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PERFORMANCE AND METABOLOMIC URINALYSIS OF HEAT-STRESSED DAIRY GOAT FED DIET SUPPLEMENTED WITH SOYBEAN OIL

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By

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

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January 2014

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Heat stress negatively affects the animal production that could result in devastating economic lossess. The benefits of supplementing dietary fats to the animals under such conditions have been extensively reviewed. However, there is a lack of information on the effects of soybean oil supplementation on the performance of the animals, particularly under heat stress conditions. In addition, the application of the state-of-the-art technology such as ^{1}H NMR-based metabolomics to study the fundamental physiological processes in animal production study is still few. The first experiment was carried out to assess the productive performance, thermoregulatory functions and milk parameters of heat stressed Murciano-Granadina goats fed diets supplemented with soybean oil. Meanwhile, the second experiment was conducted to identify possible metabolite markers as a result of the heat stress and soybean oil supplementation. The results from the first experiment showed that the heat stressed goats lost an average of 3.14 ± 2.30 kg of their body weight compared to the thermoneutral group that gained an average of 3.19 ± 2.62 kg of body weight. They also had a 39.1% lower dry matter intake (DMI), consumed 46.8% more water and produce 9.7% less milk compared to the thermoneutral group goats. Under heat stress conditions, there were significant correlations between temperature humidity index (THI) values and the water consumption (r = 0.66; P<0.01), rectal temperature (r = 0.94; P<0.001) and respiratory rate (r = 0.87; P<0.001). There were also non-significant (P>0.05) negative correlations between THI and DMI (r = -0.19) and milk production (r = -0.36). No significant effect (P>0.05) of soybean oil supplementation and its interaction with thermal conditions were found in any productive performance. Nonetheless, milk from supplemented animals were 23.2 and 1.6% higher in milk fat and protein content, respectively, besides 18.1 and 14.0% higher feed efficiency under heat stress and thermoneutral conditions, respectively. In the second experiment, partial least square – discriminant analysis (PLS-DA) model was only found significant (P<0.01) for heat stress vs. thermoneutral treatment groups comparison. Several metabolites of importance were identified which involved in various physiological response of animals to heat stress, such as increased harmful gut microbiota activity (hippurate), increased catecholamines and neurotransmitter activities (L-phenylalanine, glycine) and

decreased degradation of energy-related metabolites (acetate, isoleucine and glutamate). The urine sample of the heat stressed goats was also shown to have a higher 3-hydroxybutyrate and lower creatinine, which are important in assessing the energy status of the animals. Despite the lack statistical evidence, soybean oil supplementation was shown to have beneficial physiologic effects on the animals, especially during heat stress with the higher creatinine level, and lower isoleucine and glutamate found in the urine. The present study has proved the adverse effects of heat stress on animal production. There was also economic and physiologic benefit of soybean oil supplementation despite no significant result on the productive performance was observed. Meanwhile, metabolite assessment provides a deeper understanding on the physiological response of the animals towards environment and feeding interventions.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PRESTASI DAN ANALISIS METABOLOMIK URIN KAMBING TENUSU DI DALAM TEGASAN HABA YANG DIBERI DIET DITAMBAH MINYAK KACANG SOYA

Oleh

MOHD NAZRI BIN MD NAYAN

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Tegasan haba memberi kesan negatif kepada produksi haiwan yang boleh menyebabkan kerugian ekonomi yang teruk. Kelebihan memberi diet minyak tambahan kepada haiwan di dalam keadaan tersebut telah dikaji dengan meluas. Walaubagaimanpun, maklumat berhubung kesan penambahan minyak kacang soya ke atas prestasi haiwan, terutamanya di dalam keadaan tegasan haba, masih sedikit. Selain itu, aplikasi teknologi terkini seperti kaedah metabolomik berasaskan ^{1}H NMR bagi kajian pokok mengenai proses fisiologi di dalam produksi haiwan masih berkurangan. Ujikaji pertama telah dijalankan bagi menilai prestasi produktif, fungsi pentermokawalaturan dan parameter susu pada kambing Murciano-Granadina yang berkeadaan tegasan haba serta diberi minyak kacang soya sebagai makanan tambahan. Manakala ujikaji kedua dijalankan bagi mengenalpasti penanda metabolit yang berpotensi, kesan daripada tegasan haba dan makanan tambahan minyak kacang soya. Hasil daripada ujikaji pertama mendapati kambing di dalam keadaan tegasan haba secara puratanya telah kehilangan berat badan sebanyak 3.14 ± 2.30 kg berbanding kumpulan termoneutral yang bertambah berat badan sebanyak 3.19 ± 2.62 kg. Kambing tersebut juga mempunyai pengambilan bahan kering (DMI) yang 39.1% lebih rendah, meminum 46.8% lebih banyak air dan menghasilkan 9.7% lebih rendah susu berbanding kambing di dalam kumpulan termoneutral. Di dalam keadaan tegasan haba, terdapat korelasi yang signifikan di antara indeks kelembapan suhu (THI) dan pengambilan air (r = 0.66; P<0.01), suhu rektum (r = 0.94; P<0.001) dan kadar pernafasan (r = 0.87; P<0.001). Hasil kajian juga mendapati korelasi negatif yang tidak signifikan (P>0.05) di antara THI dan DMI (r = -0.19) dan produksi susu (r = -0.36). Tiada kesan yang signifikan (P>0.05) oleh tambahan minyak soya dan juga interaksinya yang telah didapati dalam sebarang prestasi produksi. Walaubagaimanapun, susu daripada haiwan yang diberikan diet tambahan didapati mempunyai 23.2 dan 1.6% lebih tinggi kandungan lemak dan protinnya; di samping menunjukkan kecekapan makanan yang masing-masing 18.1 and 14.0% lebih tinggi di dalam keadaan tegasan haba dan termoneutral. Di dalam ujikaji kedua, hanya model partial least square – discriminant analysis (PLS-DA) bagi perbandingan kumpulan tegasan haba vs. termoneutral yang didapati signifikan (P<0.01). Beberapa metabolit-metabolit penting telah dikenalpasti yang terlibat di

dalam pelbagai tindakbalas fisiologi, seperti peningkatan aktiviti mikrobiota usus yang berbahaya (hipurat); peningkatan aktiviti katekolamina dan neurotransmiter (L-fenilalanina, glisina); dan penurunan degradasi metabolit berkaitan tenaga (asetat, isoleusina and glutamat). Sampel urin daripada kambing di dalam tegasan haba juga didapati mengandungi 3-hidroksibutirat yang lebih tinggi dan kreatinina yang lebih rendah, yang mana adalah penting di dalam menilai status tenaga haiwan. Walaupun kekurangan bukti statistik, makanan tambahan minyak kacang soya telah menunjukkan mempunyai faedah kesan secara fisiologi ke atas haiwan, terutamanya ketika dalam keadaan tegasan haba dengan aras kretinina yang tinggi dan isoleusina dan glutamat yang rendah, yang didapati di dalam urin. Kajian semasa ini telah membuktikan kesan buruk tegasan haba ke atas produksi haiwan. Terdapat juga faedah makanan tambahan minyak kacang soya secara ekonomi dan fisiologi walaupun tiada hasil yang signifikan dilihat pada prestasi produktif. Manakala, penilaian metabolit membolehkan kefahaman yang mendalam tentang respon fisiologi hawan terhadap intervensi persekitaran dan pemakanan.

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May the sun always shine on your window pane,
May a rainbow be certain to follow each rain,
May the hand of a friend always be near you,
May God fill your heart with gladness to cheer you.

Thank you very much.

Nazri Hj. Nayan
Kuala Lumpur
January 2014

I certify that an Examination Committee has met on 10th January 2014 to conduct the final examination of Mohd Nazri Bin Md Nayan on his degree thesis entitled "Performance and Metabolomic Urinalysis of Heat-Stressed Dairy Goats Fed Diets Supplemented with Soybean Oil" in accordance with 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 Master of Science.

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
^1H	Proton
3.5% FCM	3.5% Fat Corrected Milk
ADF	Acid detergent fiber
ANOVA	Analysis of Variance
BWC	Body weight change
C	Control
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
FE	Feed efficiency
g	Gram
HS	Heat Stress
kg	Kilogram
L	Liter
LCT	Lower critical temperature
LWSI	Livestock Weather Safety Index
min	Minute
mL	Milliliter
NDF	Neutral detergent fiber
NIRS	Near-infrared spectrometer
NMR	Nuclear magnetic resonance
PBS	Phosphate buffer solution
PC	Principal component
PCA	Principal components analysis
PLS-DA	Partial least squares – discriminant analysis
ppm	Part per million
Q^2	Prediction coefficient
r	Correlation
R^2	Explained variance
RH	Relative humidity
RR	Respiratory rate
RT	Rectal temperature
S	4% soybean oil supplementation
S.E.M	Standard error of means
SD	Standard deviation
SIMCA	Soft Independent Modeling of Class Analogy
THI	Temperature humidity index
TMR	Total mixed ration
TN	Thermoneutral
TNZ	Thermoneutral zone

TSP	3-(trimethylsilyl) propionic-2,2,3,3-d4 acid
UCT	Upper critical temperature
VFA	Volatile fatty acids
VIP	Variable importance for projection
WCS	Wavelet compression spectral
δ	Chemical shift (delta)
μL	Microliter

CHAPTER 1

INTRODUCTION

1.1 Animal Production in Challenging Climates

The past decades, the world has been experiencing a continuous environmental ambiguity due to global warming. Excessive production of greenhouse gases such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_20) from human activities prevent heat from escaping the atmosphere. As a result, the earth surface temperature has increased by 0.78 °C over the past 100 years, with about 0.61°C of this warming occurring for just over the past 30 years (NRC, 2010). It is predicted with current climate models that this would continually increase, indicating a rise in global average surface temperature between 1.88ºC and 4.08ºC in the next 100 years (Intergovernmental Panel on Climate Change (IPCC), 2007).

Thus, there is a growing concern about global warming and its impact on human beings and animal agriculture which can result in devastating economic losses. Increasing awareness of the adverse impact has not only become a major concern for tropical countries, but also to those nations in the temperate regions such as subtropical-Mediterranean zones, which are annually exposed for 3 to 5 months to considerable heat stress especially during summer (Diffenbaugh et al., 2007). In recent years, natural disasters such as the 2003 heat wave in Europe which killed thousands of people have triggered even more concern about the heat stress impact in these regions.

It has been shown that heat stress is a great obstacle to the efficiency and productivity in dairy animals, especially during hot climates (Liu et al., 2008). It has resulted in millions of dollars revenue lost each year due to production losses, and in extreme cases, death (St-Pierre et al., 2003; Brown-Brandl et al., 2006). In the United States (US), estimated total annual economic losses was between USD1.69 and USD2.36 billion to livestock industries due to heat stress, where USD897 to USD1500 million of these losses occurred in the dairy industry (St-Pierre et al., 2003). Among contributing factors to this economic issue include decreased milk production, increased metabolic disorders and health problems, compromised milk quality and reduced reproductive performance (St-Pierre et al., 2003; Wheelock et al., 2010).

Heat stress can be defined as a physiological condition when a total heat load (internal production and environmental source) exceeding the capacity for heat dissipation which result in an increased core body temperature that exceeds its normal range for optimum body functions. This eventually induces physiological and behavioral responses to reduce the adverse effects (Bernabucci et al., 2010). Homeothermic animals have a thermoneutral zone where energy expenditure to maintain normal body temperature is minimal, constant and independent of environmental temperature (Yousef, 1987). When there is an additional energy required to maintain their core body temperature within the thermoneutral zone, animals are under heat stress (Lu, 1989). Heat-stressed animals will initiate physiological and behavioral responses in order to maintain their body temperature within the normal range (38.7 to 40.2°C). Reduced feed intake, growth, milk production and reproduction are commonly reported (Kadzere et al., 2002; Marai et al., 2007).

Meanwhile, the application of an advanced technology such as ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy in metabolomics study can benefit us by providing useful information to assess the underlying physiological mechanisms in heat stressed animals. This metabolic profiling of biofluid samples has the potential to characterize and enable the identification of metabolites that may have a role in animals that are affected by thermal treatment and dietary supplementation. Through this method, there is a great possibility to improve the current status of biological information related to the metabolome (Dunn et al., 2005).

1.2 Nutrition as an Option for Improvement

Nutritional management is an important aspect in maintaining high production of the animals under a challenging environment. According to Lu (1989), alleviating heat stress of animals may be only achieved marginally by nutritional manipulation alone. However, it would be more effective if combinations with other methods are also taken into consideration such as genetic selection of heat resistant breeds, housing and management modifications. There have been several reviews on nutritional management for the lactating animals in hot climates (Fuquay, 1981; Collier et al., 1982; West, 2003). According to West (2003), there are several considerations in nutritional management during heat stress which includes diet reformulation to account for reduced dry matter intake (DMI), greater nutrient requirements but avoiding nutrient excesses and heat increment from diet.

Fat supplementation has been used as a strategy to reduce the adverse effects of heat stress, as well as to manipulate milk composition into a more desirable state. Several studies have shown a positive response of heat stressed animal supplemented with vegetables fat (Liu et al., 2008; Caroprese et al., 2009). Other studies had shown changes in fatty acid profile of milk supplemented with fat, without giving any negative effects on the animal performance i.e. the dry matter intake and milk production (Gómez-Cortés et al., 2008; Heguy et al., 2006).

1.3 Problem Statements and Justifications

The adverse effects of heat stress on animal production, especially dairy animals are well justified. Among commonly reported physiological and behavioral responses to heat stress conditions are: decreased feed intake and milk production (Lu, 1989; Spiers et al., 2004; Bohmanova et al., 2007); and increased water consumption and thermoregulatory functions such as respiratory rate and rectal temperature (Srikandakumar et al., 2003; Caroprese et al., 2011). Milk composition has also been reported to be affected by heat stress especially the fat and protein contents of the milk (Staples et al., 2002; Finocchiaro et al., 2005). Meanwhile, nutritional management can be a successful approach in alleviating the effects of heat stress on animals. Several different feeding strategies have been suggested (Schneider et al., 1984; West, 2003; Bernabucci et al., 2010), including the supplementation of fat (Chilliard et al., 2003; Liu et al., 2008; Caroprese et al., 2009).

Hence, the possible benefit of soybean oil supplementation on the animal's performance under high ambient temperature is assessed. The potential benefits of soybean oil supplementation have been shown by several recent studies (Bouattour et al., 2007; Matsushita et al., 2007; Gómez-Cortés et al., 2008). Nevertheless, studies focusing on the effects of soybean oil supplementation on the physiological changes of the animals under thermal stress are scarce. Meanwhile, the applications of nuclear magnetic resonance (NMR) on animal production studies are still limited (Klein et al., 2010), as majority of the studies and reviews are carried out with regards to human research (Dunn et al., 2005; Moco et al., 2007; Zhang et al., 2011). Thus, the application of this technology would facilitate in the understanding the underlying physiological mechanisms occurring during the exposure of the animals to heat stress conditions.

1.4 Hypotheses

Null hypothesis (H_0): there will be no significant difference among all treatment means, μ (H_0: $\mu_{treatment} = \mu_{control}$).

Alternative hypothesis (H_1), one-tail: If the animals are under heat stress conditions, then the body weight, feed intake and milk production are expected to decrease ($H_1 = \mu_{treatment} < \mu_{control}$); while water consumption, rectal temperature and respiratory rate are expected to increase ($H_1 = \mu_{treatment} > \mu_{control}$). Meanwhile, if the animals are supplemented with soybean oil, then they are expected to have a more positive body weight change, higher feed intake and milk production ($H_1 = \mu_{treatment} > \mu_{control}$); and lower water consumption, rectal temperature and respiratory rate ($H_1 = \mu_{treatment} < \mu_{control}$) in both thermal conditions.

Alternative hypothesis (H_1), two-tails: Thermal and soybean oil treatments are expected to affect the metabolomic profile in the urine samples (H_1: $\mu_{treatment} \neq \mu_{control}$).

1.5 Objectives

General objective of this thesis is to study the effects of thermal conditions and soybean oil supplementation on the physiological performance of the animals. The research was conducted with specific aim:

i. To assess the effects of heat stress and soybean oil supplementation on the productive performance (body weight change, feed intake, water consumption) and thermoregulatory functions (rectal temperature and respiratory rate) of Murciano-Granadina goats;

ii. To assess the effects of heat stress and soybean oil supplementation on milk parameters (milk production, milk fat and protein content and feed efficiency) of the goats;

iii. To investigate potential metabolite markers as a result of heat stress and soybean oil supplementation, through the application of ^{1}H NMR-based metabolomic urinalysis of the goats.

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