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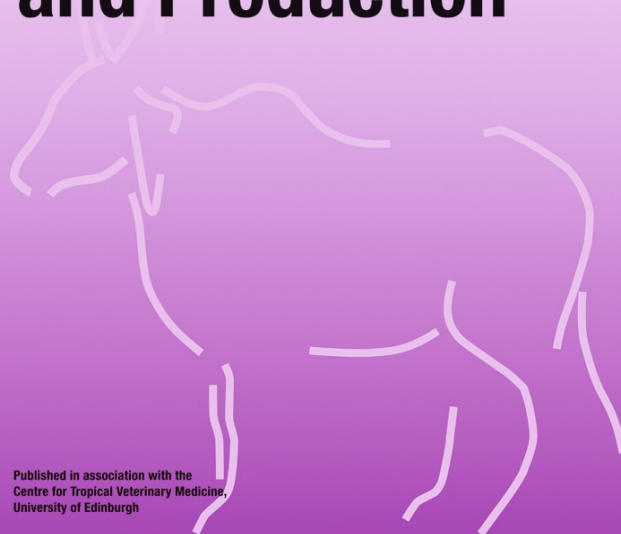
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Replacement of berseem hay by *Salix tetrasperma* on physiological performance of New Zealand White rabbits under subtropical conditions of Egypt

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Abstract Forty-eight growing New Zealand White male rabbits aged 6 weeks (874 ± 1.3 g initial body weight (BW)) were used to study effects of partial replacement of berseem hay (BH) with *Salix tetrasperma* hay (ST) on growth and physiological responses. Rabbits were allotted to one of four diets of 12 rabbits each for 75 days in a completely randomized design. The treatments were as follows: control (30 % BH), ST25 (7.5 % ST+22.5 % BH), ST50 (15 % ST+15 % BH), ST75 (22.5 % ST+7.5 % BH). Nutrient digestibility coefficients, nutritive value and N utilization of rabbits fed with the ST50 rations were higher ($P < 0.05$) than the other groups. Final live BW, average daily gain, feed intake and feed efficiency of rabbits fed ST25 and ST50 were higher ($P < 0.05$) than those fed ST75 and the control. Serum biochemical metabolites of urea, creatinine, aspartate amino transferase and alanine amino transferase concentrations varied among diets, with

the rank order ($P < 0.05$) ST75 > ST25 and ST50 > control. Glucose level was higher ($P < 0.05$) for the control than the other diets. Rabbits fed with the mixed diets of ST had lower ($P < 0.05$) total lipids, cholesterol and triglycerides levels than those fed with control. Haematological indices of packed cell volume, haemoglobin, red blood cells, white blood cells and lymphocyte counts were lower ($P < 0.05$), but monocyte was higher, in rabbits fed with the ST75 than the other groups. However, other haematological parameters were similar among diets. Since all the performance and blood parameters were within normal ranges for healthy rabbits, and there were no signs of toxicity, we conclude that partial replacement of BH by ST improves rabbit growth performance, and did not impact rabbit health.

Keywords Blood metabolic profile · Growth performance · Rabbits · *Salix tetrasperma*

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Abbreviations

ADF	Acid detergent fibre
ADL	Acid detergent lignin
ALT	Alanine amino transferase
AST	Aspartate amino transferase
BH	Berseem hay
BW	Body weight
N	Nitrogen
CT	Condensed tannins
DM	Dry matter
ME	Metabolizable energy
NDF	Neutral detergent fibre
PCV	Packed cell volume
RBC	Red blood cells
ST	<i>Salix tetrasperma</i>
WBC	White blood cells

Introduction

Salix (willow) trees have been used successfully as a source of emergency fodder for rabbits, sheep and cattle during summer/autumn feed shortages and droughts (Charlton et al. 2003). The browse on wide-spaced soil conservation trees can be a feed source for livestock during feed shortages (McWilliam 2004). The total N and ME content of willow fodder are about 26.3 g and 10.5 MJ per kg DM, respectively (ThiMui et al. 2005). Willow is moderately digestible and highly palatable for livestock and is superior to dry summer pasture, and it is a source of minerals for grazing livestock, including calcium, magnesium, potassium and zinc (Guevara-Escobar 1999).

Willow species synthesize low molecular phenolic glycosides, such as salicin (35 g/kg DM) and/or condensed tannin (CT, 38 g/kg DM) (Pitta et al. 2007). High levels of salicin may reduce its palatability to grazing livestock. Condensed tannin in forage legumes reduces microbial degradation of plant proteins in the rumen and increases the flow of undegraded dietary protein to the small intestine, thus increasing protein absorption and utilization, and enhancing amino acid absorption from the small intestine (Orians et al. 2000).

Our objective was to evaluate the influence of the partial substitution of berseem hay with *Salix tetrasperma* on growth performance, digestibility and blood serum parameters of growing rabbits.

Materials and methods

Animals, housing treatments and diets

Forty-eight New Zealand White growing male rabbits aged 6 weeks (874 ± 1.4 g initial body weight (BW)) were randomly allotted into one of four treatments of 12 rabbits each. All rabbits were kept in rooms with standardized air conditioning of 20 to 25 °C and 55 to 65 % humidity for 75 days. Rabbits were housed in galvanized wire cages (40 cm high \times 50 cm width \times 60 cm length) and fresh water was available at all times. All rabbits were kept under the same managerial, hygienic and environmental conditions. Each treatment was further subdivided into four replicates and each was weighed weekly. Four rabbits from each treatment were individually housed in metabolic cages for 7 days to allow quantitative collection of faeces, feed refusals and urine for digestibility estimation. Faeces and urine from each rabbit were collected in labelled polyethylene bags and stored at -10 °C. Feed and water were supplied ad libitum during the experimental period. Live BW and feed intake were recorded weekly during the experimental period. Average daily gain and feed efficiency were calculated.

Small stems and leaves of *S. tetrasperma* (ST) were collected from the experimental field located in New Borg El-Arab, North Coast of Alexandria, Egypt. Stems and leaves were chopped and dried. Four experimental diets were formulated as follow: control (30 % berseem hay (BH)), ST25 (7.5 % ST+22.5 % BH), ST50 (15 % ST+15 % BH), ST75 (22.5 % ST+7.5 % BH). Chemical composition (% DM) of BH and ST and experimental diets are in Tables 1 and 2.

Laboratory analysis

Samples of ST, BH, feed and faecal as well as urine were analysed. The NDF, ADF and ADL were analysed according to Van Soest et al. (1991) procedures. The AOAC (2000) techniques were used for dry matter (DM), CP, crude fibre, ether extract, ash and N in urine determinations. Values of total digestible nutrients were calculated according to Chee et al. (2013). Hemicellulose and cellulose were calculated. Mineral elements (Ca, K and Mg) of BH and ST were determined with an atomic absorption spectrophotometer (Unicam 919) while P was colorimetrically determined using molybdovanadate reagent. The CT of ST was determined according to Makkar (2003), salicin and phenolic compounds were determined using the high-performance liquid chromatographic using the procedure of Meier et al. (1988).

At the end of the experiment, blood samples were obtained from sacrificed rabbits in labelled sterile tubes. Samples were allowed to coagulate at room temperature and centrifuged at 3,000 rpm for 15 min and serum was separated and stored at -20 °C until assay. Total protein, albumin, glucose, total

Table 1 Chemical composition (g/kg) of berseem hay and *S. tetrasperma*

	Berseem hay	<i>Salix tetrasperma</i>
Organic matter	867.5	918.6
Crude protein	121.8	122.5
Total N	19.5	19.6
Ether extract	16.7	32.6
N free extract	464.5	626
Neutral detergent fibre	591.2	379.5
Acid detergent fibre	364.3	261.1
Acid detergent lignin	72.6	112.6
Phenolic compounds	ND	83.2
Condensed tannins	ND	37
Salicin (g/kg dry matter)	ND	12
Ca	16.5	126
P	2.4	45
Mg	ND	20.2
K	15	126
Metabolizable energy, MJ/kg DM	65.2	97.5

ND not determine

Table 2 Ingredients and chemical composition of experimental diets

	Control	<i>Salix tetrasperma</i> (ST)		
		ST25	ST50	ST75
Ingredients, g/kg				
Yellow corn	70	70	70	70
Wheat bran	170	170	170	170
Barley grain	200	200	200	200
Soybean meal	200	200	200	200
Berseem hay	300	300	150	75
<i>Salix tetrasperma</i>	0	0	150	225
Molasses	30	30	30	30
Salt	5	5	5	5
Limestone	10	10	10	10
Di Ca phosphate	10	10	10	10
Premix	4	4	4	4
Methionine	1	1	1	1
Chemical analysis, g/kg				
Organic matter	930.6	927.8	926.5	925.2
Crude protein	172.4	174.7	175.8	176.9
Crude fibre	152.4	140.6	133.7	116.3
Ether extract	31.2	32.1	32.9	32.6
N free extract	574.6	580.4	584.1	599.4
Neutral detergent fibre	394.5	381.1	376.6	373.1
Acid detergent fibre	271.7	269.4	265.4	262.2
Acid detergent lignin	137.6	131.4	130.2	128.6
Hemicellulose	122.8	111.7	111.2	110.9
Cellulose	134.1	138.0	135.2	133.6
Growth energy, MJ/kg	1.83	1.82	1.82	1.82

One kilogram of premix contained the following: Vit. A, 12,000,000 IU; Vit. D₃, 2,200,000 IU; Vit. E, 1,000 mg; Vit. B₁, 1,000 mg; Vit. B₂, 4,000 mg; Vit. B₆, 100 mg; Vit B₁₂, 10 mg; pantothenic acid, 3.33 g; biotin, 33 mg; folic acid, 0.83 g; Zn, 11.79 g; Mn, 5 g; Fe, 12.5 g; Cu, 0.5 g; Se, 16.6 mg; Mg, 66.7 g. Gross energy calculated according to MAFF (1975) were as follow: growth energy, MJ/kg DM=0.0226 crude protein+0.0407 ether extract+0.0192 crude fibre+0.0177 N free extract

cholesterol, triglyceride, total lipids, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined by spectrophotometer (Spectronic 21 DUSA) using commercial diagnostic kits (Combination, Pasteur Lap.), while the globulin was calculated by the deference between total protein and albumin. Heparinized blood samples were also obtained for blood cells counts. The packed cell volume (PCV) was measured by the microhaematocrit centrifuge (Mitruka and Rawnsley 1977) and haemoglobin concentration was determined by the cyanmethemoglobin technique (Mitruka and Rawnsley 1977). The white blood cells (WBC) counting method was based on the dilution of obtained blood samples with diluting fluids (Mitruka and Rawnsley 1977). Individual cells were then counted in the counting chamber (haemocytometer).

At the end of the fattening period, four rabbits were chosen randomly from each treatment and fasted for about 16 h, then individually weighed and slaughtered to determine carcass traits.

Statistical analysis

The experimental design was completely randomized using the General Linear Means of SAS (2001). Measured parameters were analyzed using the following statistical model:

$$y_{ij} = \mu + d_i + \varepsilon_{ij}$$

where y_{ij} is the value measured, μ is the overall mean effect, d_i is the i th diet effect and ε_{ij} is the random error associated with the j th rabbits assigned to the i th diet.

Significant differences of $P < 0.05$ among means were determined using Duncan's Multiple Range Test.

Results

Total N and crude protein contents for ST were similar to BH. Moderate contents of EE, organic matter, NDF and ADF were detected in ST, while ME was higher in ST than BH. No CT or salicin was detected in BH but ST had a relatively moderate CT and high salicin contents (Table 1). The ST contained substantial concentrations of secondary metabolites, including lignin, CT, salicin and other phenolic compounds. Crude fibre content increased from 12 to 15 % by substituting BH with ST in the experimental diets (Table 2).

Rabbits fed with ST50 had the highest ($P < 0.05$) DM, organic matter, crude protein, crude fibre and ether extract digestibility, but there were no differences between control and ST25 or ST75. The ST50 had better nutritive value, N balance and N digested values compared to other groups. The final BW, average daily gain and feed intake increased ($P < 0.05$) in ST25 and ST50 rabbits versus control and ST75. The feed efficiency of ST25 and ST50 rabbits was improved ($P < 0.05$) versus control and ST75 (Table 3).

The highest carcass weights occurred for rabbits fed with ST25 and ST50 compared with ST75 and control rabbits. Rabbits fed with ST50 increased dressing percent ($P < 0.05$) versus the other groups. The ST75 rabbits had the highest ($P < 0.05$) proportional liver, heart, kidney, lung and brain mass compared to ST25, ST50 and control rabbits. Control rabbits had the highest ($P < 0.05$) values of glucose, total protein, albumin, total lipids, cholesterol and triglycerides versus those fed with other diets. Glucose and triglyceride concentrations of ST25, ST50 and ST75 rabbits were lower ($P < 0.05$) than control. Globulin and cholesterol were decreased ($P < 0.05$) in ST75 rabbits versus control. Urea,

Table 3 Feed intake, digestion coefficients, N utilization and growth performance of rabbits fed with different percentage of *S. tetrasperma* of the diet berseem hay

	Control	<i>Salix tetrasperma</i> (ST)		
		ST25	ST50	ST75
Intake				
Dry matter, g/day/head	169±17.6 a	168±11.8 a	159±7.9 b	148±9.1 b
Water, mL/day/head	175±11.9 c	196±14.7 b	206±9.6 b	246±9.9 a
Digestibility coefficients, g/kg				
Dry matter	610±5.7 b	609±2.7 b	623±4.3 a	588±3.3 c
Organic matter	632±3.7 b	633±4.4 b	645±2.7 a	612±2.1 c
Crude protein	622±1.8 b	632±2.1 b	649±1.6 a	591±2.2 c
Crude fiber	501±3.9 b	518±4.8 b	529±2.4 a	392±4.1 c
Ether extract	784±1.4 b	798±3.3 b	818±2.1 a	780±1.5 c
N free extract	659±1.8	652±2.2	660±1.2	652±1.9
Nutritive value, g/kg				
Total digestible nutrients	618±2.2 b	619±2.7 b	631±1.9 a	598±2.3 c
Digestible crude protein	109±1.5 b	110±2.4 ab	115±0.9 a	104±1.1 b
N utilization				
Intake (NI), g/day	47.1±0.90	47.1±1.10	45.1±0.71	42.0±0.6
N digested (ND), g/day	29.3±0.41 a	29.7±0.81 a	29.3±0.32 a	24.8±0.4 b
N Balance (NB), g/day	20.6±0.21 b	20.8±0.40 b	21.3±0.11 a	15.5±0.2 c
NB/NI	0.44±0.01 b	0.44±0.02 b	0.47±0.02 a	0.37±0.01 c
NB/ND	0.70±0.05 b	0.70±0.03 b	0.73±0.01 a	0.63±0.02 c
Growth performance				
Initial BW, g	875±23.9	878±21.6	871±25.6	873±19.6
Final BW, g	2,676±17.5b	2,845±18.1a	2,895±16.6a	2,246±26.8c
Average daily gain	24.0±0.60b	26.2±0.51a	27.0±0.51a	18.3±0.50c
Feed intake, g/head/day	146±0.3b	152±0.3a	155±0.2a	135±0.3c
Feed efficiency, feed/gain	6.1±0.12b	5.8±0.10c	5.7±0.11c	7.4±0.10a

Means within rows with different letters (a, b, c) are significantly different ($P<0.05$)

creatinine, AST and ALT in ST75 rabbits were higher ($P<0.05$) than of those fed with control, ST25 and ST50. The WBC and lymphocyte in control rabbits were decreased ($P<0.05$) compared to the other ST groups. Whereas rabbits fed with ST75 had the highest monocyte versus ST50 and control, but did not differ from those fed ST50 (Table 4).

Discussion

Salix hay (i.e. ST) contained an adequate amount of N, DM, organic matter, crude protein, NDF, ADF and ME, which support moderate growth of livestock (McWilliam 2004). Increased digestibility and N-utilization may be due to positive impacts of ST on absorption and utilization of nutrients. The increased growth rate in rabbits fed with ST25 and ST50 may be due to increased feed utilization in these groups, and could be due to an increased digestibility and N-utilization (McWilliam 2004). Low to medium concentrations of CT (20–40 g/kg DM) occurred in willow forages, increased the efficiency of protein digestion by increasing flow of N to the intestine relative to N intake, increased flow of essential amino

acids out of the abomasum by 50–53 % and increased net absorption of essential amino acids from the small intestine by 59–63 %, with no effect on digestibility (McWilliam 2004). Rizzi and Chiericato (2008) demonstrated that the use of *Salix alba* for growing rabbits allowed a better productive performance. Al-Fataftah and Abdelqader (2013) reported that broiler birds provided with *Salix babylonica* exhibited the maximum final BW, average daily gain and feed conversion improved compared with other groups. However, these studies indicate that the responses to dietary tannin are variable and depend on the type, source and concentration of tannin used, animal species and basal diet fed.

Serum urea N and creatinine levels were within the established range of 7.4–17.6 and 0.06–0.14 mmol/L, respectively, for rabbits (Özkan et al. 2012). The elevated urea N concentration of ST75 displayed the decreased biological value of ST75 compared to ST25, ST50 and control. Increased catabolism of amino acids, when the biological value of proteins was decreased has been implicated in high plasma urea N levels (Aderolu et al. 2007). Depressed serum glucose levels of ST75 compared to ST25 rabbits, ST50 and control possibly reflects the lower energy of ST25, ST50 and control.

Table 4 Carcass organs weight, serum biochemical metabolites and haematological indices of rabbits fed with different percentage of *S. tetrasperma*, the diet berseem hay

	Control	<i>Salix tetrasperma</i> (ST)		
		ST25	ST50	ST75
Body weight, kg	2.6±0.2	2.6±0.1	2.6±0.2	2.4±0.1
Carcass weight, kg	1.8±0.2b	2.0±0.2a	2.0±0.1a	1.5±0.1c
Carcass organ, g/kg				
Dressing	688±5.4b	713±4b	773±4.4a	604±5.2c
Liver	22±0.7c	22±0.9b	24±0.5b	29.2±0.5a
Kidney	5.0±0.1c	5.1±0.2b	5.4±0.1b	6.4±0.1a
Lung	4.5±0.2b	4.6±0.1b	4.7±0.1b	5.8±0.3a
Brain	2.6±0.1b	2.6±0.1b	2.7±0.1ab	2.9±0.2a
Heart	2.1±0.1c	2.5±0.1b	2.6±0.1b	2.9±0.2a
Spleen	0.6±0.1	0.6±0.1	0.6±0.1	0.8±0.1
Testes	1.7±0.1	1.7±0.1	1.7±0.1	1.7±0.1
Serum biochemical metabolites				
Urea, mmol/L	7.3±0.10c	7.8±0.33b	7.9±0.24b	8.4±0.11a
Creatinine, mmol/L	0.08±0.01c	0.09±0.01b	0.09±0.01b	0.1±0.04a
Glucose, mmol/L	7.0±0.25a	6.44±0.15b	6.29±0.36b	5.44±0.16c
Total protein, g/dL	7.0±0.44a	7.0±0.21ab	6.4±0.63b	6.0±0.33c
Albumin, g/dL	3.6±0.12a	3.2±0.15b	3.0±0.16b	2.9±0.11c
Globulin, g/dL	3.4±0.66a	3.5±0.09a	3.4±0.11b	3.1±0.15c
Total lipids, g/dL	3.5±0.24a	3.4±0.15b	3.3±0.17b	3.1±0.21c
Cholesterol, mg/dL	252±9.1a	236±4.0ab	212±4.0b	200±7.0c
Triglycerides, mg/dL	128±3.9a	120±2.89b	112±2.68b	99±6.65c
AST, IU/L	69.0±2.0c	72.0±1.8b	74.0±1.0b	80.0±1.6a
ALT, IU/L	40.1±0.6c	42.0±0.4b	45.0±0.9b	50.0±0.2a
Haematological indices				
Packed cell volume, %	34.5±0.12b	38.0±0.24a	38.3±0.17a	32.0±0.22c
Haemoglobin, g/L	110±0.1a	111±0.71a	111±0.51a	101±0.3b
Red blood cells, ×10 ¹² /L	6.4±0.15b	7.6±0.31a	8.0±0.31a	5.4±0.1c
White blood cells, ×10 ⁹ /L	7.3±0.23b	9.9±0.11a	10.9±0.14a	11.1±0.09a
Lymphocyte	52.1±0.35b	60.1±0.21a	59.8±0.28a	58.4±0.22a
Monocyte	1.94±0.16b	2.08±0.21b	2.42±0.19ab	2.87±0.2a
Neutrophils	43.1±0.63	44.5±0.09	45.0±0.69	45.8±1.64
Basophils	0.14±0.02	0.13±0.04	0.16±0.01	0.13±0.06
Eosinophils	1.45±0.11	1.51±0.10	1.52±0.09	1.43±0.09

Means with different letters (a, b, c) in the same row vary significantly ($P<0.05$)

ALT alanine amino transferase, AST aspartate amino transferase

Glucose levels were within the range indicated for healthy rabbits (Özkan et al. 2012), and it appears plausible to infer that the depressed serum glucose was due to tannic acid intoxication and because the animals were not surviving at the expense of body tissues.

Decreased total serum protein and albumin of rabbits fed with ST75 was an indication of the relatively poor protein quality and due to the level and availability of the dietary protein. However, total protein and albumin were within the ranges for healthy rabbits (Ajayi and Raji 2012). Higher values obtained for the ST25 and ST50 show that the tannin level of ST was safe and beneficial because tannins at low levels improved protein availability and utilization.

Serum levels of AST and cholesterol are those conventionally used for diagnosing domestic animal hepatic damage, specifically, and are used to detect bile obstruction (i.e. mild and progressive damage to the liver). Normal ranges for AST and ALT are 42.5–98.0 and 48.5–78.9 IU/L (Ajayi and Raji 2012). That none of these blood metabolites differed among diets, and that all of them fell within the normal ranges for rabbits, suggest that no damage had occurred in the liver. A lack of increase in these metabolites above normal values suggests that necrotic damage to the kidney did not occur; results are similar to previous observations (Silanikove et al. 1996).

Rabbits, particularly those consumed ST which contained CT, did not show clinical signs of morbidity or signs of tannin

toxicity. Absence of signs of ill health and mortality in rabbits that consumed ST75, in which CT were more concentrated, confirms the non-toxic level of CT in ST. Reduction in PCV usually suggested the presence of a toxin which had adverse effects on blood formation (Oyawoye and Ogunkunle 1998). The decreased PCV and red blood cells (RBC) in ST75 rabbits versus control could be attributed to anti-nutritional factors in ST, particularly phenols and CT (Rubanza et al. 2005). The normal RBC values elucidated the absence of haemolytic anaemia and depression of erythropoiesis. The lack of a difference of haemoglobin concentrations was within the reported range (104–140 g/L) for rabbits. Poljičak-Milas et al. (2009) suggested the absence of microcytic hypochromic anaemia occasioned by iron deficiency and improper utilization for the formation of haemoglobin.

Higher WBC and lymphocyte counts for rabbits fed with ST75 may be connected with the diet CT concentration. Values of WBC, lymphocyte and monocyte counts were within the ranges for healthy rabbits as reported by Poljičak-Milas et al. (2009). This could be an indication of the health status of the experimental rabbits and confirms the earlier conjecture that the concentration of CT in this diet was lower than the level that could induce toxicity or morbidity. Monocytes are essential to the immune system as they are precursors of macrophages and lymphocytes essential for humoral and cell-mediated immunity responses. Generally, toxic substances in feed tend to suppress haemopoietic tissues with consequent production of a high WBC count. Our results agree with Solaiman et al. (2010), who indicated decreased WBC but increased lymphocyte counts with increasing dietary tannin concentrations.

Conclusions

Feeding of *S. tetrasperma* to growing rabbits, with 25 and 50 % of the diet berseem hay, improved their physiological performance without reversible effects or stimulating an immune response. Moreover, the findings of all the haematological and serum metabolites were within the established and normal ranges for healthy rabbits under drought conditions

Conflict of interest The authors declare that they have no conflict of interest.

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