UNIVERSITI PUTRA MALAYSIA

EFFECTS OF IODINE AND SELENIUM SUPPLEMENTATION ON GROWTH, CARCASS CHARACTERISTICS AND MEAT QUALITY OF CROSSBRED KACANG GOATS

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

May 2013

DEDICATIONS

THIS THESIS IS DEDICATED IN MEMORY OF MY LATE MOTHER AND FATHER

AND

TO MY FAMILY WITH LOVE AND RESPECT

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

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Chairman : Awis Qurni Bin Sazili, PhD

Faculty : Agriculture

Unlike in sheep and cattle, research work in relation with the effects of trace elements such as selenium (Se) and iodine (I) on metabolism and meat quality in goats are rather limited. In most parts of the world, there are still Se and I deficiencies which justify continuous efforts to fortify compound animal feeds with Se and I for the enhancement of growth and health of goats as well as an intervention to produce Se and I enriched meat and organs for human consumption.

The objective of this study was to investigate the impact of dietary supplementation of iodine, selenium and a combination of both at a level of 0.6mg/kg DM on growth performance, carcass characteristics, myosin heavy chain-slow and fast proteins distribution and meat quality of local Kacang crossbred male goats. A total of twenty four, 7 to 8 months old animals with a mean live weight of 22.00 ± 1.17 kg were randomly assigned to either one of four dietary treatments groups each consisting of 6 animals. The four dietary treatments were: T1 (control) – basal diet without supplementation; T2 – basal diet with 0.6 mg Se/kg DM; T3 – basal diet with 0.6 mg I/kg DM; T4 – basal diet with combination of 0.6 mg Se/kg DM and 0.6 mg I/kg DM. The inorganic selenium was given in the form of sodium selenite while

inorganic iodine was in the form of potassium iodide. The basal diet offered was a concentrate mix based on palm kernel cake, corn and rice bran. The amount of concentrate offered was based on 1% of body weight with *ad libitum* amount of fresh guinea grass. After 100 days of feeding, the animals were slaughtered following which s*upraspinatus* (SS), *longissimus lumborum* (LL), *semitendinosus* (ST) muscles were sampled from each carcass. Representative muscle samples were dissected at 3 specific periods, that is, immediately after evisceration (30 min), 24 h and 7 days *post mortem* to represent the unconditioned (pre-rigor), 1 day-conditioned (chilled) and 1 week-conditioned samples, respectively.

The animals in T4 group indicated a higher total weight gain ($p<0.05$) and average daily weight gain (ADWG) ($p<0.05$) than the T1 and T2 animals. Significant improvement in the feed conversion ratio (FCR) was only noted in group T4 compared to the T1 (control) animals. In comparison with those of control, significant differences in carcass dressing percentage were presented by the T2, T3 and T4 animals. Results from this study indicated that serum of the animals subjected to T2, T3 and T4 were significantly higher ($p<0.001$) in Se and I concentrations than the control group. Furthermore, the increases ($p<0.05$) in total I and Se contents in the selected muscles (SS, LL and ST), liver and kidney of the supplemented goats have reflected positive response towards the dietary supplementations. Meanwhile, thyroid follicular epithelial cells height of the animals supplemented with I (T3) and combination of I and Se (T4) were significantly greater than those of control (T1) and Se supplemented (T2) groups. Besides, free triiodothyronine (FT_3) of the animals supplemented with combination of I and Se (T4) was higher ($p<0.05$) than the animals supplemented with I (T3) alone. However, the dietary supplementation of

Se, I and their combination did not affect the distribution of myosin heavy chain-slow and fast proteins in the SS, LL and ST muscles.

With regard to meat quality, lower (p<0.05) drip loss was indicated by the muscles of T2 and T4 animals. The n-3 polyunsaturated fatty acids in the muscles of all supplemented groups were significantly higher than the control group. The concentrations of malondialdehyde in SS, LL and ST muscles obtained from T2 and T4 animals were significantly lower than those of T1 and T3 at day 7 *post mortem*. Additionally, significantly higher activities of glutathione peroxidase enzyme in serum, liver and muscles (SS, LD and ST) were also exhibited by the T2 and T4 animals.

Generally, the present study demonstrated that the combined dietary supplementation of Se and I has not only improved growth performance but also meat oxidative stability and water holding capacity of meat in goats.

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

KESAN SUPLEMENTASI IODIN DAN SELENIUM KE ATAS PERTUMBUHAN, CIRI KARKAS DAN KUALITI DAGING KAMBING KACANG KACUKAN

Oleh

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Tidak seperti dalam biri-biri dan lembu, kerja-kerja penyelidikan berhubung dengan kesan unsur surih seperti selenium (Se) dan iodin (I) pada metabolisme dan kualiti daging kambing adalah terhad. Di kebanyakan tempat di dunia, masih terdapat kekurangan Se dan I yang mewajarkan usaha berterusan bagi mempertingkatkan kandungan Se dan I di dalam makanan haiwan kompaun untuk meningkatkan pertumbuhan dan kesihatan kambing serta pengubahsuaian bagi menghasilkan daging dan organ-organ yang diperkaya dengan Se dan I untuk kegunaan manusia.

Objektif kajian ini adalah untuk menyiasat kesan suplemen pemakanan iodin, selenium dan gabungan kedua-duanya pada tahap 0.6mg/kg DM kepada pertumbuhan prestasi, ciri-ciri karkas, komposisi protein dan kualiti daging daripada kambing Kacang kacukan tempatan jantan. Sebanyak dua puluh empat ekor haiwan, berumur di antara 7 hingga 8 bulan dengan berat purata sebanyak 22.00 ± 1.17 kg telah diagihkan secara rawak kepada salah satu daripada empat kumpulan rawatan pemakanan di maan setiap satu terdiri daripada 6 ekor haiwan. Empat rawatan pemakanan tersebut adalah: T1 (kawalan) - diet asas tanpa tambahan; T2 - diet asas

dengan tambahan sebanyak 0.6 mg Se / kg DM; T3 - diet asas dengan tambahan sebanyak 0.6 mg I / kg DM; T4 - diet asas dengan gabungan tambahan sebanyak 0.6 mg Se / kg DM dan 0.6 mg I / kg DM. Mineral selenium bukan organik telah diberi dalam bentuk natrium selenite manakala iodin bukan organik pula dalam bentuk kalium iodida. Diet asas yang ditawarkan adalah terdiri dari campuran kek isirong sawit, jagung dan beras. Jumlah konsentrat yang ditawarkan adalah berdasarkan kepada 1% daripada berat badan haiwan dengan pemberian rumput guinea segar tanpa had. Selepas tempoh 100 hari rawatan pemakanan, penyembelihan haiwan dilakukan dan disusuli dengan pensampelan otot *supraspinatus* (SS), *longissimus lumborum* (LL) dan *semitendinosus* (ST) dari setiap karkas. Sampel dari setiap otot diambil mengikut 3 jangkamasa berbeza, iaitu, selepas pembersihan karkas (30 min), 24 jam dan 7 hari post mortem, masing-masing bagi mewakili tempoh sebelum kejang mayat, 1 hari dan 7 hari tempoh penuaan.

Haiwan dalam kumpulan T4 menunjukkan jumlah berat badan ($p < 0.05$) dan berat badan harian purata yang lebih tinggi ($p < 0.05$) daripada haiwan di dalam kumpulan T1 dan T2. Peningkatan yang ketara dalam nisbah penukaran makanan hanya dicatatkan dalam kumpulan T4 berbanding T1 (kawalan). Berbanding haiwan dari kumpulan kawalan, perbezaan yang signifikan dalam peratusan karkas bersih telah ditunjukkan oleh haiwan dari kumpulan T2, T3 dan T4. Hasil kajian ini menunjukkan bahawa kandungan Se di dalam serum dari haiwan yang telah berikan rawatan diet T2, T3 dan T4 adalah lebih tinggi ($p < 0.001$) berbanding dengan kumpulan kawalan. Tambahan pula, peningkatan kandungan I dan Se ($p < 0.05$) di dalam otot terpilih (SS, LL dan ST), hati dan buah pinggang kambing turut menunjukkan tindak balas positif haiwan terhadap rawatan pemakanan yang diberikan. Sementara itu, ketinggian sel epitelium bagi folikel tiroid juga bertambah

melalui rawatan I (T3) dan gabungan I dan Se (T4) berbanding dengan kumpulan T1 (kawalan) dan T2. Selain itu, paras triiodothyronine bebas (FT3) di dalam haiwan yang dirawat dengan gabungan I dan Se (T4) adalah lebih tinggi ($p < 0.05$) berbanding dengan haiwan yang diberikan rawatan diet I (T3) semata-mata. Walau bagaimanapun, rawatan diet Se, I dan gabungan keduanya tidak memberikan sebarang kesan ke atas komposisi protein myosin di dalam otot SS, LL dan ST. Berhubung dengan kualiti daging, kehilangan air melalui titisan adalah lebih rendah ($p < 0.05$) pada otot haiwan yang di telah berikan diet T2 dan T4. Asid lemak tidak tepu n-3 pada otot bagi semua kumpulan rawatan (T2, T3 dan T4) adalah jauh lebih tinggi berbanding dengan kumpulan kawalan (T1). Kandungan malondialdehid di dalam otot SS, LL dan ST yang diperoleh daripada haiwan T2 dan T4 adalah jauh lebih rendah berbanding dengan T1 dan T3 pada hari ke-7 *post mortem*. Selain itu, aktiviti enzim glutathione peroxidase yang jauh lebih tinggi juga telah dikenalpasti di dalam serum, hati dan otot (SS, LD dan ST) dari haiwan T2 dan T4.

Secara umumnya, kajian ini telah menunjukkan bahawa gabungan suplemen pemakanan Se dan I bukan sahaja dapat memperbaiki prestasi pertumbuhan haiwan tetapi juga dapat meningkatkan kestabilan oksidatif dan daya membendung air daging kambing.

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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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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

ZEIAD AMJAD ABDULRAZZAK AGHWAN

Date: 31 May 2013

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ADF	acid detergent fibre
ADWG	average daily weight gain
ANOVA	analysis of variance
APS	ammonium persulphate
ATP	adenosine triphosphate
BSA	bovine serum albumin
^{o}C	degrees celcius
CP	crude protein
CLA	conjugated linoleic acid
cm2	square centimetre
d	day
ddH_2O	deionized distilled water
DIT	diiodotyrosine
DM	dry matter
DMI	Dry matter intake
EDTA	ethylenediamine tetra-acetic acid
FA	fatty acid
FCR	feed conversion ratio
FT_3	free triiodothyronine hormone
FT_4	free thyroxine hormone
g	gram
GLM	general linear model
GSHPx	glutathione peroxidase enzyme
h	hour
HDL	high density lipoprotein
I	iodine
IgG	immunoglobulin G
IMCT	intramuscular connective tissue
IMF	intramuscular fat
kg	kilogram
l	litre

µl	micro litre
LDL	low density lipoprotein
LL	longissimus lumborum
mA	milliamperage
Mcal	megacalorie
MDA	malondialdehyde
ME	metabolisable energy
mg	milligram
µg	microgram
MHC	myosin heavy chain
MHC-f	fast-myosin heavy chain
MHC-s	slow-myosin heavy chain
min	minute
MIT	monoiodotyrosine
µm	micrometer
µM	micromole
mM	millimole
ml	millilitre
MUFA	monounsaturated fatty acid
n-3:n-6 ratio	Total n-3 PUFA to Total n-6 PUFA ratio
NDF	neutral detergent fiber
OM	organic matter
pHu	ultimate pH
pmol/l	pico mole per litre
PUFA	polyunsaturated fatty acid
PUFA:SFA ratio	Total PUFA to Total SFA ratio
PVDF	polyvinylidene diflouride
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
Se	selenium
sec	second
SFA	Saturated fatty acid

SS	*Supraspinatus*
ST	*Semitendinosus*
T_3	triiodothyronine hormone
T_4	thyroxine hormone
TBS-T	tris buffered saline-tween 20
TCA	trichloroacetic acid
Tris	2 amino-2-(hydroxymethyl)-propane-1,3-diol
USF	unsaturated fatty acid
UFS:SFA ratio	Total UFA to Total SFA ratio
v/v	volume per volume
WHC	water holding capacity
wk	week
w/v	weight per volume

CHAPTER I

GENERAL INTRODUCTION

Sheep and goats are among farm animals which are commonly implicated in the global livestock industry, and together, they represent more than 52% of the domesticated ruminants (buffaloes, camels, cattle, goats, and sheep) in the world. The goat population has increased steadily since 2001, with goats emerging as a main ruminant animal used for meat and milk production in the coming years (Tedeschi *et al*., 2010). The acceptance of goat meat around the world has increased over the past few years, mainly because it is leaner than beef and mutton (Mahgoub *et al*., 2002) and with lower cholesterol than other ruminants (Naud´e & Hofmeyer, 1981) as well as increasing ethnic populations

Growth performance in ruminants is important for producers since it reflects the feed conversion ratio and rate of weight gain to obtain a carcass with good conformation and meat products with acceptable quality traits. Dietary supplementations of trace elements such as selenium (Se) and iodine (I) to farm animals is crucial to maintain the proper functions of their body as well as to enhance metabolic and defence mechanisms, which results in improvement of growth (Potter *et al*., 1980; McDowell, 2003; Kumar *et al*., 2009) and meat eating quality (Kaufmann & Rambeck, 1998; Franke *et al*., 2008; Juniper *et al*., 2009; Shi et al., 2011). Several studies reported that both Se and I are important for thyroid gland function and thyroid hormone biosynthesis (Arthur *et al*., 1990; Todini, 2007; Schomburg & Kohrle, 2008; Qin *et al*., 2011).

Supplementation of I up to 10 mg/kg diet tended to reduce the daily gain and increased the weight of thyroid gland in bulls (Meyer *et al*., 2008). Calves supplemented with 50 mg I/kg DM diet as calcium iodate shown reduced feed intake and weight gain (Newton *et al*., 1974). Excessive iodine intake by ewes in late pregnancy reduced plasma immunoglobulin G concentration in their lambs (Boland *et al*., 2004). Studies in pigs (Schone *et al.*, 2006; Franke *et al.*, 2008; Li *et al.*, 2011), bulls (Meyer *et al.*, 2008) and broiler chickens (Rottger *et al.*, 2011) showed that iodine supplementation resulted in higher I levels in their blood, muscle and organs with no effect on growth rate and carcass characteristics.

Adequate selenium intake supports thyroid gland metabolism, thyroidal hormone synthesis and protect the thyroid gland from the risk of oxidative damage to lipids of the cellular membrane and proteins in the cell (Zimmermann & Kohrle, 2002). Recent studies demonstrated inconsistent findings on the effects of Se supplementation on growth rate in farm animals. Studies in calves (Wichtel *et al.*, 1996), lambs (Kumar *et al.*, 2009) and goats (Yue *et al*., 2009; Shi *et al*., 2011) reported that dietary Se supplementation has positively affected the growth rate while the other related works documented no response in calves (Skrivanova *et al.*, 2007), lambs (Vignola *et al.*, 2009) and goats (Chung *et al*., 2007). Moreover, earlier studies concluded that dietary supplementation of Se in the diets of calves (Skrivanova *et al.*, 2007), lambs (Qin *et al*., 2007; Vignola *et al*., 2009), and goats (Shi *et al*., 2011) increased Se content of meat and edible organs.

In the meat industry, emphasis has been continuously given on the production of leaner meat through a better understanding on the role of muscle fibres in

determining growth, carcass characteristics and meat quality. The increase in muscle fibre number and size, and fibre type transformation from small, slow-twitch oxidative fibres to large, fast-twitch glycolytic fibres has been associated with increased muscle mass (Wegner *et al*., 2000). It has been reported that increases in lean tissue growth rate and composition in carcass were positively related with both glycolytic and oxidative metabolisms in pig *Longissimus dorsi* muscle (Karlsson *et al*., 1993; Henckel *et al*., 1997). Findings from previous studies (Ashmore, 1974; Rahelic & Puac, 1981; Ruusunen & Puolanne, 2004) suggested that the shift in skeletal muscle metabolism from red oxidative to white glycolytic type could explain the increased growth rate and lean meat in domesticated animals compared with wild animals. Furthermore, studies in pigs (Brocks *et al*., 1998) and sheep (Kadim *et al*., 1993) showed a positive relationship between oxidative fibres (type I) percentage and carcass fatness. Recently, Wimmers *et al*. (2008) reported a positive association between the abundance of myosin heavy chain type IIB (fast-twitch glycolytic) and muscularity in several diverse pig breeds.

The consumption of goat meat has recently increased and this could be due to the fact that it contains lower fat and cholesterol compared to other red meats which have been implicated with high incidence of cardiovascular diseases, particularly in developed countries (Park *et al*., 1991; Banskalieva *et al*., 2000; Mushi *et al*., 2008). Despite being low in lipid content, goat meat has a high proportion of unsaturated fatty acids as well as being a source of conjugated linoleic acid (Webb *et al*., 2005). However, goat meat has generally been perceived as less tender meat than other types of red meat such as beef, lamb or pork (Smith *et al*., 1974; Schonfeldt *et al*., 1993).

Despite the available data on the iodine content in pork (He *et al*., 2002) and veal (Meyer *et al*., 2008), the effect of dietary I on meat quality is yet to be investigated. This may be explained by the lower carry-over of iodine into meat as compared to milk and eggs (Franke *et al*., 2008). Winger *et al.* (2008) documented that in meat products involving iodine and its salts may potentially increase oxidative reactions, thus reducing shelf life. Additionally, it may also reduce the bioavailability of other nutritionally important substances. However, García-Iniguez de Ciriano *et al.* (2010) reported that no oxidation was detected using iodized salt with selenium, n-3 PUFA and natural antioxidant as an ingredient in dry fermented sausages formulations. More recently, Hes *et al*. (2012) reported no significant differences in the content of thiobarbituric acid reactive substances (TBARS) of iodized (KI) and non-iodized salt fresh pork after 60 d of storage.

Oxidative damage is the major non-microbial factor responsible for quality deterioration of muscle foods (Descalzo *et al*., 2005). Lipid oxidation during handling, processing and storage of fresh meat conduce to discoloration, drip losses, off-flavour and off-odour development as well as the possibility to produce toxic compounds (Gray *et al*., 1996). Malondialdehyde (MDA) is the oxidative stress final metabolic product of lipid peroxides that helps to be a directory of antioxidant status (Descalzo *et al*., 2005). Meat oxidative stability can be maintained through the balance between pro-oxidant and antioxidant components in the muscle (Jensen *et al*., 1998; Descalzo & Sancho, 2008). The polyunsaturated fatty acids, particularly those in the phospholipid fraction of cell membranes (Gray *et al*., 1996) as well as oxygen, and haem proteins (Kanner *et al*., 1988) are the main pro-oxidant substrates needed for meat deteriorative reaction. It has been reported that selenium is

important for the intra- and extracellular antioxidant systems in the body (Surai & Dvorska, 2002), and that muscle Se content is an important regulator of GSHPx activity (Skrivanova *et al*., 2007; Zhan *et al*., 2007; Juniper *et al*., 2008a; Wang *et al*., 2011). Glutathione peroxidase plays vital defence function and delays the commencement of oxidation reactions, which affects meat quality characteristics (Morrissey *et al*., 1998). Findings of several studies showed that dietary Se supplementation in the diet reduced drip loss in pork (Mahan *et al*., 1999; Mateo *et al*., 2007; Zhan *et al*., 2007; Li *et al*., 2011) and poultry (Wang *et al*., 2011) during *post mortem* aging period. Drip loss is among the important parameters of fresh meat that influence consumer's perception and the final weight of meat (Den Hertog-Meischke *et al*., 1997). Dietary supplementation of selenium to pigs and broiler chickens enhanced meat discolouration throughout *post mortem* aging by improving antioxidant ability and protecting against deteriorative reactions during lipid peroxidation (Daun & Akesson, 2001; Daun & Akesson, 2004; Zhan *et al*., 2007; Wang *et al*., 2011). However, studies in calves (Skrivanova *et al*., 2007) and lambs (Vignola *et al*., 2009; Ripoll *et al*., 2011) demonstrated lack of effect of dietary Se on the meat colour. Moreover, the antioxidant status in goats was markedly improved following Se supplementation (Yue *et al*., 2009; Shi *et al*., 2011). It has been reported that the inconsistent findings related with the effect of dietary Se on meat oxidative stability could be explained by differences in sources of Se and species of animals (Wang *et al*., 2011).

Unlike in sheep and cattle, research work and publications in relation with the effects of trace elements such as Se and I on metabolism and meat quality in goats are rather limited (Haenlein & Anke, 2011). In most parts of the world, there are still Se

(Rayman, 2004; Thomson, 2004) and I (Kaufmann & Rambeck, 1998) deficiencies which justifies continuous efforts to fortify compound animal feeds with Se and I to enhance the growth and promote health of goats and also as an intervention to produce Se and I enriched meat and organs for human consumption.

It was hypothesized that the dietary supplementations of Se or I and their combination above requirement levels in goats may improve growth performance, carcass characteristics and meat quality with changes in the distribution of myosin heavy chain proteins in the skeletal muscles. Hence, this study was conducted in an attempt to achieve the following objectives:

1. To determine the effects of iodine and selenium dietary supplementation and a combination of both at a level of 0.6 mg/kg DM on the growth performance of Kacang crossbred male goats.

2. To determine the effects of iodine and selenium dietary supplementation and a combination of both at a level of 0.6 mg/kg DM on the carcass characteristics of Kacang crossbred male goats.

3. To determine the effects of iodine and selenium dietary supplementation and a combination of both at a level of 0.6 mg/kg DM on the distribution of myosin heavy chain protein in three major skeletal muscles of Kacang crossbred male goats.

4. To determine the effects of iodine and selenium dietary supplementation and a combination of both at a level of 0.6 mg/kg DM on the eating quality of goat meat.

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