFHO dan SWS. Ciri-ciri karkas dan kualiti daging perlu diambil kira apabila penilaian terhadap keberkesanan sistem renjatan hendak dilakukan. Pada jam ke 12 postmortem, LFHO, LFHB dan HFHB mempamerkan glikogen dan pH yang lebih rendah ($p<0.05$), laktat dan nilai potensi glikolitik yang lebih tinggi berbanding dengan SWS. Kadar penurunan pH yang lebih cepat ($p<0.05$) telah ditemui dalam LFHO, LFHB dan HFHB berbanding SWS. pH, glikogen, laktat, potensi glikolitik, kehilangan air menerusi titisan, warna, daya potongan, panjang sarkomer dan indeks fragmentasi miofibril pada hari ke 1, 7 dan 14 pasca mortem tidak berbeza di antara rawatan. LFHO, LFHB dan HFHB menunjukkan kehilangan air menerusi memasak yang lebih tinggi ($p <0.05$) berbanding SWS pada hari ke 7 dan 14 postmortem. Insiden bintik kecil dan percikan darah pada bahu, pinggang dan kaki daripada kumpulan yang direnjat dengan elektrik adalah lebih tinggi ($p<0.05$) berbanding SWS. Namun begitu, HFHB mempunyai bintik kecil dan percikan darah yang lebih rendah ($p<0.05$) daripada LFHB dan LFHO. Kambing-kambing dari SWS, LFHO dan HFHB mempunyai kehilangan darah yang lebih tinggi ($p<0.05$) dan sisa hemoglobin dalam otot yang lebih rendah

berbanding LFHB. Daging LFHB mempunyai nilai TBARS dan jumlah bilangan bakteria yang lebih tinggi ($p<0.05$) berbanding rawatan lain pada hari ke 7 dan 14 postmortem. Analisis immunoblot, karbonil dan penentuan kandungan thiol mendedahkan bahawa pengoksidaan protin meningkat selari dengan jangkamasa penuaan, namun tidak terjejas dengan kaedah penyembelihan yang berbeza. Ungkapan rantaian berat miosin dan troponin-T berubah apabila masa penuaan semakin meningkat, aktin bagaimanapun kekal stabil. Penemuan semasa ini menunjukkan bahawa kaedah sembelihan menyebabkan kesan tekanan fisiologi yang besar di mana ia boleh menjejaskan kebajikan haiwan dan kualiti daging, dimana semua tahap parameter biokimia darah yang diperhatikan jatuh dalam julat fisiologi yang normal untuk kambing. Tambahan lagi, HFHB boleh digunakan sebagai gantian kepada kaedah renjatan elektrik konvensional memandangkan ia memenuhi kedua-dua syarat dan keperluan Islam terhadap penyembelihan haiwan dalam keadaan hidup sewaktu penyembelihan dan juga keperluan kebajikan haiwan terhadap ketidaksedaran haiwan semasa penyembelihan.

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I certify that a Thesis Examination Committee has met on 06 June 2016 to conduct the final examination of Azad Behnan Sabow on his thesis entitled "Welfare, Physiological Stress Responses, and Carcass and Meat Quality in Goats Subjected to Different Slaughter Methods" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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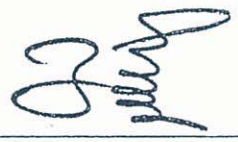

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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A	Amperage
a*	Redness
AC	alternating current
APS	ammonium persulfate
AS	slaughter following minimal anesthesia
ATP	adenosine triphosphate
b*	Yellowness
BSA	bovine serum albumin
C*	Chrom
CaCl2•2H2O	calcium chloride dihydrate
CD	correlation dimension
cfu	colony forming units
CK	creatine kinase
cm	Centimeter
CNS	central nervous systems
CO2	carbon dioxide
d	Day
DAB	3,3-diaminobenzidine
ddH2O	deionized distilled water
DFD	dark, firm and dry
DTNP	2, 2-dithiobis (5-nitropyridine)
EC	European Community Council Regulations
ECG	Electrocardiogram
ECT	electroconvulsive therapy
EDTA	ethylene diamine tetraacetic acid
EEG	Electroencephalogram
EFSA	European Food Safety Association
ETHal	end tidal halothane
F50	median frequency
FAO	Food and Agricultural Organization
g	Gram
h	Hour
Hb	Hemoglobin
H2O2	hydrogen peroxide
HFHB	high frequency head-to-back electric stun

Hº	Hue
HO•	hydroxyl radicals
HPA	hypothalamic pituitary adrenal
HS	halal slaughter
HSA	Humane Slaughter Association
Hz	Hertz
IgG	immunoglobulin G
K3Fe(CN)6	potassium ferricyanide
KCl	potassium chloride
kg	Kilogram
KH2PO4	monopotassium phosphate
kHz	Kilohertz
l	Liter
L*	Lightness
LDH	lactate dehydrogenase
LFHB	low frequency head-to-back electric stun
LFHO	low frequency head-only electric stun
LL	Longissimus lumborum
LW	body weight pre-slaughter
M	Mole
mA	Milliampere
MAC	minimum alveolar concentration
Mb	Myoglobin
MDA	Malondialdehyde
MES	2-(N-morpholino) ethanesulfonic acid
MFI	myofibrillar fragmentation index
MgCl2	magnesium chloride
MgCl2•6H2O	magnesium chloride hexahydrate
MHC	myosin heavy chain
min	Minute
µg	micro gram
µl	Microliter
µl	micro liter
µm	Micrometer
µM	Micromole
µmole	Micromole
ml	Milliliter

mM	Millimole
mmole	Millimole
ms	Millisecond
µV	Microvolt
MTT	thiazolyl blue tetrazolium bromide
Na2HPO4	disodium phosphate
NaCl	sodium chloride
NAD	β-nicotinamide adenine dinucleotide
ng	Nanogram
m	Nanometer
nmol	Nanomole
ºC	degree Celsius
OD	optical density
OIE	World Organi ation for Animal Health
%	Percent
pH0	pre-rigor pH
pH12h	12 hours post ortem pH
pH45min	45 min postmortem pH
pHu	ultimate Ph
PSE	pale, soft and exudative
Ptot	total power
PVDF	polyvinylidene diflouride
RD	reflective density
RMS	root mean square
ROS	reactive oxygen species
s	Second
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SER	somatosensory evoked responses
ST	Semitendinosus
SWS	slaughter without stunning
TBARS	thiobarbituric acid-reactive substances
TBST	tris buffered saline-tween 20
TCA	trichloroacetic acid
TCA	trichloroacetic acid
TEMED	tetramethylethylenediamine
Tris	2-amino-2-(hydroxymethyl)-propane-1,3-diol

USDA	United States Department of Agriculture
V	Voltage
v/v	volume per volume
v/v	volume per volume
VER	visual evoked responses
w/v	weight per volume
WHC	water holding capacity

CHAPTRE ONE

GENERAL INTRODUCTION

Goat meat (Chevon) is gaining popularity among meat consumers (Ilie *et al.*, 2012). Thanks to its low intramuscular fat, mainly saturated fatty acid, and cholesterol level when compared to similar cuts of beef and mutton (Adam *et al.*, 2010; Madruga & Bressan, 2011), which is proven by the increase in goat meat production from 4.90 million tons in 2008 to 4.99 million tons in 2011 (FAO, 2011). This prompts the gradual expansion of goat meat industries in most part of the world to improve the availability of goat meat in order to meet the demands of consumers (Dhanda *et al.*, 2003) through the use of automated slaughter techniques.

Slaughter, despite its short duration is a critical point in the meat production chain, with potential risks and its mishandling can ruin the efforts made by producers during the longer growing and fattening phases (Anil, 2012b; Farouk *et al.,* 2014; Grandin, 2010). Slaughter procedures are usually regulated by legislation, codes of practice and species-specific recommendations (Agbeniga, 2012; Anil, 2012a).

Halal slaughter without stunning is legally recognized as the appropriate method for slaughtering animals intended for consumption by Muslims (Farouk *et al.*, 2014). However, halal slaughter has remained controversial from an animal welfare point of view in the last few decades (Anil, 2012b; Grandin, 2010). The welfare issues during slaughter without stunning include the stress during handling and restraining, pain during the neck cut, and unnecessary distress during bleeding out (Gregory, 2005; Rosen, 2004). Nonetheless, it has been argued that the use of an exquisitely sharp knife produces minimal behavioral reactions in animals and therefore that such a neck cut is not perceived by the animal as painful (Regenstein, 2012; Rosen, 2004). However, there are few neurophysiological and physiological evidences to support this argument. Until now, it is not clear whether the slaughter of conscious animals causes pain or distress. This may be due to the complexities of measuring pain in animals (Mellor *et al.*, 2000; Rutherford, 2002) and limitations in the interpretation of behavioral and physiological responses to slaughter by neck cut alone.

The minimally anesthesia model is now widely used in all farm animals the cerebrocortical responses to noxious stimuli during neck cut and surgical operations without compromising the welfare of the animal (Johnson *et al.*, 2009). Animals under minimal anesthesia model continued to show electroencephalographic (EEG) responses from the cerebral cortex, and demonstrate normal physiological cardiovascular functions to nociceptive stimulation that are similar to those seen in fully conscious animals (Murrell & Johnson, 2006). However, as emotion and conscious awareness also contributed significantly to the process of nociception in animals, there could be differences in

response between awake and minimally anesthetized animals, particularly on parameters that are under the influence of the autonomic nervous system. Thus, assessing the effects associated only with sensory pain during slaughter in minimally anesthetized animals, versus both sensory and affective pain when animals are slaughtered fully conscious without any form of stunning is warranted. This study examined the blood biochemistry, electroencephalographic changes and meat quality characteristics associated with the neck cut without stunning in non-anesthetized and minimally anesthetized goats.

Stunning is applied on animals to make them unconscious and insensible to pain for durations long enough for death to occur whilst the animal is still in the state of unconsciousness (EFSA, 2006; Linares & Vergara, 2012). Currently, permitted methods for stunning are mechanical (captive bolt pistol) stunning, electrical stunning and gas stunning (Lambooij *et al.*, 2012; Nakyinsige *et al.*, 2013a; OIE, 2008). Electrical stunning which is accomplished by passage of a sufficient amount of current through the central nervous system is the most common stunning and slaughter application by the meat industry (Farouk, 2013). It works by producing brain dysfunction and unconsciousness either temporarily, in which case the animal dies as a result of bleed out (exsanguination); head-only electrical stunning or with subsequent killing by cardiac arrest; head-to-back electrical stunning. The head-only electrical stunning method is the only approved method for halal slaughter (Farouk, 2013; Lambooij *et al.,* 2012; Nakyinsige *et al.,* 2013a). However, conventional head-only electrical stunning (the use of a frequency of 50 Hz) method has been shown to have adverse effects on carcass and meat quality which has become the main challenge in the industry (Farouk *et al.*, 2014; Llonch *et al.*, 2015). Thus, a development in the use of high frequency electrical currents to stun and immobilize red meat animals pre-slaughter has been introduced (Simmons *et al.*, 2006).

The technique uses similar voltage and amperes settings as the traditional head-only electrical stunning settings, but uses higher frequencies (> 300 Hz). The settings used in the traditional system when applied to full animal body stop the heart, but the modified system at higher frequency does not and thus can be applied from head-to-back instead of head-only as used in the traditional system (Farouk, 2013; Farouk *et al.,* 2014). The merits of using a high frequency head-to-body component include: (i) the procedure complies with halal requirements since it does not cause cardiac arrest in animals; (ii) muscle activity associated with convulsions is avoided or reduced; (iii) bleeding efficiency and meat quality are enhanced, and (iv) the method reduces carcass damage (Agbeniga, 2012; Simmons *et al.,* 2006). It also minimizes one of the greatest potential welfare concerns, i.e. the length of time the animal is conscious after the cut and therefore the time it could potentially be experiencing pain. However, the claim made in the review article is yet to be proven through a comprehensive scientific study.

The global demand for meat and meat products from animals' slaughtered using halal slaughter method without stunning practiced by Muslims is substantial and growing (Anil 2012a). Thus, it is necessary for the meat industries to meet the religious and nutritional demands of consumers with respect to meat while maintaining and/or improving quality, wholesomeness and safety in addition to maintaining good animal welfare (Castro-Giráldez *et al.*, 2011). Thus, this study was conducted in an attempt to assess physiological stress responses, carcass characteristics and meat quality in anesthetized goats subjected to slaughter without stunning and slaughter following different electrical stunning methods.

It is hypothesized that minimizing stress on animals during slaughtering improves welfare and meat quality with positive economic as well as qualitative influences. Hence, the specific objectives of this study were:

1. To determine blood biochemical and electroencephalographic changes associated with slaughter in goat subjected to conscious halal slaughter and slaughter following minimal anesthesia.
2. To evaluate bleeding efficiency, physicochemical characteristics and shelf life of meat goat subjected to conscious halal slaughter and slaughter following minimal anesthesia.
3. To assess the effects of slaughter without stunning and slaughter following different methods of electrical stunning on blood biochemical and electroencephalogram (EEG) changes associated with stress in goats.
4. To determine carcass and meat quality characteristics in goats subjected to slaughter without stunning and slaughter following different electrical stunning methods.
5. To compare bleeding efficiency, lipid and protein oxidation and microbiological quality of goat meat obtained by slaughter without stunning and slaughter following different electrical stunning methods.

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