UNIVERSITI PUTRA MALAYSIA

EFFECTS OF Brachiaria decumbens STAPF IN SHORT- AND LONG-TERM FEEDING ON In Vitro RUMEN FERMENTATION AND GROWTH PERFORMANCE IN SHEEP

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By

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Brachiaria species have been planted on more than 80% of improved farming pastures with *Brachiaria decumbens* as the most favoured species. It is important because of its high productivity under intensive use, and its tolerance of low fertility soil. However, the presence of steroidal saponins in *B. decumbens* which had become a limiting factor led to the outbreaks of photosensitivity in ruminants. This current study focused on the effects of *B. decumbens* in short and long-term feeding on *In Vitro* gas production, rumen fermentation, nutrient digestibility and growth performance in sheep. A total of 30 (6-month-old) male Dorper cross sheep were divided randomly into 3 treatment groups with 10 sheep per treatment. This study was conducted in 2 phases which is the short-term (7 days) and long-term (90 days) stages excluding the 2 weeks adaptation period. Treatment 1 (control) sheep were fed with *Pennisetum purpureum* and concentrates as the basal diet, whereas Treatment 2 and 3 sheep were fed with low (10%) and high (60%)) levels of *B. decumbens* respectively according to 3% kg/feed per body weight. The digestibility trial was done at day 7 during the short-term and at day 90 during the long-term. The amount of feed offered and refusals were recorded daily while body weight gain and body measurement were recorded weekly to determine the feed efficiency. The *In Vitro* gas production showed no significant changes in net gas production and gas production kinetics during the 48 hours on incubation. However, the gas production significantly decreased as the level of *B. decumbens* increases, with T3 diet demonstrated poorest gas production. The concentration of ammonia and pH showed no significant difference among treatments but decreasing as *B. decumbens* level increases. The same goes with all individual VFA except for acetic acid and total VFA ($p<0.05$) The sheep digestibility of organic matter, neutral detergent fiber, and crude protein except dry matter showed no significant differences ($p>0.05$) during the short-term, while DM, CP, NDF and acid detergent fiber decreased significantly ($p<0.05$) during the long-term. For the growth performance and feed intake, there were also no significant differences during the short-term.

Meanwhile, there were significant differences in total weight gained each week, average daily gain (ADG), total feed intake and daily feed intake recorded daily during the long-term. T3 sheep fed with the highest concentration of *B. decumbens* exhibited the worst growth performance than other treatments. Significant differences ($p<0.05$) were detected on day 7, 30, 60, and 90 among treatment sheep. T1 showed the highest growth hormones compare to other treatments and T3 sheep revealed the highest reduction in growth hormone concentration. Despite the ADG and weight gain result for long-term and growth hormones, there were no significant differences on the body measurement during both terms including the body indexes. Overall, feeding sheep with the high level of *B. decumbens* showed inferior in the gas production and rumen fermentation, growth performance and digestibility as compared to the control group fed with *P. purpureum* with 0% of *B. decumbens.* The effects of both low and high saponin levels of *B. decumbens* on *In Vitro* study and growth performance on sheep from this study may contribute to future research of *B. decumbens.*

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KESAN JANGKA PENDEK DAN JANGKA PANJANG PEMBERIAN MAKAN *Brachiaria decumbens* STAPF PADA FERMENTASI RUMEN *In Vitro* DAN PRESTASI PERTUMBUHAN TERHADAP BIRI – BIRI

Oleh

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Spesies *Brachiaria* telah ditanam lebih daripada 80% padang rumput pertanian yang telah diperbaik pulih dengan *Brachiaria decumbens* sebagai spesies yang paling digemari. Hal ini penting kerana produktivitinya yang tinggi dalam penggunaan intensif, dan toleransi yang tinggi terhadap masalah kesuburan tanah. Walau bagaimanapun, saponin steroid dalam *B. decumbens* menjadi factor pembatas dalam menyebabkan masalah fotosensitisasi pada ruminan. Kajian semasa ini fokus pada kesan jangka pendek dan jangka panjang pemberian makan *Brachiaria decumbens* pada fermentasi rumen, produksi gas *In Vitro*, kebolehcernaan nutrien dan prestasi pertumbuhan tahap rendah terhadap biri – biri. Sebanyak 30 ekor bebiri (Dorper cross) jantan berusia 6 bulan dibahagikan secara rawak kepada 3 kumpulan dengan 10 ekor bebiri setiap rawatan. Kajian ini dilakukan pada 2 fasa: jangka pendek (7 hari) dan jangka panjang (90 hari) tidak termasuk tempoh penyesuaian 2 minggu. Bebiri kumpulan 1 (kawalan) diberi makan *Pennisetum purpureum* dan pellet sebagai diet basal, manakala bebiri kumpulan 2 dan 3 diberi makan dengan tahap *B. decumbens* yang rendah (10%) dan tinggi (60%) menurut 3% kg/makanan per berat badan. Percubaan pencernaan dilakukan pada hari ke-7 semasa jangka pendek dan pada hari ke-90 semasa jangka panjang. Jumlah makanan yang ditawarkan dan baki direkod secara harian manakala kenaikan berat badan dan pengukuran badan dicatat secara mingguan untuk menentukan kecekapan makanan. Pengeluaran gas *In Vitro* tidak menunjukkan perubahan ketara dalam pengeluaran gas bersih dan pengeluaran gas kinetik selama 48 jam semasa inkubasi. Walaubagaimanapun, pengeluaran gas menurun dengan ketara apabila tahap *B. decumbens* meningkat, dengan diet kumpulan 3 menunjukkan pengeluaran gas paling rendah. Konsentrasi amonia dan pH tidak menunjukkan perbezaan yang signifikan antara kumpulan bebiri tetapi menurun apabila tahap *B. decumbens* meningkat. Hal yang sama berlaku untuk semua individu VFA kecuali asid asetik dan jumlah keseluruhan VFA ($p<0.05$). Pencernaan nutrisi

bebiri: bahan organik, fiber detergen neutral, dan protein mentah kecuali bahan kering tidak menunjukkan perbezaan yang signifikan (p> 0,05) semasa jangka pendek, sementara DM, CP, NDF dan fiber detergen asid menurun dengan ketara (p <0,05) semasa jangka panjang. Bagi prestasi pertumbuhan dan pengambilan makanan, tidak ada perbezaan yang signifikan semasa jangka pendek. Sementara itu, terdapat perbezaan yang signifikan dalam jumlah kenaikan berat badan, kenaikan harian purata (ADG), jumlah pengambilan makanan dan pengambilan makanan harian semasa jangka panjang. Bebiri kumpulan 3 yang diberi konsentrasi *B. decumbens* tertinggi menunjukkan prestasi pertumbuhan terburuk daripada kumpulan lain. Perbezaan yang ketara (p <0.05) dikesan pada hari ke-7, 30, 60, dan 90 di antara bebiri. Kumpulan menunjukkan hormon pertumbuhan tertinggi dibandingkan dengan kumpulan lain manakala bebiri kumpulan 3 menunjukkan penurunan konsentrasi hormon pertumbuhan tertinggi. Walaupun terdapat signifikan pada ADG dan kenaikan berat badan untuk jangka panjang dan penurunan hormon pertumbuhan, tiada perbezaan yang signifikan didapati pada pengukuran badan selama kedua - dua jangka termasuk indeks badan. Secara keseluruhan, pemberian *B. decumbens* di konsentrasi tertinggi sebagai makanan kepada bebiri menunjukkan penurunan dalam prestasi pertumbuhan, pencernaan dan pengeluaran gas dan fermentasi rumen berbanding kumpulan kawalan yang diberi makan *P. purpureum* dengan 0% kandungan *B. decumbens*. Kesan tahap konsentrasi saponin rendah dan tinggi dalam *B. decumbens* bagi kajian *In Vitro* dan prestasi pertumbuhan pada bebiri dari kajian ini boleh menyumbang kepada penyelidikan *B. decumbens* di masa hadapan.

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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AMT	*Astragalus membranaceus root*
APS	*Astragalus polysaccharide*
B. brizantha	*Brachiaria brizantha*
B. decumbens	*Brachiaria decumbens*
B. humidicola	*Brachiaria humidicola*
B. mutica	*Brachiaria mutica*
B. ruziziensis	*Brachiaria ruzizensis*
M. uniflorum	*Macrotyloma uniflorum*
P. chartarum	*Pithomyces chartarum*
P. maximum	*Panicum maximum*
P. purpureum	*Pennisetum purpureum*
Q. Saponaria	*Quillaja Saponaria*
T. terrestris	*Tribulus terretris*
Y. schidigera	*Yucca schidigera*
%	Percent
°C	Celsius
µL	Microlitre
ADF	Acid detergent fibre
ADG	Average daily gain
BL	Body length
BLI	Body length index
cm	Centimetre
CO2	Carbon dioxide
CP	Crude protein

DM	Dry matter
DMI	Dry matter intake
EE	Ether extract
FE	Feed efficiency
g	Gram
GH	Growth hormone
GRF	Growth hormone - releasing factor
h	Hour
HG	Heart girth
HGI	Heart girth index
IGF-1	Insulin – like growth factor
IVDMD	In vitro dry matter digesitibility
kg	Kilogram
L	Litre
mg	Milligram
min	Minute
mL	Millilitre
mM	Millimolar
NDF	Neutral detergent fibre
NH3-N	Ammonia nitrogen
nm	Nanometre
OM	Organic matter
OMD	Organic matter digestibility
ppm	Parts per million
rpm	Revolution per minute

SAS	Statistical analysis system
SI	Somatic index
SRIH	Somatostatin
ST	Somatotropin
v/v	Volume/volume
v/w	Volume/weight
VFA	Volatile fatty acid
w/w	Weight/weight
WH	Whither height

CHAPTER 1

INTRODUCTION

1.1 *Brachiaria decumbens* intoxication

The ruminant sector is one of the main livestock industries in Malaysia with feed being the main factor influencing both large and small ruminant production (Yusoff et al., 2016). One of the most important grasses for ruminant production is *Brachiaria spp*. Owing to the hot and humid climatic condition in Malaysia, *B. decumbens* is considered to have the potential as an excellent pasture species for the ruminant industries (Assumaidaee and Mustapha 2012). Other species of *Brachiaria spp*. namely *B. brizantha, B. humidicola, B. mutica*, and *B. ruziziensis* are also widely used as ruminant feed. Livestock production on this pasture system has been variable even when the quality and quantity of the pasture are high (Low, 2015). *B. decumbens* originally came from the highlands of Central and Eastern Africa. It is now widespread in the tropics and sub-tropics. Initially, *Pithomyces chartarum* was suspected as the primary cause of the outbreak of photosensitivity due to the presence of spores. However, several authors have shown that none of the *P. chartarum* isolates produce sporodesmin that cause hepatotoxicity (Graydon et al., 1991; Salam Abdullah et al., 1992). Further study has revealed that the crystal-associated cholangiohepatophaty lesions in the liver tissue of sheep are similar to that found in *Panicum spp*. and *Tribulus terretris* poisoning. These forages contain lithogenic saponins known to cause photosensitization (Bridges et al., 1987; Miles et al., 1993). The toxicity symptoms were reproduced and confirmed in sheep through oral dosing of crude saponins extracted from the plant *T. terrestris* (Zygophyllaceae) (Lascano and Euclides, 1996).

Similarly, Lemos et al. (1998) suggested that *Brachiaria* spp poisoning was also due to lithogenic saponins contained in the plant. Cruz et al. (2001) have induced cholangiopathy in lambs by administering *B. decumbens* extracts showing that the poisoning was caused by lithogenic saponins contained in the grass. Furthermore, Salam Abdullah et al. (1992) claimed that steroidal saponins were found in the rumen contents of poisoned sheep fed with *B. decumbens*. Supporting this, a study has confirmed the presence of plant saponins in the rumen of sheep suffering from *B. decumbens* toxicity (Lajis et al., 1993). The presence of steroidal saponins in *B. decumbens* is the main cause of photosensitivity in goats, sheep, llama, buffaloes, deer, and cattle (Low, 2015).

Although feeding the animals with *B. decumbens* has improved their overall performance, the risk of toxicity has become a limiting factor (Muniandy et al., 2020). Even though most of the outbreaks related to hepatogenous photosensitization are caused by *B. decumbens*, other *Brachiaria* species such as *B. brizantha, B. humidicola,* and *B. ruziziensis* could also cause poisoning in cattle, sheep, goats, and buffalo (Riet-Correa et al., 2011). The toxicity of *B.*

decumbens is due to the presence of steroidal saponins and intoxication occurs when the grass is fed as the main source of feed for grazing animals (Assumaidaee and Mustapha, 2012). Clinical symptoms such as liver damage in histopathology, changes in haematological results, hepatic jaundice, and photosensitization on the skin especially around the eyelid and muzzle have been observed in sheep fed with *B. decumbens*, indicating hepatotoxicity due to toxic damage (Abas et al., 1983). Souza et al. (2010) stated that *B. decumbens* poisoning in sheep can occur at any time of year and any maturity stage of the plant.

According to Riet-Correa et al. (2011), sheep are more susceptible to *B. decumbens* poisoning than goats and other ruminants. Wina et al. (2006) mentioned that goats are categorized as browsers, so they tend to confront more secondary metabolite plants compared to sheep. Hence, a higher proportion of entodiniomorphid protozoa, which aid the digestion process was found in the rumen of a goat than in sheep which led to the rumen micro-organisms of goats being more adaptable to these substances than those of sheep. This was supported by studies conducted by Abas et al. (1983) and Low (1993) who recorded the absence of photosensitization in goats grazing together with sheep suggesting that goats are more resistant than sheep. Furthermore, lamb and naïve sheep are more susceptible to toxicity poisoning as compared to experienced flocks (Faccin et al., 2014). In short, this study contributes to the distribution of *B. decumbens* during emergency for a while or taking in livestock that is previously exposed of *B. decumbens*.

1.2 Problem Statements

1. Many studies had only reported the clinico-pathology effects of *B. decumbens* in both grazing and experimental sheep.

2. Limited data is available on the effect of feeding *B. decumbens* on the nutrient composition, *In Vitro* gas production, and rumen fermentation characteristic of sheep.

3. No study has been conducted to justify the impact of feeding different levels of *B. decumbens* diets on the growth performance and digestibility of sheep during the short and long-terms feeding.

1.3 Hypotheses

1. It was hypothesized that there will be responses in both *In Vitro* and *In Vivo* studies using different levels of *B. decumbens* diets and at different time phases.

2. The *In Vitro* gas production and rumen fermentation characteristic will be decreased significantly at a higher level of *B. decumbens* diet.

3. Sheep fed with a high level of *B. decumbens* will have the lowest growth performance and digestibility.

4. Sheep fed with a high level of *B. decumbens* will be severely affected physical and morphologically during the long-term feeding.

1.4. Objectives

1. The general aim of this study was to investigate the effect of different levels of *B. decumbens* diets on the *In Vitro* rumen fermentation and performances of sheep at different time phases. The specific objectives were:

2. To evaluate the effects of different *B. decumbens* level on the nutrient composition, *In Vitro* gas production, and rumen fermentation characteristic of sheep.

3. To study the effects of different *B. decumbens* levels on the feed intake and nutrient digestibility of sheep during the short and long-terms.

4. To determine the effects of different levels of *B. decumbens* on the growth performance, growth hormone concentration, and body measurement of sheep during the short and long-terms.

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