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PLASMA FATTY ACID PROFILE COMPARISONS BETWEEN THE LESSER MOUSE DEER (*TRAGULUS JAVANICUS*) AND THE COMMON MUNTJAC (*MUNTIACUS MUNTJAK*)

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SUMMARY

The Lesser Mouse Deer and the Common Muntjac are native to the South-east Asian region. The main aim of this study was to compare the extent of plasma fatty acid unsaturation between these two species. Dietary unsaturated fatty acids are expected to undergo extensive biohydrogenation and saturation in the gut of the Common Muntjac, as is expected for a 'true' ruminant. Being a 'partial' ruminant, the Lesser Mouse Deer is expected to allow some dietary unsaturated fatty acids to escape rumen biohydrogenation, thus allowing accumulation of more unsaturated fatty acids in the plasma. Six Lesser Mouse Deer and six Common Muntjac, kept for recreational purposes, were used in this study. Plasma fatty acids were extracted from blood and feed samples, methylated using 14 % boron triflouride and separated using gas liquid chromatography. It was found that both species had higher unsaturated fatty acid content in their plasma compared to saturated fatty acids. This is different from domesticated ruminants where saturated fatty acids usually are about 60 % of total plasma fatty acids. The plasma unsaturated fatty acid content of the Lesser Mouse Deer is marginally higher (52.9 %) than the Common Muntjac (51.8 %). However, the Lesser Mouse Deer had higher plasma n-6 polyunsaturated fatty acids (PUFA) at 16 %, and a more balanced n-3 PUFA content. This resulted in better and lower n-6 PUFA : n-3 PUFA ratios in the Lesser Mouse Deer (4.2). These findings could be attributed to the reduced biohydrogenation and destruction of dietary unsaturated fatty acids in the gut of Lesser Mouse Deer, thus enabling more of these fatty acids to be absorbed by the body. In conclusion, true and partial wild ruminants found to have different plasma fatty acid profile as a result of their differences in gut function and morphology.

Keywords: Plasma, fatty acids, Lesser Mouse Deer (*Tragulus javanicus*), Common Muntjak (*Muntiacus muntjak*).

INTRODUCTION

The relationship between dietary intake and plasma fatty acid profiles is well described for most domesticated ruminants (Rajion *et al.*, 2001; Wiklund *et al.*, 2001) while Sinclair *et al.* (1982) and Miller *et al.* (1986) have published their findings on the fatty acid profiles for wild ruminants in the early eighties. Among true ruminants, it is a generally well accepted fact that domestication resulted in much higher saturated fatty acids (SFA) content compared to polyunsaturated fatty acids (PUFA) content in both tissue and plasma (Harfoot and Hazlewood, 1988, Rajion *et al.*, 2001; Wiklund *et al.*, 2001). This phenomenon is attributed to the complete biohydrogenation of PUFA in the rumen by rumen microbes as PUFA is known to be toxic to rumen micro-organisms and could potentially disrupt nutrient digestion in the ruminant animal (Jenkins, 1997). Domestication of true ruminants such as sheep, goats and cattle is thought to contribute to a significantly higher SFA content in their tissues not only through biohydrogenation alone, but also as a direct result of higher adipose fat deposition which is generally made up of saturated fats. Among partial ruminants such as deer, it was shown that feeding commercial concentrates to these animals generally resulted in increased PUFA accumulation in body fat stores. This is presumably due to the ability of PUFA to bypass rumen biohdrogenation in partial ruminants such as deer (Meyer *et al.*, 1998). The interest in PUFA is driven by the fact that these fatty acids have been associated with benefits to human health when they are supplied in a balanced manner (Tisch, 2005). It is generally accepted that PUFA should be supplemented at the balanced ratio of n-6 PUFA (or omega-6 fatty acids) to n-3 PUFA (omega-3 fatty acids) between 1:1 to 6:1 for maximum benefits (Haumann, 1997). Therefore, diets with a higher PUFA and balanced n-6:n-3 ratio are highly desirable to sustain health in human populations.

Mouse deer (*Tragulus spp.*) are among the smallest ruminants known. The lesser mouse deer (*Tragulus javanicus*) of Southeast Asia is probably the smallest; an adult stands only 20 cm high and weighs a mere 1-2.5 kg. The lesser mouse deer is a partial ruminant indigenous to South-east Asia and though protected, its meat is well sought after by the indigenous people. It can be found in the Peninsular Malaysia and Java. These animals inhabit equatorial forests and mixed secondary tropical forests. They generally live among undergrowth on the edges of dense lowland rainforests. They especially haunt rivers and swampy bush areas, seeking escape by water when in danger (National Research Council, 1991). Although they look vaguely like tiny deer, mouse deer differ in several particulars. The stomach is simpler and has three instead of four effective compartments; rumen, reticulum,

abomasums. Rumination occurs but mouse deer are the most primitive of all ruminants. Indeed, they share a number of characteristics with non ruminants including lack of horns, antlers, continually growing, tusk-like upper canines in males, sharp-crowned premolars and four fully developed toes. Essentially vegetarians, they feed chiefly on fruits and leaves and the premolars of mouse deer are designed for piercing and chopping rather than chewing (National Research Council, 1991).

The Common Muntjac (*Muntiacus muntjak*) belongs to the *Cervidae* family. The Common Muntjac was thought to originate between 15 to 35 million years ago, from an area now situated on the borders of present-day Laos and Vietnam (National Research Council, 1991). Mature animals may attain a height of 43 – 46 cm at the shoulder and weigh between 11 to 16 kg, with females being smaller. Muntjac bucks have long pedicles from which relatively small antlers grow, where a single backward curved antler is up to 10 cm in length. Unlike Lesser Mouse Deer, both sexes of the Common Muntjac have canine teeth or tusks, and are predominantly browsers. The prefer foods lower in fibre but rich in protein and other nutrients (National Research Council, 1991). Unlike the Lesser Mouse Deer, *M. muntjak* are true ruminants and would therefore expected to be able to perform complete biohydrogenation of fatty acids in their rumen.

The objective of this paper was to investigate the possible differences in plasma fatty acid profiles as a function of ruminal biohydrogenation, between domesticated *M. muntjak* and domesticated *T. javanicus* fed commercial concentrates. The information derived would enable ruminant nutritionists to better understand the dynamics of ruminal fatty acid biohydrogenation in both true and partial wild ruminants. The data would prove invaluable to devise effective dietary manipulation techniques to improve meat contents of PUFA in domesticated ruminants (Goh *et al.*, 2001; Rajion *et al.*, 2001).

MATERIALS AND METHODS

Animals

Six Lesser Mouse Deer (*T. javanicus*) and six Common Muntjac (*M. muntjak*) of both sexes from the states of Perak and Selangor were used for this study. All animals were fed with mainly beef cattle concentrate pellets and supplemented with grasses, fruits and leaves.

Blood sampling

About 0.5 – 1.0 mL of blood was collected from the jugular vein. The blood was put in a sodium ethyldiaminetetraacetate (EDTA) tube (Venoject™, Becton Dickinson, USA), stored at 0 – 4°C and transported to the Faculty of Veterinary Medicine, Universiti Putra Malaysia for processing. The feed samples were also taken during blood sampling. At the laboratory, plasma was separated by centrifuging the blood at 1500G for 15 min. The plasma was then decanted into Eppendorf™ vials and stored at -20°C freezer until total plasma fatty acid extraction was carried out. Feeds were also stored at -20°C until fatty acid extraction was performed.

Total lipid extraction

The total fatty acids were extracted from plasma and feeds using chloroform-methanol 2:1 (v/v) (Merck AG, Damstadt, Germany) solvent system as described by the Folch extraction method (Folch *et al.*, 1957). The frozen plasma was left to thaw at room temperature. The plasma was then homogenised in a 50 mL stoppered tube filled with chloroform-methanol (2:1, v/v) at 1 part sample to 50 parts choloroform methanol. Nitrogen was flushed into and the tube capped to prevent oxidation. The mixture was shaken for 10 min vigorously and left to stand for 12 h with occasional shaking. The mixture was filtered through a number 1 Whatman filter paper into a 250mL separating flask. 10mL of chloroform-methanol (2:1, v/v) were used to wash the residue on the filter paper and funnel. Then, 10mL of normal saline was added into the separating flask to facilitate phase separation. The flask was capped tightly and shaken vigorously for 1 min before leaving it to stand for at least 4 h. The addition of normal saline resulted in the formation of two phases: the lower phase containing lipid components and a upper phase containing non-lipid contaminants. After the phase separation, the lower phase was collected into a round flask bottle. Evaporation of solvent from lipid extracts was conducted under negative pressure suction in a rotary evaporator at 70 - 75°C. The lipid extracts were then re-diluted with chloroform-methanol to make up a total volume of 5 mL before being transferred to a Teflon™-lined methylating tube. 100 øL of heneicosanoic acid (4 mg/mL) (Sigma Chemical Co. St Louis, MI) was added as an internal standard to each sample prior to drying under purified nitrogen (99.999 % purity, MOX Sdn Bhd, Malaysia).

Total fatty acid extraction from feed samples was performed by homogenising 0.6 g pellet in 40 mL of chloroform-methanol (2:1 v/v) in a 50 mL stoppered tube. The subsequent procedures were similar to those described for plasma sample except for the quantity of heneicosanoic acid (internal standard) added, which was 200 *mL*.

Fatty acid methyl esters (FAME) preparation

The dried lipid extracts were saponified with the addition of 2 mL of 0.66N methanolic potassium hydroxide (R & M Chemicals, UK). The methylation tube was capped and the mixture heated in a boiling water bath at 100°C for 10 min with occasional shaking. After the mixture had

Table 1: Fatty acid composition of feedstuffs fed to *T. javanicus* and *M. muntjak*

Fatty acids	*T. javanicus* feeds		*M. muntjak* feeds	
	Mean (mg/100g)	% of totalfatty acids	Mean (mg/100g)	% of totalfatty acids
Palmitic Acid (16:0)	146.1	17.1	165.3	19.9
Palmitoleic Acid (16:1)	15.9	1.9	15.3	1.8
Stearic Acid (18:0)	282	33.0	226.6	27.3
Oleic Acid (18:1)	281.4	32.9	293.7	35.4
Linoleic Acid (18:2 n-6)	96.6	11.3	103.6	12.5
Linolenic Acid (18:3 n-3)	9.7	1.1	8.6	1.0
Arachidic Acid (20:0)	19.8	2.3	14.7	1.8
Eicosaenoic Acid (20:1)	1.2	0.1	1.6	0.2
Eicosadienoic Acid (20:2)	2.3	0.3	1.1	0.1

Note : All values were not significantly different from each other ($p>0.05$).

cooled down, 2 mL of 14% boron trifluoride in methanol (Sigma Chemical Co., St Loius, MI) was added. The tube was sealed and reheated for 20 min with occasional shaking. The mixture was then allowed to cool down to room temperature at the end of the 20 min period. At this point, all the fatty acids were transmethylated into fatty acid methyl esters (FAME). The last stage was the retrieval and cleansing of the FAMEs. 4 mL of distilled water and 4 mL of petroleum ether (R & M Chemicals, UK) were added. The mixture was shaken for 1 min and then centrifuged at 1000 G for 10 min. The upper petroleum ether phase was transferred into another test tube containing 0.5 g of anyhydrous sodium sulphate to dry the sample. The petroleum ether containing the FAME was finally transferred into a 7 mL vial, flushed with nitrogen and stored at 4°C until gas-liquid chromatography (GLC) analysis.

Gas-liquid chromatography analysis

Separation of FAME was performed with a 5890 Hewlett Packard Gas Liquid Chromatograph (GLC) equipped with a Supelco SP 2330 series bonded phase fused silica capillary column (30m, 007 Carbowax/BTR, 0.25 μm film thickness) and Flame Ionization Detector (FID). The temperatures of both the injector and detector were set at 220°C. The oven temperature was programmed at 100 to 190°C with an increment rate of 7.2°C/min. Purified nitrogen was used as carrier gas at 40 mL/min. Peak integration was done using a Hewlett Packard integrator (HP 3220) for accurate estimation of individual fatty acid content in the sample. Identification of the FAME was done using the authentic commercial external standards from Sigma Chemical Co., St Louis, MI, USA.

All chemicals used for extraction, fatty acid methyl esters preparation and gas chromatography runs were of analytical grade.

Data analysis

All tests were conducted at 95% confidence level. Plasma fatty acid comparisons between the Common Muntjak and Lesser Mouse Deer were only evaluated for percentages and ratios instead of the absolute amounts as per accepted convention for fatty acid analysis. Percentages and ratios were analysed using the Mann-Whitney U Test, a *t*-test equivalent procedure for non-parametric data sets.

Feed fatty acid profiles (Table 1) showed that both species of animals were fed with diets that had similar fatty acid composition. The main difference is the quantity offered to each animal species due to the disparity of their body weights. In general, the saturated fatty acids (SFA) content of the feeds ranged between 49 – 52 %, with the remainder being unsaturated fatty acids (UFA). The n-3: n-6 ratio was 9.9 and 12.0 for *T. javanicus* and *M. muntjak*, respectively. This indicated that the Common Muntjac is actually being fed with slightly higher ($p>0.05$) amounts of n-6 PUFA, while the reverse is true for the Lesser Mouse Deer.

It is evident from Table 2 that both species surveyed in this study had higher amounts of UFA vis-à-vis SFA in their plasma. In fact, the plasma SFA content for *T. javanicus* at about 53 % is slightly higher ($p>0.05$) than that of the Common Muntjac. Percentages of myristoleic, oleic and linoleic acids were significantly different between the two species. The Lesser Mouse Deer had lower proportion of myristolec acid at 9.3 %, but significantly higher percentages of oleic and linoleic acids at 13.3 % and 16 %, respectively. The plasma linoleic acid in the Lesser Mouse Deer is at least 50 % more (both in terms of absolute amount and percentage value) than that from the Common Muntjac. In fact, total plasma PUFA (i.e. n-6 PUFA plus n-3 PUFA) is 19.8 % for the Lesser Mouse Deer, and only 12.9 % for the Common Muntjac.

Table 2: Plasma fatty acid profiles of *T. javanicus* and *M. muntjak*

	T. javanicus		*M. muntjak*	
Fatty acids	Mean (mg/100g)	% of totalfatty acids	Mean (mg/100g)	% of totalfatty acids
Myristic Acid (14:0)	20.2	7.5	31.3	11.1
MyristoleicAcid (14:1)	25.1	9.3[a]	57.3	20.4[b]
Palmitic Acid (16:0)	60.8	22.6	49.2	17.5
Palmitoleic Acid (16:1)	25.7	9.5	22.2	7.9
Stearic Acid (18:0)	42.2	15.7	45.6	16.2
Oleic Acid (18:1)	35.9	13.3[a]	24.5	8.7[b]
Linoleic Acid (18:2 n-6)	43	16.0[a]	30.7	10.9[b]
Linolenic Acid (18:3 n-3)	10.2	3.8	5.6	2.0
Arachidic Acid (20:0)	3.7	1.4	9.6	3.4
Eicosaenoic Acid (20:1)	2.5	0.9	3.8	1.3
Eicosadienoic Acid (20:2)	nd	nd	1.7	0.6
Total Saturated Fatty Acids (TSFA)	126.9	47.1	135.7	48.2
Total UnsaturatedFatty Acids (TUFA)	142.4	52.9	145.8	51.8
Total n-6 PUFA	43	16.0[a]	30.7	10.9[b]
Total n-3 PUFA	10.2	3.8	5.6	2.0
n-6:n-3 ratio	4.2		5.5	
Total fatty acids	269.3	100	281.5	100

Note : nd = not detected
TSFA = Myristic, Palmitic, Stearic & Arachidic acids
TUFA = Total Fatty Acids – TSFA
Values within rows with different superscripts differed significantly at $p<0.05$, all other values were not significantly different from each other.

These are clear indications that partial ruminants like *T. javanicus* allow more dietary PUFA to escape ruminal biohydrogenation, and thus more is available for hind gut absorption. Although the n-6:n-3 ratio was not significantly different between the two species. It is clear that *T. javanicus* had a lower ratio which is highly desirable and beneficial for human health.

DISCUSSION

True ruminants such as goats, sheep and Common Muntjacs have higher levels of saturated fatty acids than partial ruminants. The opposite can be said for partial ruminants, where the UFA are usually observed to be higher in their plasma (Meyer *et al.*, 1998). The rumen is the major site for biohydrogenation and destruction of UFA as these compounds are usually toxic to the rumen organism (Doreau and Ferlay, 1994; Jenkins, 1997). UFA are chemically reactive and would therefore harm the normal physiological and fermentation processes mediated by these ruminal bacteria. Thus, in order to survive, these rumen microbes usually produce lipases for the hydrolysis of the fatty acids and reductive enzymes to saturate the UFA into chemically inert SFA (McDonald, 1995). In fact, up to 85 % of linoleic acid and more than 95 % of linolenic acids in the diet are reported to be destroyed in the rumen during digestion (Doreau and Ferlay, 1994). The ultimate product of rumen biohydrogenation is usually the 18-carbon stearic acid, which are normally stored as subcutaneous fats throughout the body. In partial ruminants, the UFA are allowed to bypass the rumen fermentation with moderate amounts of chemical saturation, thus enabling more PUFA and other unsaturated fats to be absorbed into the blood stream. In fact, work by Yeom *et al.* (2004) in domesticated ruminants proved that was the case when unsaturated fats were allowed to escape rumen biohydrogenation. Ultimately, these were clearly reflected by the almost two-fold increase in total n-3 and n-6 PUFA in the blood plasma of Lesser Mouse Deer compared to that of the Common Muntjac.

Apart from the ability of unsaturated fats to bypass enzyme saturation in the rumen of partial ruminants, shorter retention time of ingesta in rumen as in the case of Lesser Mouse Deer would also contribute to the increased availability unsaturated fats in the hind gut (Harfoot and Hazlewood, 1988). Another factor that logically contributes to higher UFA in this study compared to that of sheep and goats (Rajion *et al.*, 2001) is the type of feed offered to the Lesser Mouse Deer and Common Muntjac. Other than pellet, they were also given fresh grass that contained high amounts of PUFA. Leafy plants are known to hold sizable amounts of PUFA in their leaves. It is also clear from Tables 1 and 2 that dietary

fatty acid only had limited effects on the plasma fatty acid profiles of both species. Evidence suggested that both the Lesser Mouse Deer and Common Muntjac are able to selectively enrich their plasma with more unsaturated fats. Another reason why both species had more UFA compared to domesticated ruminants such as sheep and goats is because, being 'concentrate selectors' (National Research Council, 1991 ; Meyer *et al.*, 1998), both of these species tend to have lower fat synthesis rate in their liver (Meyer *et al.*, 1998) and thus do not re-synthesise much fats *in vivo*, which are commonly SFA. These factors allowed both Lesser Mouse Deer and the Common Muntjac to accumulate lesser amounts of SFA in their plasma. It is therefore logical to postulate that higher plasma UFA and PUFA content in these species would ultimately result in meats and carcases which had less undesirable SFA content. Apart from the SFA content, the plasma n-6: n-3 fatty acid ratio is very important in all mammalian animals (Lands, 1992). Any imbalance or dominance of either one of these fatty acids will cause severe physiological derangements to the individual (Gurr *et al.*, 2002). Generally, higher n-6 PUFA content is associated with pro-inflammatory responses and an over-reactive immune system, whereas higher n-3 PUFA is known to be anti-inflammatory, suppressing not only the immune and reproductive functions, but may potentially delay blood clotting cascades as well. The ideal ratio of n-6: n-3 fatty acids is thought to be 1:1 to 1:6 (Haumann, 1997). The plasma n-6: n-3 ratio of both species falls within this range, a further testimony that tissues from wild ruminants is a healthier and viable alternative to meats from domesticated ruminants, provided that the meat from these animals are harvested in a sustainable and responsible way.

Although there are significant differences for plasma oleic and myristoleic acids between the two species, changes in these fatty acids are more difficult to interpret since they are regulated by the action of tissue desaturases, and are also synthesised in vivo by the heptocytes (Jenkins and Thies, 1997). The same was also true for comparisons for palmitic and stearic acids. Furthermore, it should also be noted that apart from ruminal biohydogenation, plasma fatty acid profiles for ruminants are also to some extent, the results of interaction between various intervening extrinsic and intrinsic factors within the animal itself (Chilliard, 1993; Sampelayo *et al.*, 2006), such as sex (Brindley, 1985), hormone (Watkins and German, 1998) and even age. However, due to limitation in sample numbers, these factors could not be investigated in detail.

CONCLUSIONS

In conclusion, plasma fatty acid profiles in wild ruminants depended not only on the dietary fatty acid supply alone, but also on the extent of ruminal biohydrogenation afforded by the differences in rumen structure and functions.

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