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SHORT COMMUNICATIONS

The influence of feeding low and high level of *Brachiaria decumbens* diets on the hematology, serum biochemistry, and acute phase proteins of sheep

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Abstract

The present study aims to determine the hematology, serum biochemistry, and acute phase proteins (APPs) responses of both serum and cerebrospinal fluid (CSF) in sheep fed with low and high levels of *Brachiaria decumbens* (*B. decumbens*) diets at different time phases. A total of 30 6-month-old male Dorper cross sheep were randomly divided into three treatment groups consisted of 10 sheep each. Treatment 1 (control) sheep were fed with *Pennisetum purpureum* and concentrates as the basal diet, whereas Treatments 2 and 3 sheep were fed with low (10%) and high (60%) level of *B. decumbens*, respectively. The hematology results revealed that there were significant differences ($p < 0.05$) in the red blood cells, mean corpuscular volume, mean corpuscular hemoglobin concentration, white blood cells, neutrophils, monocytes, eosinophils, basophils, platelets, and plasma proteins between groups. Except for packed cell volume, there were also significant differences in all hematology parameters at different time phases. All biochemistry parameters except creatinine revealed significant differences among treatment groups. However, there were significant differences in all parameters between time. On the other hand, APPs results showed significant differences in the serum haptoglobin and serum amyloid A in both serum and CSF between groups and time.

Keywords *Brachiaria decumbens* · Hematology · Biochemistry · Haptoglobin · Serum amyloid A · Serum · Cerebrospinal fluid · Sheep

Introduction

Even though *Brachiaria decumbens* (*B. decumbens*) is a significant feed source for grazing livestock, there have been many incidences of toxicity in grazing ruminants including sheep due to the presence of steroidal saponins (Chung et al. 2018). Sheep are more susceptible than other ruminants, and the young are more predisposed than adults (Riet-Correa et al. 2011; Gracindo et al. 2014). Protodioscin is the major steroidal saponin present in *B. decumbens* which is related to secondary hepatogenous photosensitization in ruminants (Low 2015). Additionally, young leaves of *Brachiaria* spp. have greater saponin concentration than older plants (Castro et al. 2011; Riet-Correa et al. 2011). Jaundice, weight loss, dehydration, anorexia, skin lesions at the photosensitized areas, nervous signs during the chronic stage, and gross lesions in the liver, as well as the kidneys, are some vital signs of *B. decumbens* intoxication (Graydon et al. 1991; Muniandy et al. 2020). Previous studies on *B. decumbens*

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reported that increased liver enzymes such as bilirubin levels, serum aspartate aminotransferase (AST), and serum gamma-glutamyltransferase (GGT) are the cause of impaired liver functions (Castro et al. 2011). Those elevations are linked to cholestasis, hepatic dysfunction, and hemolysis which can be an indicator of the hepatic lesion due to *B. decumbens* toxicity (Cardona-Álvarez et al. 2016). The high degree of renal susceptibility will then be caused by high bilirubin levels due to ischemia. As a result, the serum urea and creatinine will be elevated which is an indicator of renal impairment (Lelis et al. 2018). In the meantime, acute phase proteins (APPs) are blood proteins produced by the liver during acute phase responses (Jesse et al. 2019). This is the primary defense system that is triggered by infection and inflammation, stress, or trauma. In ruminants, haptoglobin (Hp) and serum amyloid A (SAA) are the major APPs identified as a marker during infection and inflammation, being more specific and sensitive than blood analysis (Ceciliani et al. 2012). However, there is a dearth of knowledge on hematology and APPs concerning *B. decumbens* toxicity. Besides, the acute and chronic effects from the feeding of this signal grass also warrant further investigation. Therefore, the present study aims to determine the hematology and serum biochemistry changes, as well as the APPs responses of both serum and cerebrospinal fluid (CSF) in sheep fed with low and high levels of *B. decumbens* diets and at different time phases.

Materials and methods

Experimental design

The experimental protocols and ethics were conducted according to the Institutional Animal Care and Use Committee (IACUC) of Universiti Putra Malaysia (UPM) (Approval number: UPM/IACUC/AUP-R046/2019). A total of 30 6-month-old male Dorper cross sheep were purchased and randomly divided into three treatment groups consisted of 10 sheep each. Treatment 1 (control) sheep were fed with *Pennisetum purpureum* and concentrates as the basal diet, whereas Treatments 2 (T2) and 3 (T3) sheep were fed with low (10%) and high (60%) of *B. decumbens*, respectively. The determination of the saponin concentration was carried out by using an established method to determine the low and high levels of *B. decumbens* (Yuliana et al. 2014). The low and high levels were determined by evaluating the saponin concentration of *B. decumbens* at different percentages mixed with *P. purpureum* (Fig. 1). This study was conducted in two phases consisted of the acute (7 days) and chronic (90 days) stages. Blood and CSF samples were collected via jugular venipuncture and lumbar puncture at the lumbosacral site correspondingly at day 0, 7, and 90 for complete blood cell count, Hp, and SAA analyses.

Blood and acute phase proteins analysis

The blood samples were submitted to the Hematology and Biochemistry Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia for hematology and biochemistry analysis. Hematocrit and white blood cell counts were conducted using the Cell-Dyn 3700 Automatic Analyzer. Serums were processed by an automated chemistry analyzer (HITACHI 902 Automatic Analyzer®, Japan) to obtain the biochemistry parameters. The parameters analyzed were aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), total bilirubin, creatinine, urea, phosphorus, total protein, albumin, globulin, and albumin:globulin (A:G) ratio. The concentrations of both SAA and Hp in each blood serum and CSF samples were measured using enzyme-linked

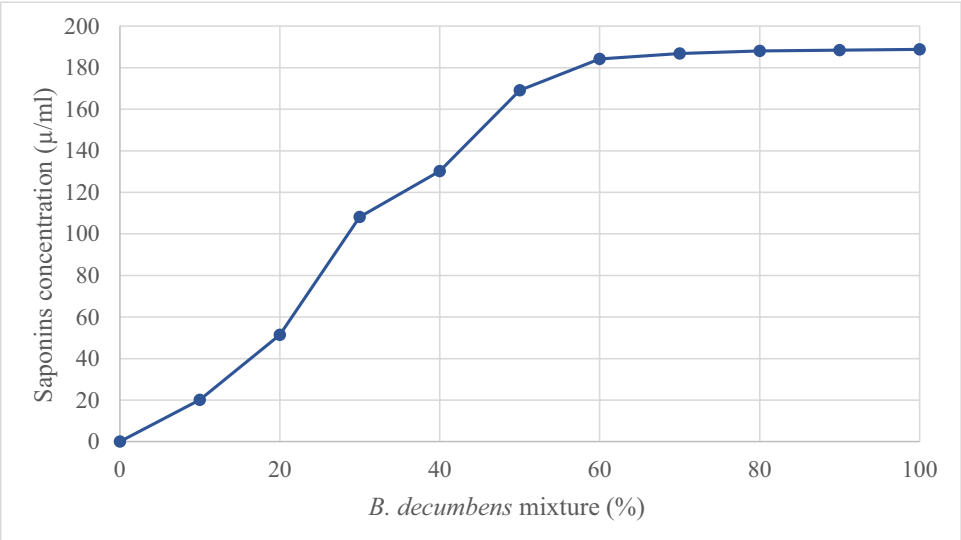

Fig. 1 Saponin concentration of different percentages of *B. decumbens* mixed with *P. purpureum*

immunosorbent assay (ELISA) kits (BT-Laboratory, Shanghai).

Statistical analysis

Based on the output parameter of the G*power analysis, the number of animals used was sufficient to obtain an actual power of 0.8. All data collected were analyzed using Statistical Analysis Software version 9.4 (SPSS Inc.). Shapiro–Wilk test was used to check for normality of data where $p > 0.05$ was considered as normally distributed data. Then, the data was further subjected to a two-way analysis of variance (ANOVA) to determine the effects of *B. decumbens* levels and time on the treatments. Tukey Kramer (Honest Significant Difference) post-hoc test was used to compare the mean differences among groups at a 5% level of significance. The data were considered significant at $p < 0.05$.

Results

Hematology

The hematology results of sheep fed with low and high levels of *B. decumbens* diets at different time phases are shown in Table 1. The horizontal values are comparing among the treatment groups, while the vertical values are comparing among the time periods. Among treatment groups, significant differences ($p < 0.05$) were observed in the red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC), neutrophils, monocytes, eosinophils, basophils, platelets, and plasma proteins. There were significant increases in the MCV and eosinophils parameters. Nonetheless, significant decreases were presented in the RBC, MCHC, WBC, neutrophils, monocytes, basophils, platelets, and plasma proteins among treatment sheep. Except for packed cell volume (PCV), there were also significant differences ($p < 0.05$) in all hematology parameters at different time phases. T2 sheep showed the lowest MCHC, band neutrophils, and platelets count during the acute phase, which continues to maintain low until the chronic phase. Similarly, the plasma protein of T3 sheep also was the lowest throughout the whole study period. The lymphocytes of T3 sheep were the highest during the acute phase. During the chronic phase, the WBC, monocytes, and basophils were the lowest in T2 sheep. Conversely, T3 sheep indicated the lowest RBC and hemoglobin (Hb), as well as the highest MCV and eosinophils values during the chronic phase.

Biochemistry

Table 2 displays the impact of different levels of *B. decumbens* diets on the biochemistry changes of sheep throughout the study period. All biochemistry parameters except creatinine revealed significant differences ($p < 0.05$) among treatment groups. Among treatment groups, there were significant increases in the AST and albumin to globulin (A:G) ratio. Besides, significant decreases were observed in the total bilirubin, urea, and phosphorus parameters. In addition, GGT, total protein, albumin, and globulin demonstrated both increases and decreases among the treatments throughout the study duration. Also, there were significant differences ($p < 0.05$) in all parameters at different time phases. During the acute phase, T2 sheep exhibited the highest phosphorus concentration among treatments. T3 had the highest GGT, total protein, albumin, and globulin concentrations, while the total bilirubin was the lowest, which maintained low until the chronic phase. During the chronic phase, the creatinine concentration and A:G ratio were the lowest and highest respectively in T2 sheep. Meanwhile, T3 sheep displayed the lowest urea and phosphorus concentrations. Despite the highest concentrations of GGT, total protein, albumin, and globulin, which were reported during the acute phase, those parameters plunged significantly to be the lowest concentration during the chronic phase in T3 sheep.

Acute phase proteins

The APPs of serum and CSF in sheep fed with different levels of *B. decumbens* are documented in Table 3. Among the treatment groups, there were significant differences ($p < 0.05$) in the serum Hp, serum SAA, and CSF SAA. T2 and T3 sheep fed with different levels of *B. decumbens* diets presented a significant decrease in the serum Hp and significant increases in both serum SAA and CSF SAA as compared to the control group. The same parameters also showed significant differences ($p < 0.05$) at different time phases. T3 sheep fed with high levels of *B. decumbens* diet depicted the lowest serum Hp but the highest Serum SAA and CSF SAA concentrations during the chronic phase.

Discussions

Hematology

The association between *B. decumbens* toxicity and hematology parameters has never been reported before. The closest work was performed to evaluate the toxicological influence of dioscin in rats, which resulted in significant changes in hematology parameters such as RBC, PCV, and MCHC (Xu et al. 2012). In this present study, there

Table 1 Hematology changes in sheep fed with low and high levels of *B. decumbens* diets at different time phases

Parameter	Day	T1 (Control)	T2 (10%)	T3 (60%)
RBC ($\times 10^{12}$/L)	0	12.58 ± 0.49^{Aa}	12.70 ± 0.13^{Aa}	12.53 ± 0.47^{Aa}
	7	12.79 ± 0.23^{Aa}	13.65 ± 0.92^{Aab}	12.85 ± 0.13^{Aa}
	90	12.52 ± 0.17^{Aa}	11.34 ± 0.25^{Bb}	10.40 ± 0.69^{Bb}
Hb (g/L)	0	108.33 ± 3.39^{Aa}	111.67 ± 3.19^{Aa}	112.33 ± 2.79^{Aab}
	7	108.67 ± 2.35^{Aa}	120.67 ± 7.97^{Aab}	110.00 ± 2.56^{Aa}
	90	105.67 ± 3.39^{Aa}	105.33 ± 2.74^{Ab}	104.00 ± 2.28^{Ab}
PCV (L/L)	0	0.31 ± 0.01^{Aa}	0.31 ± 0.01^{Aa}	0.32 ± 0.00^{Aa}
	7	0.32 ± 0.00^{Aa}	0.33 ± 0.02^{Aa}	0.32 ± 0.00^{Aa}
	90	0.31 ± 0.01^{Aa}	0.30 ± 0.01^{Aa}	0.30 ± 0.01^{Aa}
MCV (fL)	0	24.67 ± 1.05^{Aa}	23.33 ± 0.33^{Ab}	24.67 ± 1.05^{Aab}
	7	25.00 ± 0.73^{Aa}	25.67 ± 0.56^{Ba}	24.67 ± 0.42^{ABa}
	90	27.00 ± 0.26^{Aa}	26.33 ± 0.42^{ABa}	29.67 ± 1.38^{Ab}
MCHC (g/L)	0	353.00 ± 6.76^{Aa}	356.33 ± 5.12^{Aa}	350.67 ± 6.99^{Aa}
	7	349.33 ± 7.35^{Aa}	343.67 ± 3.11^{Ab}	343.67 ± 4.62^{Aa}
	90	340.67 ± 1.05^{Aa}	348.00 ± 4.77^{ABab}	343.00 ± 0.97^{Ba}
WBC ($\times 10^{9}$/L)	0	8.15 ± 1.22^{Aa}	8.77 ± 0.74^{Aa}	8.49 ± 0.77^{Aa}
	7	7.50 ± 0.42^{Aa}	8.32 ± 0.70^{Aa}	7.36 ± 0.71^{Aab}
	90	5.85 ± 0.52^{Aa}	4.42 ± 0.56^{Ab}	6.06 ± 0.18^{ABb}
Band neutrophils ($\times 10^{9}$/L)	0	0.08 ± 0.01^{Aa}	0.11 ± 0.01^{Aa}	0.10 ± 0.02^{Aa}
	7	0.08 ± 0.01^{Aa}	0.08 ± 0.01^{Ab}	0.07 ± 0.01^{Aa}
	90	0.06 ± 0.01^{Aa}	0.05 ± 0.01^{Ac}	0.07 ± 0.01^{Aa}
Neutrophils ($\times 10^{9}$/L)	0	3.06 ± 0.16^{Aa}	3.01 ± 0.32^{Aa}	3.12 ± 0.19^{Aa}
	7	3.11 ± 0.26^{Aa}	4.09 ± 0.71^{Aa}	3.01 ± 0.32^{Aa}
	90	2.90 ± 0.25^{Aa}	1.69 ± 0.08^{Bb}	2.59 ± 0.24^{Aa}
Lymphocytes ($\times 10^{9}$/L)	0	3.17 ± 0.15^{Aa}	2.81 ± 0.26^{Aa}	2.69 ± 0.33^{Aa}
	7	3.72 ± 0.29^{Aa}	3.55 ± 0.26^{Aa}	3.84 ± 0.30^{Ab}
	90	3.18 ± 0.48^{Aa}	2.39 ± 0.44^{Aa}	2.93 ± 0.07^{Aa}
Monocytes ($\times 10^{9}$/L)	0	0.42 ± 0.05^{Aa}	0.41 ± 0.02^{Aa}	0.41 ± 0.02^{Aa}
	7	0.43 ± 0.04^{Aa}	0.43 ± 0.06^{Aab}	0.35 ± 0.04^{Aab}
	90	0.30 ± 0.04^{Aa}	0.21 ± 0.04^{Ab}	0.32 ± 0.01^{ABb}
Eosinophils ($\times 10^{9}$/L)	0	0.06 ± 0.02^{Aa}	0.06 ± 0.02^{Aa}	0.04 ± 0.02^{Aab}
	7	0.09 ± 0.01^{Aa}	0.12 ± 0.03^{Aa}	0.03 ± 0.02^{Aa}
	90	0.07 ± 0.02^{Aa}	0.05 ± 0.02^{Ba}	0.12 ± 0.02^{ABb}
Basophils ($\times 10^{9}$/L)	0	0.06 ± 0.01^{Aa}	0.05 ± 0.01^{Aa}	0.05 ± 0.01^{Aa}
	7	0.07 ± 0.03^{Aa}	0.06 ± 0.02^{Aa}	0.05 ± 0.02^{Aa}
	90	0.07 ± 0.01^{Ba}	0.01 ± 0.01^{Bb}	0.02 ± 0.01^{ABa}
Platelets ($\times 10^{9}$/L)	0	733.33 ± 69.89^{Aa}	811.67 ± 90.41^{Ab}	888.33 ± 43.45^{Aa}
	7	685.67 ± 57.57^{Aa}	550.67 ± 51.62^{Aa}	554.33 ± 2.64^{Ab}
	90	822.00 ± 57.97^{Aa}	558.00 ± 123.86^{ABab}	706.67 ± 15.54^{Bc}
Plasma protein (g/L)	0	68.67 ± 1.84^{Aa}	70.00 ± 1.93^{Ab}	71.33 ± 1.12^{Ab}
	7	68.00 ± 0.73^{Aa}	65.33 ± 1.12^{Aa}	64.00 ± 1.26^{Ba}
	90	68.33 ± 0.56^{Aa}	65.33 ± 0.42^{Ba}	61.33 ± 1.12^{Ca}

All values were expressed as mean ± standard error. Means with different superscript (^{ABC}) within the same row denotes significance ($p < 0.05$) among treatments, while means with different superscript (^{abc}) within the same column denotes significance ($p < 0.05$) among time phases. T: Treatment, RBC: Red blood cells, Hb: Hemoglobin, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration, WBC: White blood cells

were significant changes in the hematology parameters of sheep fed with different toxic levels of *B. decumbens* diets. There was a decrease in RBC and MCHC with an increase in MCV indicating macrocytic hypochromic anemia. The decreased in RBC proves that saponins from *B. decumbens* have the potential to damage red blood cells that are linked

Table 2 Biochemistry changes in sheep fed with low and high levels of *B. decumbens* diets at different time phases

Parameter	Day	T1 (Control)	T2 (10%)	T3 (60%)
AST (U/L)	0	119.00 ± 1.67^{Aa}	120.00 ± 18.63^{Aa}	121.00 ± 8.27^{Aa}
	7	124.33 ± 5.92^{Aa}	140.00 ± 14.43^{ABa}	157.00 ± 11.59^{Bb}
	90	120.00 ± 22.89^{Aa}	125.33 ± 4.46^{Aa}	144.67 ± 27.70^{Aab}
GGT (U/L)	0	58.00 ± 1.46^{Aa}	62.67 ± 4.97^{Aa}	68.00 ± 6.49^{Aa}
	7	57.67 ± 4.04^{Aa}	79.33 ± 7.38^{Ba}	91.33 ± 6.22^{Bb}
	90	52.00 ± 1.67^{Aa}	30.67 ± 4.62^{Bb}	21.67 ± 1.48^{Bc}
Total bilirubin (umol/L)	0	4.60 ± 0.62^{Aa}	2.97 ± 0.58^{Aa}	4.10 ± 0.91^{Aa}
	7	4.20 ± 0.51^{Aa}	1.70 ± 0.06^{Ba}	1.47 ± 0.18^{Bb}
	90	4.37 ± 0.46^{Aa}	2.50 ± 0.77^{ABa}	0.90 ± 0.06^{Bc}
Creatinine (umol/L)	0	25.67 ± 4.85^{Aa}	36.33 ± 8.10^{Aab}	32.00 ± 8.85^{Aa}
	7	26.00 ± 5.06^{Aa}	29.33 ± 2.49^{Aa}	32.00 ± 7.04^{Aa}
	90	19.33 ± 0.84^{Aa}	20.33 ± 1.48^{Ab}	20.67 ± 1.69^{Aa}
Urea (mmol/L)	0	5.00 ± 0.81^{Aa}	5.40 ± 0.77^{Aa}	4.30 ± 0.38^{Aa}
	7	4.77 ± 0.21^{Aa}	4.63 ± 0.13^{Aa}	4.90 ± 0.17^{Aa}
	90	5.17 ± 0.20^{Aa}	2.17 ± 0.15^{Bb}	1.53 ± 0.11^{Cb}
Phosphorus (mmol/L)	0	2.00 ± 0.24^{Aa}	1.83 ± 0.27^{Aa}	2.00 ± 0.24^{Aa}
	7	2.13 ± 0.11^{Aa}	2.60 ± 0.10^{Ba}	2.10 ± 0.13^{Aa}
	90	2.20 ± 0.18^{Aa}	1.10 ± 0.04^{Bb}	0.90 ± 0.06^{Bb}
Total protein (g/L)	0	63.60 ± 1.11^{Aa}	66.47 ± 1.83^{Aa}	66.93 ± 1.57^{Aa}
	7	65.00 ± 1.93^{Aa}	73.30 ± 1.79^{Bb}	74.20 ± 1.37^{Bb}
	90	62.97 ± 3.22^{Aa}	32.27 ± 0.88^{Bc}	24.73 ± 2.02^{Cc}
Albumin (g/L)	0	30.40 ± 0.69^{Aa}	29.67 ± 0.74^{Aa}	30.53 ± 0.61^{Aa}
	7	31.90 ± 0.39^{Aa}	35.07 ± 0.92^{Bb}	34.90 ± 0.78^{Bb}
	90	32.07 ± 0.75^{Aa}	19.23 ± 1.13^{Bc}	14.70 ± 1.11^{Cc}
Globulin (g/L)	0	33.20 ± 0.61^{Aa}	36.80 ± 1.84^{Aa}	36.40 ± 2.02^{Aa}
	7	33.10 ± 2.31^{Aa}	38.23 ± 0.93^{Aa}	40.10 ± 1.57^{Bb}
	90	30.90 ± 2.74^{Aa}	13.03 ± 1.76^{Bb}	10.03 ± 0.92^{Bc}
A:G (Unit)	0	0.93 ± 0.02^{Aa}	0.83 ± 0.04^{Aa}	0.87 ± 0.06^{Aa}
	7	1.00 ± 0.07^{Aa}	0.92 ± 0.02^{Aa}	0.87 ± 0.04^{Aa}
	90	1.03 ± 0.07^{Aa}	1.67 ± 0.29^{ABb}	1.50 ± 0.04^{Bb}

All values were expressed as mean ± standard error. Means with different superscript (^{ABC}) within the same row denotes significance ($p < 0.05$) among treatments, while means with different superscript (^{abc}) within the same column denotes significance ($p < 0.05$) among time phases. T: Treatment, AST: Aspartate aminotransferase, GGT: Gamma-glutamyl transferase, A:G: Albumin to globulin ratio

with hemolytic activity (Sparg et al. 2004) and were more severe at the higher toxic level and chronicity. Moreover, the WBC, neutrophils, monocytes, eosinophils, and basophils parameters were also reduced significantly in sheep fed with different levels of *B. decumbens* diets. The reduction of WBC parameters and platelet counts may be contributed by the suppressive effect due to interference with bone marrow activity during toxicity (Zubair and Martyniuk 2018). Eosinophilia in this study may be due to the chemotactic factors during inflammation in which the eosinophils responded to chemotaxins by phagocytizing the particular matter in response to venoms, dextrans, kinins, or physical trauma (McEwen 1992). Although there was no significant change in the PCV, the plasma protein was significantly decreased in all treatment sheep, and this may be attributed to a decrease in synthesis due to hepatic disease (Nagy et al. 2015).

Biochemistry

In the present study, there were significant increases in AST and GGT in both T2 and T3 sheep during the acute phase. Previous studies on *B. decumbens* toxicity demonstrated that the increase in bilirubin levels, AST, and GGT suggest reduced liver functions (De Oliveira et al. 2013). GGT in the present work demonstrated a significant decrease during the chronic phase which might be due to some compensatory mechanism (Riet-Correa et al. 2011). Furthermore, the total bilirubin was also reduced significantly in both treatment groups despite findings reporting hyperbilirubinemia in *B. decumbens* toxicity cases (Assumaidaee et al. 2010). According to previous work, the serum bilirubin levels could be reduced due to the inhibition of heme oxygenase activity and oxidative degradation of bilirubin (Roy-Chowdhury

Table 3 Acute phase proteins concentrations of both serum and cerebrospinal fluid in sheep fed with low and high levels of *B. decumbens* diets at different time phases

Parameters	Day	T1 (Control)	T2 (10%)	T3 (60%)
Serum Hp (μg/ml)	0	196.00 ± 3.54^{Aa}	200.73 ± 0.44^{Aa}	193.80 ± 4.78^{Aa}
	7	199.00 ± 0.77^{Aa}	220.42 ± 27.11^{Aab}	206.10 ± 8.63^{Aa}
	90	193.00 ± 4.57^{Aa}	154.32 ± 5.07^{Bb}	147.78 ± 7.13^{Bb}
Serum SAA (μg/ml)	0	12.59 ± 1.66^{Aa}	10.81 ± 0.43^{Aa}	13.82 ± 1.90^{Aa}
	7	13.52 ± 2.14^{Aa}	12.43 ± 0.82^{Aa}	16.03 ± 1.76^{Aa}
	90	10.79 ± 0.44^{Aa}	14.67 ± 2.89^{ABa}	18.64 ± 0.93^{Bb}
CSF Hp (μg/ml)	0	235.40 ± 4.44^{Aa}	235.77 ± 1.22^{Aa}	232.43 ± 3.61^{Aa}
	7	233.92 ± 4.10^{Aa}	263.85 ± 17.59^{Aa}	287.53 ± 33.84^{Aa}
	90	236.73 ± 3.46^{Aa}	241.70 ± 18.03^{Aa}	237.97 ± 19.48^{Aa}
CSF SAA (μg/ml)	0	17.74 ± 0.34^{Aa}	17.60 ± 0.40^{Aa}	17.50 ± 0.42^{Aa}
	7	17.62 ± 0.39^{Aa}	19.22 ± 1.89^{Aa}	20.72 ± 1.40^{Aab}
	90	17.31 ± 0.33^{Aa}	21.71 ± 1.51^{Ba}	24.04 ± 0.79^{Bb}

All values were expressed as mean ± standard error. Means with different superscript (^{ABC}) within the same row denotes significance ($p < 0.05$) among treatments, while means with different superscript (^{abc}) within the same column denotes significance ($p < 0.05$) among time phases. T: Treatment, D: Day, Hp: Haptoglobin, SAA: Serum amyloid A, CSF: Cerebrospinal fluid

et al. 2020). Moreover, elevated kidney parameters such as serum urea and creatinine are also signs of ruminants intoxicated with *B. decumbens* (Gracindo et al. 2014). However, in this study, the creatinine concentration changed very little, maybe due to the amount of *B. decumbens* toxin and/or the period of intoxication that was insufficient to damage the kidneys (Driemeier et al. 2002). The decrease in urea level in *B. decumbens* intoxicated sheep was probably due to the hepatic insufficiency and a reduction in total protein that lead to a decrease in urea synthesis (Fernández et al. 1996). On the other hand, the total protein, albumin, and globulin were elevated during the acute phase of *B. decumbens* intoxicated groups which may be due to some host cell responses (Tothova et al. 2016). However, during the chronic phase, there were hypoproteinemia, hypoalbuminemia, and hypoglobulinemia with an increased A:G ratio which is likely to be due to the damage of hepatocytes tissues as this has been proposed in some earlier toxicity studies (Zubair and Martyniuk 2018).

Acute phase proteins

The present study showed that the serum Hp levels increased slightly during the acute phase and reduced significantly at day 90 in both *B. decumbens* intoxicated groups. Hp will increase acutely and remain elevated for two weeks for the healing process as well as immune-modulatory functions to reconstitute equilibrium (Jesse et al. 2019). On the other hand, both serum and CSF SAA showed significant increases and remained high throughout the study period where T3 sheep had the highest serum SAA and CSF SAA during the chronic phase. This finding was consistent with Chung et al. (2019) who mentioned that SAA stimulation was related to chronic inflammatory diseases and there will be an increase during late inflammation. According to Jesse et al. (2019), the increase in SAA is associated with the inhibitory effects of inflammatory cells to prevent chronic inflammatory states, tissue damages, and even diseases.

Conclusion

In summary, the usability of *B. decumbens* grass in ruminants especially sheep is risky by the existence of steroidal saponins. In the present study, most hematology indicators except PCV, all biochemistry parameters, serum Hp, serum SAA, and CSF SAA of sheep were affected differently by the different levels of *B. decumbens* diets and the duration of feeding. Valuable information from the current study could be useful in identifying new indicators in sheep during *B. decumbens* intoxication.

Author contribution ELTC, MFHR, AS, and FFAJ postulated the experimental design. KVM, MSJ, and MHMH performed work associated with this study. KVM and ELTC performed the statistical analysis and prepared the manuscript. All authors reviewed manuscript upon submission.

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Data availability Not applicable.

Code availability Not applicable.

Declarations

Ethics approval All experimental procedures were conducted in accordance with the Institutional Animal Care and Use Committee

(IACUC) of Research Policy at Universiti Putra Malaysia (UPM) (Approval number: UPM/IACUC/AUP-R046/2019).

Consent to participate Not applicable.

Consent for publication The content of the manuscript was read by all authors and recommended it worthy of publication.

Conflict of interest The authors declare no competing interests.

References

Assumaidaee, A.A., Zamri-Saad, M., Jasni, S., and Noordin, M.M., 2010. The role of oxidative stress in *Brachiaria decumbens* toxicity in sheep. Pertanika Journal of Tropical Agricultural Science, 33(1), 151-157.

Cardona-Álvarez, J., Vargas-Vilória, M., and Paredes-Herbach, E., 2016. Clinical and histopathological study of the phototoxic dermatitis in Zebu calves in grazing of *Brachiaria decumbens*. Revista Medicina Veterinaria Zootecnia Córdoba, 21(2), 5366-5380.

Castro, M.B., Santos Jr., H.L., Mustafa, V.S., Gracindo, C.V., Moscardini, A.C.R., Louvandini, H., Paludo, G.R., Borges, J.R.J., Haraguchi, M., Ferreira, M.B., and Riet-Correa, F., 2011. *Brachiaria spp*. Poisoning in Sheep in Brazil: Experimental and Epidemiological Findings. Poisoning by Plants, Mycotoxins and Related Toxins, 1, 110–117.

Ceciliani, F., Ceron, J.J., Eckersall, P.D., and Sauerwein, H., 2012. Acute phase proteins in ruminants. Journal of Proteomics, 75, 4207–4231.

Chung, E.L.T., Predith, M., Nobilly, F., Samsudin, A.A., Jesse, F.F.A., and Loh, T.C., 2018. Can treatment of *Brachiaria decumbens* (signal grass) improve its utilisation in the diet in small ruminants? —a review. Tropical Animal Health and Production, 50, 1727–1732.

Chung, E.L.T., Jesse, F.F.A., Marza, A.D., Ibrahim, H.H., Abba, Y., Zamri-Saad, M., Haron, A.W., Lila, M.A.M., Saharee, A.A., and Bakar, M.Z.A., 2019. Responses of pro-inflammatory cytokines, acute phase proteins and cytological analysis in serum and cerebrospinal fluid during haemorrhagic septicaemia infection in buffaloes. Tropical Animal Health and Production, 51(6), 1773-1782.

De Oliveira, C.H.S., Barbosa, J.D., Oliveira, C.M.C., Bastianetto, E., Melo, M.M., Haraguchi, M., Freitas, L.G.L., Silva, M.X., and Leite, R.C., 2013. Hepatic photosensitisation in buffaloes intoxicated by *Brachiaria decumbens* in Minas Gerais state, Brazil. Toxicon, 73, 121–129.

Driemeier, D., Colodel, E.M., Seitz, A.L., Barros, S.S., and Cruz, C.E., 2002. Study of experimentally induced lesions in sheep by grazing *Brachiaria decumbens.* Toxicon, 40, 1027–1031.

Fernández, A., Ramos, J.J., Sanz, M.D.C., Saez, T., and Luco, D.F.D., 1996. Alterations in the performance, haematology and clinical biochemistry of growing lambs fed with aflatoxin in the diet. Journal of Applied Toxicology, 16(1), 85-91.

Gracindo, C.V., Louvandini, H., Riet-Correa, F., Barbosa-Ferreira, M., and de Castro, M.B., 2014. Performance of sheep grazing in pastures of *Brachiaria decumbens, Brachiaria brizantha, Panicum maximum,* and *Andropogon gayanus* with different protodioscin concentrations. Tropical Animal Health and Production, 46(5), 733–737.

Graydon, R.J., Hamid, H., Zahari, P., and Gardiner, C., 1991. Photosensitisation and crystal- associated cholangiohepatopathy in sheep grazing *Brachiaria decumbens*. Australian Veterinarian Journal, 68(7), 234–236.

Jesse, F.F.A., Chung, E.L.T., Abba, Y., Muniandy, K.V., Tan, A.H.A.R., Maslamany, D., Bitrus, A.A., Lila, M.A.M., and Norsidin, M.J., 2019. Establishment of lung auscultation scoring method and responses of acute phase proteins and heat shock proteins in vaccinated and non-vaccinated goats. Tropical Animal Health and Production, 51, 289–295.

Lelis, D.L., Rennó, L.N., Chizzotti, M.L., Pereira, C.E.R., Silva, J.C.P., Moreira, L.G.T., Carvalho, F.B.P., and Chizzotti, F.H.M., 2018. Photosensitization in naïve sheep grazing signal grass (*Brachiaria decumbens*) under full sunlight or a silvopastoral system. Small Ruminant Research, 169, 24-28.

Low, S., 2015. Signal grass (*Brachiaria decumbens*) toxicity in grazing ruminants. Agriculture, 5(4), 971–990.

McEwen, B.J., 1992. Eosinophils: a review. Veterinary Research Communications, 16(1), 11-44.

Muniandy, K.V., Chung, E.L.T., Jaapar, M.S., Hamdan, M.H.M., Salleh, A., and Jesse, F.F.A., 2020. Filling the gap of *Brachiaria decumbens* (signal grass) research on clinico-pathology and haemato-biochemistry in small ruminants: A review. Toxicon, 174, 26-31.

Nagy, O., Tothovam C., Nagyova, V., and Kovac, G., 2015. Comparison of serum protein electrophoretic pattern in cows and small ruminants. Acta Veterinaria Brno, 84(2), 187-185.

Riet-Correa, B., Castro, M.B., Lemos, R.A., Riet-Correa, G., Mustafa, V., and Riet-Correa, F., 2011. *Brachiaria spp*. poisoning of ruminants in Brazil. Pesquisa Veterinária Brasileira, 31(3), 183–192.

Roy-Chowdhury, N., Wang, X., and Roy-Chowdhury, J., 2020. Bile pigment metabolism and its disorders. In Emery and Rimoin's Principles and Practice of Medical Genetics and Genomics (pp. 507–553). Academic Press United States.

Sparg, S., Light, M.E., and Van Staden, J., 2004. Biological activities and distribution of plant saponins. Journal of Ethnopharmacology, 94(2-3), 219–243.

Tothova, C., Nagy, O., and Kovac, G., 2016. Serum proteins and their diagnostic utility in veterinary medicine: a review. Veterinární Medicína, 61(9), 475-496.

Xu, T., Zhang, S., Zheng, L., Yin, L., Xu, L., and Peng, J., 2012. A 90-day subchronic toxicological assessment of dioscin, a natural steroid saponin, in Sprague–Dawley rats. Food and Chemical Toxicology, 50(5), 1279-1287.

Yuliana, P., Laconi, E.B., Wina, E., and Jayanegara, A., 2014. Extraction of tannins and saponins from plant sources and their effects on in vitro methanogenesis and rumen fermentation. Journal of the Indonesian Tropical Animal Agriculture, 39(2), 91-97.

Zubair, M. and Martyniuk, C.J., 2018. A review on hemato-biochemical, accumulation and patho-morphological responses of arsenic toxicity in ruminants. Toxin Reviews, 38(3), 176-186.

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