EFFECTS OF DIETARY OILS DIFFERING IN FATTY ACID PROFILES ON RUMEN FERMENTATION, MICROBIAL POPULATION AND NUTRIENT DIGESTIBILITY IN GOATS

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By

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Chairman: Professor Abdul Razak Alimon, PhD
Institute: Institute of Tropical Agriculture and Food Security

Study have been conducted to investigate the effects of oils with different fatty acid profiles on rumen fermentation, microbial population and nutrient digestibility in goats. Sixteen male goats fitted with rumen cannula were randomly assigned to four experimental dietary treatments. The first group acted as control (CNT) and was given basal diet; whereas the second, third and fourth groups were supplemented with 6% of olive oil (OL), palm olein oil (PO) and sunflower oil (SF) respectively. The feed was analysed for proximate analysis. The rumen content was collected for enumeration of protozoa, analysis of fibre degrading bacteria and methanogens using qPCR, and chemical analysis for ammonia and VFA. For the second experiment, sixteen male goats were used for digestibility experiment. Approximately, 10% of total faecal collection was sampled for chemical analysis of nutrient and their digestibility was calculated. The GLM procedure from SAS was used to analyse the parameters. The mean pH of the rumen fluid pH values were significantly affected by day of sampling ($P < 0.01$). Higher mean of ammonia concentrations was observed in CNT (42.6 mg/L) compared to others and was significantly affected by diet ($P < 0.01$), day of sampling ($P < 0.05$) and diet × day of sampling interaction ($P < 0.05$). The mean of total VFA concentration (mmol) was higher in SF and OL groups and significantly affected by diet treatments ($P < 0.05$). Molar proportion of acetate, propionate and isobutyrate were affected by diet ($P < 0.05$) and day of sampling ($P < 0.05$). Molar proportion of butyrate and valerate were affected by day of sampling ($P < 0.05$). The mean number of protozoa was lower ($P < 0.05$) in treatment groups compared to CNT. There were significant differences in apparent digestibility of CP ($P < 0.05$) and EE ($P < 0.01$) of treatment groups compared to CNT. Real-time PCR have been used to quantify three cellulolytic bacteria species which are, *F. succinogens*, *R. albus* and *R. flavefaciens*, together with protozoa, methanogen archeae and total bacteria for day 0, day 18 and day 30 with significant observed on *R. albus* ($P < 0.05$) and protozoa population ($P < 0.01$). As a conclusion, supplementation of dietary oils improved rumen fermentation as total VFA production increased whereas ammonia and protozoa counts decreased significantly. Therefore, the results suggest that vegetables oil reduced and maintain low level of rumen protozoa population during supplementation in goats and help

savings on protein supplements, reduce the excretion of nitrogen and its negative environmental impact.

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

KESAN MINYAK DALAM MAKANAN YANG BERLAINAN PROFIL ASID LEMAK KEPADA PENAPAIAN RUMEN, POPULASI MIKROB DAN PENGHADAMAN NUTRISI OLEH KAMBING

Oleh

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Suplemen minyak sayuran dan minyak bijian dalam makanan boleh mengurangkan bilangan protozoa dalam rumen dan mengekalkan populasi protozoa yang rendah sepanjang tempoh suplementasi, menjimatkan protein tambahan serta mengurangkan penyingkiran nitrogen dan kesan negative terhadap alam sekitar. Enam belas kambing jantan yang dilengkapi dengan rumen kanula dibahagi secara rawak kepada empat rawatan diet eksperimen. Kumpulan pertama adalah kumpulan kawalan (CNT) dan telah diberikan diet asas. Kumpulan kedua, ketiga dan keempat masing-masing menerima diet eksperimen yang ditambah dengan 6% minyak zaitun (OL), minyak olein (PO) dan minyak bunga matahari (SF). Makanan dianalisis untuk analisis proksimat. Kandungan rumen kambing diambil untuk kiraan protozoa, analisis serat degradasi bakteria dan methanogen menggunakan RT-PCR serta analisis kimia untuk ammonia dan asid lemak meruap. Untuk eksperimen kedua, enam belas kambing jantan untuk eksperimen penghadaman. Sekitar 10% daripada jumlah kutipan telah disampel untuk analisis kimia bagi nutrien dan peratus pencernaan telah dikira. Prosedur GLM dari SAS telah digunakan untuk menganalisis semua parameter. Purata pH cecair rumen telah terjejas dengan ketara oleh hari persampelan ($P < 0.01$. Purata kepekatan ammonia ynag lebih tinggi diperhatikan dalam CNT (42.6 mg/L) berbanding dengan kumpulan lain dan berkait secara siknifikan dengan diet ($P < 0.01$) dan interaksi diet × hari persampelan ($P < 0.05$). Purata bagi jumlah kepekatan VFA (mmol) adalah lebih tinggi dalam kumpulan OL dan SF dipengaruhi dengan ketara oleh diet ($P < 0.05$). Kadar molar asid asetat, asid propionic dan asid isobutyrik dipengaruhi oleh diet ($P < 0.05$) dan hari persampelan ($P < 0.05$). Kadar molar asid butyrik dan asid valerat dipengaruhi oleh hari persampelan ($P < 0.05$). Purata bilangan protozoa adalah lebih tinggi ($P < 0.05$) dalam CNT berbanding dengan kumpulan lain. Terdapat perbezaan siknifikan pada penghadaman CP ($P < 0.05$) dan EE ($P < 0.01$) dalam kumpulan rawatan berbanding CNT. RT-PCR telah digunakan untuk mengkaji tiga spesies bakteria sellulolitik iaitu *Fibrobacter succinogens*, *Ruminococcus Albus* dan *Ruminococcus flavefaciens*, bersama-sama dengan protozoa, methanogen dan jumlah mikrob untuk hari 0, hari 18 dan hari 30 dan terdapat berbezaaan yang ketara dalam populasi *R. albus* ($P < 0.05$) dan protozoa ($P < 0.01$). Sebagai konklusi, suplemen

minyak dalam makanan memperbaiki penapaian rumen apabila penghasilan asid lemak meruap meningkat manakala ammonia dan protozoa menurun dengan ketara. Oleh itu, suplemen minyak dalam makanan berpotensi untuk dianggap sebagai suplemen makanan mikrob dalam ruminan.

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I certify that a Thesis Examination Committee has met on 6 March 2015 to conduct the final examination of Nur Atikah binti Ibrahim on her thesis entitled "Effects of Dietary Oils Differing Fatty Acid Profiles on Rumen Fermentation, Microbial Population and Nutrient Digestibility in Goats" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the University 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

ADF	Acid detergent fibre
ANOVA	Analysis of variance
AOAC	Association of analytical community
ATP	Adenosine triphosphate
CO_2	Carbon dioxide
CP	Crude protein
DM	Dry matter
EE	Ether extract
GLM	General linear model
LCFA	Long-chain fatty acid
N	Nitrogen
NDF	Neutral detergent fibre
OM	Organic matter
qPCR	Quantitative polymerase chain reaction/ real-time polymerase chain reaction
SAS	Statistical analysis system
VFA	Volatile fatty acid

CHAPTER 1

INTRODUCTION

Ruminants have an important role in agriculture because of their unique ability to convert roughages that are unsuitable for man, efficiently. They can effectively utilize all fibrous parts of plant including by-products produced from food for human consumption. As a result, these by-products, for example cereal by-products, stalk and straw can enter human food chain.

Understanding the nature of ruminant nutrition and digestion are essential in order to improve the management and production at farm. Among many approaches, manipulating ruminant nutrition through feed formulation and feeding management is in particular being practiced and researched over the last decade. The used of plant extract, or plant containing secondary compound such as condensed tannins and saponins as well as plant oils supplementation have been reported by researchers (Cowan, 1999; Kamra et al., 2008; Sallam et al., 2009, Bodas et al., 2012).

Digestion in ruminant is characterized by fermentation in the rumen by rumen microbial population, constituting mainly bacteria, protozoa and fungi (Takenaka and Itabashi, 1995). The breakdown of ingested feed in rumen by rumen microbes produces products that are utilized by the host animal such as short chain fatty acid (acetate, propionate, butyrate and others) and ammonia.

Rumen microbes produce enzymes that digest cellulose, hemicellulose and other fibrous components, which are not digested by enzymes of host animals. Proportionately rumen microbe composed of bacteria, protozoa and fungi (Kamra, 2005; Hungate, 1966) with bacteria representing 10^{10} bacteris, 10^{7} protozoa, 10^{6} fungi and yeast per ml of rumen liqour (Buccioni *et al*., 2012).

However, protozoa gives negatives effects towards in term of nutrition as they are predator of bacteria and their consumption and digestion of bacteria is a wasteful process that contributes to the undesirable recycling of nitrogen in rumen. Since protozoa are not essential for the animals, numerous chemical and physical methods were applied experimentally to eliminate protozoa from the rumen (Hegarty, 1999).

Oils and fats have 2.25 times higher digestible energy compared to carbohydrates in grains, generating concentrated sources of energy when added to animal feeds. The inclusion of oils and fats in animals' feeds tend to improve the physical characteristics of the feeds such as reducing the dustiness of the feed as well as improving palatability. Plant-based oils that are rich in unsaturated fatty acids are known to modify rumen fermentation, depending on their fatty acids composition, origin and degree of saturation (Szumacher-Strabel *et al.*, 2009), where unsaturated fatty acids are found to

be more toxic than saturated ones and can inhibit rumen fermentation more extensively (Jenkins, 1993).

The majority of dietary vegetable oil supplements reduces protozoa, however also contain medium-chain fatty acids that are known to have a negative effect on cellulolytic bacteria and the consequently, fibre digestion. In recent years some oilseeds crops (for example; safflower, sunflower and flaxseed) have been genetically modified to produce high concentration of one of the C_{18} fatty acids such as oleic (C18:1), linoleic (C18:2) or linolenic (C18:3) acid in the oil. The C18:2 and C18:3 fatty acids are known to be beneficial to rumen cellulolytic bacteria, mainly the dietary linoleic acid which has increased the concentration of total cellulolytic bacteria species in the rumen by over 80%. Such oils will also become in demand by the ruminant production industry as dietary supplements when their benefits become widely known. However, the effects of the locally produced oils from oil palms such as palm olein on the rumen metabolism and gastrointestinal digestion of dietary fibre is not known.

Currently, the role of livestock production in increasing global warming has been reported. The important issue highlighted was the emission of greenhouse gasses released from livestock industry, in particular methane and carbon dioxide production through fermentation in the rumen. Furthermore, ruminal protozoa have been shown to exert an effect on environmental pollutant by providing hydrogen for ruminal synthesis of methane by methanogens (methane producing organism) and excretion of nitrogen. Thus, improvement and modification of rumen microbial environment through elimination of protozoa was a major concern in this study.

The objectives of this study are to investigate the effects of dietary oils differing in fatty acid composition on rumen fermentation, rumen microbial population and nutrient digestibility in goats. The specific objectives were as follow:

1. to determine the effect of olive oil, palm olein oil and sunflower oil supplementation on rumen fermentation characteristic in goats;
2. to investigate the effect olive oil, palm olein oil and sunflower oil supplementation on digestibility of protein and fiber and other nutrients in goats;
3. to quantify cellulolytic bacteria, total bacteria, total protozoa and total archaea population in the rumen of goats receiving different type of oil supplementation.

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