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DIGESTION, GROWTH PERFORMANCE AND CAECAL FERMENTATION IN GROWING RABBITS FED DIETS CONTAINING FOLIAGE OF BROWSE TREES

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Abstract: This study aimed to evaluate the effect of feeding dried foliage (leaves and petioles) of *Acacia saligna, Leucaena leucocephala* or *Moringa oleifera* on the performance, digestibility, N utilisation, caecal fermentation and microbial profiles in New Zealand White (NZW) rabbits. One hundred weaned male NZW rabbits weighing 819.2±16.6 g and aged 35±1 d were randomly allocated into 4 groups of 25 rabbits each. Rabbits were fed on pelleted diets containing 70% concentrate mixture and 30% Egyptian berseem (*Trifolium alexandrinum*) hay (Control diet) or one of the other 3 experimental diets, where 50% of berseem hay was replaced with *A. saligna* (AS), *L. leucocephala* (LL) or *M. oleifera* (MO). Compared to Control diet, decreases in dry matter (DM; P=0.004), organic matter (P=0.028), crude protein (CP; P=0.001), neutral detergent fibre (P=0.033) and acid detergent fibre (P=0.011) digestibility were observed with the AS diet. However, DM and CP digestibility were increased by 3% with the MO diet, and N utilisation was decreased (P<0.05) with AS. Rabbits fed AS and LL diets showed decreased (P=0.001) average daily gain by 39 and 7%, respectively *vs.* Control. Feed conversion was similar in Control and MO rabbits, whereas rabbits fed AS diet ate up to 45% more feed (P=0.002) than Control rabbits to gain one kg of body weight. Caecal ammonia-N was increased (P=0.002) with LL, while acetic acid was decreased (P=0.001) with AS diet *vs.* other treatments. Caecal *E. coli* and *Lactobacillus* spp. bacteria counts were decreased with MO by about 44 and 51%, respectively, *vs.* Control. In conclusion, under the study conditions, tree foliage from *M. oleifera* and *L. leucocephala* are suitable fibrous ingredients to be included up to 150 g/kg in the diets of growing rabbits, and can safely replace 50% of berseem hay in diets of NZW rabbits without any adverse effect on their growth performance. Foliage from *M. oleifera* had a better potential as a feed for rabbits than that from *L. leucocephala.* Although foliage from *A. saliga* may be also used at 150 g/kg in the diets of growing rabbits, this level of inclusion may result in reduced feed digestibility and growth performance.

Key Words: caecal fermentation, digestion, rabbits, tree foliage.

INTRODUCTION

The acute shortage of meat supply can be bridged by the farming of highly prolific animals with short production cycles, such as rabbits. However, feed shortages in many countries can affect rabbit productivity negatively, raising the interest of nutritionists in the search for alternative sources of non-conventional, cheap and readily available feedstuffs. Tree and shrub leaves are important sources of roughage for small ruminants (Ahmed *et al.,* 2015a,b; Alsersy *et al.,* 2015; Kholif *et al.,* 2015), but the use of locally available tree foliage in rabbit nutrition is a topic relatively unexplored in tropical countries. Foliage of tropical plants is available nearby and at low cost, in contrast with

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the scarcity and often high costs of commercial feeds. Egypt, as a developing country, is facing a shortage of animal protein due to poor performance of farm animals resulting in increased costs of livestock and food of animal origin. Nutritional solutions have now become even more important to resolve these demands, and this can be achieved by taking full advantage of alternative feed resources, such as tropical plants, in rabbit diets (AbuHafsa *et al.,* 2014).

Feeding foliage from browse trees and shrubs may be of importance in animal production because these resources do not compete with human food and can provide significant amounts of nutrients. Rabbit is increasingly becoming an important meat source and its production is recommended in countries that are experiencing meat shortages, as it presents the best productive advantages to bridge the protein deficiency gap (Abdel-Aziz *et al.,* 2015).

Acacia spp. is a perennial legume shrub that yields green forage all year round and is considered a relatively palatable browse shrub rich in protein (Ahmed *et al.,* 2015a,b). *Leucaena leucocephala* is a leguminous tree found throughout the tropics, subtropics and arid regions and can be an excellent source of Ca, P, and other dietary minerals (Mtenga and Laswai, 1994; Ruiz-Feria *et al.,* 1998). The leaves of *L. leucocephala* have been widely used in Egypt as a valuable forage supplement to ruminants consuming low-protein diets. Recommended inclusion levels range between 24 to 40% for growing or fattening rabbits fed on fresh *L. leucocephala* leaves (Nieves *et al.,* 2004). *Moringa oleifera*, a non-leguminous multi-purpose tree, is one of the fastest growing trees in the world, with high crude protein (CP) in the leaves and negligible contents of secondary metabolites (Kholif *et al.,* 2015, 2016). Its leaves and fresh green pods are used as vegetables by humans and are rich in vitamins A, B and C, with a good profile of amino acids and minerals (Ca, P, and Fe) (Djakalia *et al.,* 2011). There has been increasing interest in the use of *M. oleifera* as a protein source for livestock (Kholif *et al.,* 2015, 2016). Safwat *et al.* (2014), in their review, reported that *Moringa* leaf meal at levels of inclusion of up to 20% of the diet is non-toxic and improves the productive performance of weaning rabbits.

The current study aimed to evaluate the effect of feeding growing rabbits with *A. saligna, L. leucocephala* and *M. oleifera* foliage (leaves and petioles) as a partial replacement of berseem hay on growth performance, nutrient digestibility, N utilisation and caecal fermentation.

MATERIAL AND METHODS

Experimental site and plant collection

The experiment was conducted in the livestock unit of the City of Scientific Research and Technological Applications, New Borg El-Arab (Egypt) and Animal Production Research Institute, Agricultural Research Centre (Egypt). Foliage (petioles and leaves) of *A. saligna, L. leucocephala* and *M. oleifera* were harvested around the experimental site. The leaves and petioles of each browse tree species were pooled and air-dried by spreading the material thinly on plastic sheets under shading for a period of 2-3 d, and then bagged for later feeding and chemical analysis (Table 1).

Table 1: Chemical composition (g/kg dry matter basis) of berseem hay and the tested plants.

	Berseem hay	*A. saligna*	*L. leucocephala*	*M. oleifera*
Dry matter (as fed)	896	366	322	311
Organic matter	924	914	925	928
Crude protein	123	126	207	173
Crude fibre	278	258	190	179
Ether extract	17	18	29	33
Nitrogen free extract	506	512	499	543
Neutral detergent fibre	411	615	347	328
Acid detergent fibre	307	447	268	258
Acid detergent lignin	49	107	84	80
Total phenols	19	126	106	94
Condensed tannins	17	78	30	18

Animals, diets and laboratory analyses

One hundred weaned male New Zealand white (NZW) rabbits, aged 35±1 d and with a body weight of 819±17 g, were used in the study. Rabbits were housed in pens (5 rabbits per pen), which were the experimental units for the intake/performance study. The experimental treatments (4 pelleted diets with different levels of inclusion of berseem and leaves from 3 browse trees) were randomly assigned to the experimental units (5 pens and 25 rabbits per dietary treatment). Each diet was fed for 60 d, the total duration of the experiment. Feed and fresh drinking water were offered *ad libitum*. Feed was provided in 2 equal meals at 08:00 and 17:00 h daily. A basal diet with 70% concentrate mixture and 30% Egyptian berseem (*Trifolium alexandrinum*) hay was considered as a Control. Three other experimental diets were formulated, instead of the Control diet; where 50% of berseem hay was replaced, on DM basis, with *A. saligna* (AS), *L. leucocephala* (LL) or *M. oleifera* (MO) foliage as shown in Table 2. The 4 experimental diets were formulated to be nearly isonitrogenous and isoenergetic. Dried foliage of each tree species or berseem hay were finely ground and stored in an airtight container. Dried ground foliage and/or berseem hay were mixed with the other ingredients in the proportions described in Table 2, and then compound diets were pelleted, thus preventing feed selection.

Table 2: Ingredients and chemical composition (g/kg dry matter) of experimental diets.

	Experimental diets*			
	Control	AS	LL	MO
Ingredients				
Barley	320	320	360	360
Soybean meal	180	180	100	110
Wheat bran	152	152	192	182
Berseem hay	300	150	150	150
A. saligna	0	150	0	0
L. leucocephala	0	0	150	0
M. oleifera	0	0	0	150
Molasses	20	20	20	20
Calcium carbonate	5	5	5	5
Di-calcium phosphate	15	15	15	15
Salt (NaCl)	5	5	5	5
Premix†	1	1	1	1
DL-methionine	2	2	2	2
Chemical composition				
Dry matter (g/kg feed)	899	897	885	881
Organic matter	928	927	928	928
Crude protein (CP)	162	163	166	164
Crude fibre	142	141	137	132
Ether extract (EE)	31	32	37	35
Nitrogen free extract	593	591	589	597
Neutral detergent fibre (NDF)	347	395	334	315
Non-structural carbohydrates (NSC)	388	337	391	414
Acid detergent fibre	245	278	230	211
Acid detergent lignin	97	107	81	76
Digestible energy (MJ/kg)‡	11.9	11.4	12.1	12.3

*Diets contained 70% concentrates mixture+30% berseem hay (Control); 70% concentrates mixture+15% berseem hay+15% *A. saligna* (AS); 70% concentrates mixture+15% berseem hay+15% *L. leucocephala* (LL); 70% concentrates mixture+15% berseem hay+15% *M. oleifera* (MO).

†Each 1 kilogram of premix contains: Vit. A 12000000 IU, Vit. D_3 2000000 IU., Vit. E 10000 mg, Vit. K_3 2000 mg, Vit. B_1 1000 mg, Vit. B_2 5000 mg, Vit. B_6 1500 mg., Vit B_{12} 10 mg., Biotin 50 mg., Choline Chloride 250000 mg, Pantothenic acid 10000 mg, Nicotinic acid 30000 mg, Folic acid 1000 mg, Manganese 60000 mg, Zinc 50000 mg., Iron 30000 mg, Copper 10000 mg, Iodine 1000 mg, Selenium 100 mg, Cobalt 100 mg, $CaCO_3$ 3000 mg.

‡calculated as: 0.013CP+0.036EE+0.017NSC+0.006NDF according to Villamide *et al.* (2009).

All rabbits were kept under the same managerial, hygienic and environmental conditions in rooms with standard air conditioning and with 20-25°C temperature, 55-65% relative humidity and 12 h lighting. Rabbits were treated with coccidiostats once at the beginning of the experiment (Sulphadimidine Sodium BP solution Dimi-Vet® injection; Square Pharmaceuticals, Dhaka, Bangladesh) at 1 mL/rabbit administered subcutaneously. During the performance trial, rabbits (5 per pen) were housed in galvanised wire cages (40×50×60 cm high×width×length). Body weight and feed intake of rabbits were recorded weekly, while mortality rate was recorded daily. Individual average daily gain was calculated. Feed conversion was calculated as the feed intake (kg) to weight gain (kg) ratio.

At the end of the experiment, 5 rabbits from each experimental group were selected at random (one from each pen) and placed in individual metabolic cages (galvanised wire cages) with free access to fresh drinking water and feed. There was an adaptation period of 7 d followed by a collection period of 7 d during which feed intake was measured and total faecal and urinary outputs from each rabbit were collected (Perez *et al.,* 1995) for the determination of digestibility and N balance. Feeders were filled at 08:00 and 17:00 h daily to allow for *ad libitum* feeding. Feed left out in the feeders and total faecal and urinary outputs were collected daily during the last 7 d of the digestibility/balance study. Metabolic cages were equipped with a system for separate collection of faeces and urine. Faeces were collected from each rabbit in labelled polyethylene bags according to the European reference method for rabbit digestion trials (Perez *et al.,* 1995). Urine was quantitatively collected as described by Kurien *et al.* (2004), and stored at –10°C. Dried samples of feed (hay, foliage or mixed diets), orts and faeces were ground (sieve of 2 mm diameter, Wiley mill, Model 4, Thomas Scientific, Swedesboro, NJ) and analysed for dry matter (DM; 930.15), CP (N; 954.01), crude fibre (CF; 962.09), ether extract (EE; 920.39) and ash (942.05) according to AOAC (1997). Nitrogen concentration in the urine collected from each rabbit was determined using the Kjeldahl method (AOAC, 1997). Neutral detergent fibre (NDF), acid detergent fibre and acid detergent lignin were determined in mixed diets, berseem hay, tree foliage (*A. saligna*, *L. leucocephala* or *M. oleifera*) and faecal samples according to the sequential procedures of Van Soest *et al.* (1991) using an ANKOM^{200} Fibre Analyser unit (ANKOM Technology Corporation, Macedon, NY, USA). Total phenol and tannin concentrations in berseem hay, and in the foliage from *A. saligna*, *L. leucocephala* and *M. oleifera*, were determined according to Makkar (2003).

Calculations of N balance were as in Pellet and Young (1980). Total daily amounts of N ingested (g N/d), excreted in faeces and in urine were used to calculate apparently digested N (N intake–faecal N) and retained N (N intake–[faecal N+urinary N]). Efficiency of protein utilisation was calculated as kg of protein ingested per kg of body weight gain, based on the protein evaluation approaches described by Pellet and Young (1980).

Caecal parameters

To evaluate the effect of dietary treatments on caecal size and fermentation parameters, 10 rabbits per treatment (2 per pen) were randomly selected from the 4 dietary groups on the last day of the experiment. To facilitate the evisceration process and minimise the impact of feed ingestion at different times before slaughter on organ size and caecal fermentation, any feed was withdrawn from troughs 16 h before slaughter, with access to water only (Nizza and Moniello, 2010). Rabbits were euthanised between 07:00 and 08:00 h by cervical dislocation. Thereafter, the caeca were isolated by tying off the 2 extremities with a nylon string to prevent movement of digesta. The weights of each caecum (full and empty) were recorded. The pH of caecal contents was measured using a portable pH-meter (HI98127 pHep®4 pH/Temperature Tester, Hanna® instrument, Italy). Samples of caecal contents were taken for volatile fatty acid (VFA; 1 g in 1 mL 0.5 M H_3PO_4 plus 50 mM crotonic acid) and ammonia (1 g in 1 mL 0.2 N HCl) determinations, and for bacterial counts. Concentrations of acetic, propionic and butyric acids in caecal contents were quantified using crotonic acid as the internal standard using gas chromatography (model 5890, Hewlett Packard, Little Falls, DE, USA) with a capillary column (30 m length ×0.25 mm inner diameter, 1 m phase thickness, Supelco Nukol; Sigma–Aldrich, Mississauga, ON, Canada), and flame ionisation detection. Temperature was adjusted to 100°C for 1 min, and then ramped up by 20°C/min to 140°C, and then to 200°C at 8°C/min, and held at this temperature for 5 min. The injector temperature was 200°C and the detector temperature was 250°C, with He as a carrier gas. Ammonia-N was analysed using an automatic distillation unit. Part of the caecal contents was collected in sterile McCartney bottles and kept in an ice bath for bacterial counts. Caecal bacterial counts were determined using the pour plate technique for total *E. coli*, *Bacteroides* spp., and *Lactobacillus* spp. colony counts (Maturin and Peeler, 1998). Ten-fold dilutions were prepared from each sample in peptone water. Three empty sterile Petri plates were inoculated by transferring 1 mL

from each dilution into the plates. The inoculum was thoroughly mixed with sterile molten plate medium, containing agar Chromogenic Coliform medium for *E. coli*, phenyl ethyl alcohol agar broth for *Bacteroides* spp., or MRS broth for *Lactobacillus* spp., previously held in a water bath at 50°C. The agar plates were allowed to solidify and then incubated at 37°C for 24 h. Bacterial colonies were counted in plates using an optical counter.

Statistical analyses

With the exception of bacterial counts and mortality rates, data collected were analysed by one-way ANOVA using the PROC GLM of SAS (2006). The diet (Control, AS, LL or MO) was the only source of variation (fixed effect). In the intake/performance study, pen (each containing 5 rabbits) within each treatment was the experimental unit, whereas in the digestibility study rabbit within each treatment was the experimental unit. In both cases there were 5 replicates per treatment. When diet effects were significant, the means were compared using Duncan's Multiple Range Test (Duncan, 1955). Bacterial count values were log transformed and then a Poisson regression model was fitted using the PROC GENMOD of SAS, with log of bacterial counts as the response variables, and diet (Control, AS, LL or MO) as the factor explanatory variable, assuming that the random residual variance follows a Poisson distribution. A logistic regression model for binary data (PROC GENMOD of SAS) was used to analyse mortality data. Significance was declared when $P<0.05$ and a trend if $P\leq0.10$.

RESULTS

With very similar organic matter (OM), CP and fat contents, there were some differences among diets in the fibre and lignin contents (Table 2), most likely determined by the differences among berseem hay and the 3 foliage materials in NDF, ADF and lignin (Table 1). Therefore, AS diet had the greatest and MO diet the lowest NDF, ADF and lignin contents. As a result, there were also some differences in the calculated digestible energy concentration of the diets (the range was 7.6% of the Control value).

Feed intake, growth performance and feed efficiency

Feed intake tended ($P=0.066$) to be decreased for rabbits fed on AS compared to Control diets. The final body weights were reduced ($P=0.001$) in AS and LL rabbits compared to MO and Control rabbits (Table 3). Average daily gain was decreased by more than 35% ($P=0.001$) in AS compared to Control, LL and MO rabbits. Mortality was not significantly ($P=0.648$) different among experimental groups (4, 12, 8 and 4% for Control, AS, LL and MO diets, respectively). Feed conversion as feed intake/weight gain was increased ($P=0.002$) in AS and LL treatments compared to MO and Control treatments (Table 3).

Nutrient digestibility and N utilisation

Decreased digestibility coefficients for DM ($P=0.004$), OM ($P=0.028$) CP ($P=0.001$), NDF ($P=0.033$) and ADF ($P=0.011$) were observed in rabbits fed on AS diet; whereas DM and CP digestibilities were increased with MO

Table 3: Feed intake, growth performance, and feed efficiency of rabbits fed the experimental diets*.

	Experimental diets†					
	Control	AS	LL	MO	SEM	*P*-value
Feed intake (g/head d)	100	89	99	103	4	0.066
Initial body weight (g)	821	821	819	816	17	0.743
Final body weight (g)	2121^{c}	1615^{a}	2025^{b}	2176^{c}	23	0.001
Average daily gain, (g/head per day)	22^{b}	13^{a}	20^{b}	23^{b}	1.5	0.001
Feed conversion ratio (kg feed ingested/kg of body weight gain)	4.6^{a}	6.7^{c}	4.9^{b}	4.5^{a}	0.2	0.002

*Five replicates (pens each with 5 rabbits) per treatment.

†Diets contained 70% concentrates mixture+30% berseem hay (Control); 70% concentrates mixture+15% berseem hay+15% *A. saligna* (AS); 70% concentrates mixture+15% berseem hay+15% *L. leucocephala* (LL); 70% concentrates mixture+15% berseem hay+15% *M. oleifera* (MO).

SEM: standard error of mean.

^{abc}Means in the same row with different superscripts differ significantly ($P<0.05$).

Table 4: Digestibility and N utilisation in rabbits fed the experimental diets*.

	Experimental diets†					
	Control	AS	LL	MO	SEM	*P*-value
Digestibility (g digested/kg ingested)						
Dry matter (DM)	598[b]	530[a]	594[b]	618[c]	17	0.004
Organic matter	626[b]	562[a]	622[b]	644[b]	19	0.028
Crude protein	625[b]	562[a]	631[b]	647[c]	16	0.001
Crude fibre	449	365	435	449	17	0.054
Ether extract	806	763	819	823	21	0.072
Nitrogen free extract	660	597	651	676	28	0.084
Neutral detergent fibre	527[b]	468[a]	521[b]	533[b]	29	0.033
Acid detergent fibre	448[b]	394[a]	440[b]	450[b]	21	0.011
Digestible nutrient contents (g/kg feed DM)						
Total digestible nutrients	611[b]	551[a]	615[b]	634[b]	19	0.045
Digestible protein	101[b]	91[a]	105[b]	106[b]	7	0.041
Nitrogen utilisation						
Ingested (NI; g/d)	3.2[b]	2.9[a]	3.2[b]	3.2[b]	0.1	0.016
Digested (ND; g/d)	2.0[b]	1.6[a]	2.0[b]	2.1[b]	0.2	0.009
Retained (NR; g/d)	1.0[b]	0.6[a]	1.0[b]	1.1[b]	0.1	0.011
NR % of NI	32[b]	22[a]	33[b]	35[b]	2	0.027
NR % of ND	51	40	52	54	2	0.052
Efficiency of protein utilisation (kg of protein ingested per kg of body weight gain)	0.75[a]	1.09[c]	0.81[b]	0.75[a]	0.02	0.001

*Five rabbits (replicates) per treatment.

†Diets contained 70% concentrates mixture+30% berseem hay (Control); 70% concentrates mixture+15% berseem hay+15% *A. saligna* (AS); 70% concentrates mixture+15% berseem hay+15% *L. leucocephala* (LL); 70% concentrates mixture+15% berseem hay+15% *M. oleifera* (MO).

SEM: standard error of mean.

[abc]Means in the same row with different superscripts differ significantly ($P< 0.05$).

diet compared to Control (Table 4). Total digestible nutrients (P=0.045) and digestible CP (P=0.041) contents were significantly reduced in the AS diet (Table 4). Nitrogen intake (P=0.016), N digested (P=0.011), N retained (P=0.009) and N retained as a percent of N intake (P=0.027) were decreased in rabbits fed AS diet compared to Control, with

Table 5: Caecal size and fermentation parameters of rabbits fed the experimental diets*.

	Experimental diets†					
	Control	AS	LL	MO	SEM	*P*-value
Caecal weight						
Empty caecum weight as % of body weight	1.46[b]	1.37[a]	1.46[b]	1.49[b]	0.06	0.039
Caecal content weight as % of body weight	4.8	4.1	4.8	5.0	0.2	0.055
Fermentation parameters						
pH	6.0	6.1	6.0	6.0	0.1	0.073
Ammonia-N (mmol/L)	10.1[b]	9.6[a]	12.8[c]	10.4[b]	0.3	0.002
Total volatile fatty acids (VFA; mmol/L)	68	60	66	68	3	0.062
Acetic acid (mmol/mol VFA)	803[bc]	768[a]	793[b]	824[c]	38	0.001
Propionic acid (mmol/mol VFA)	16	7	14	17	4	0.083
Butyric acid (mmol/mol VFA)	94	100	99	94	7	0.944

*Ten rabbits (replicates) per treatment.

†Diets contained 70% concentrates mixture+30% berseem hay (Control); 70% concentrates mixture+15% berseem hay+15% *A. saligna* (AS); 70% concentrates mixture+15% berseem hay+15% *L. leucocephala* (LL); 70% concentrates mixture+15% berseem hay+15% *M. oleifera* (MO).

SEM: standard error of mean.

[abc]Means in the same row with different superscripts differ significantly ($P<0.05$).

Table 6: Caecal bacterial counts (log colony forming units/g; mean and confidence limits) of rabbits fed the experimental diets.

	Experimental diets*				
	Control	AS	LL	MO	*P*-value
E. coli	5.6[b]	5.4[b]	3.9[ab]	3.1[a]	0.023
	(4.12-7.04)	(3.93-6.81)	(2.66-5.10)	(2.03-4.23)	
Bacteroides spp.	5.0	4.9	4.5	4.2	0.854
	(3.56-6.34)	(3.54-6.28)	(3.22-5.86)	(2.95-5.49)	
Lactobacillus spp.	3.2[b]	3.3[b]	2.2[ab]	1.6[a]	0.034
	(2.11-4.33)	(2.18-4.43)	(1.29-3.13)	(0.78-2.32)	

*Diets contained 70% concentrates mixture+30% berseem hay (Control); 70% concentrates mixture+15% berseem hay+15% *A. saligna* (AS); 70% concentrates mixture+15% berseem hay+15% *L. leucocephala* (LL); 70% concentrates mixture+15% berseem hay+15% *M. oleifera* (MO).

[ab]Means in the same row with different superscripts differ significantly ($P<0.05$).

no significant differences between control and the other treatments. N retained as a percentage of N digested tended ($P=0.052$) to be decreased in AS compared to Control rabbits (Table 4).

Caecal weight and fermentation

Empty caecum weight as a percentage of body weight was reduced ($P=0.039$) in AS rabbits compared to Control, without significant differences between other treatments. Caecal content weight as a percent of body weight tended ($P=0.055$) to follow the same trend of empty content weight (Table 5). Caecal pH values tended ($P=0.073$) to be higher for AS compared to the other treatments, whereas increased ($P=0.002$) caecal ammonia-N concentration was observed with LL compared to other diets. Increased acetic acid concentration ($P=0.001$) was observed in the caecal contents of MO rabbits, whereas total volatile fatty acids ($P=0.062$) and propionic acid ($P=0.083$) concentrations tended to be reduced in the caeca of rabbits fed the AS diet compared to Control (Table 5). Decreased bacterial counts of *E. coli* ($P=0.023$) and *Lactobacillus* spp. ($P=0.034$) were observed with MO diet compared to Control and AS diets, without significant differences ($P>0.05$) among treatments in the counts of *Bacteroides* spp. (Table 6).

DISCUSSION

Feed intake and nutrient digestibility

Berseem hay was used as the forage component of the control diet because it is often considered a reference forage in rabbit feeding, with a nutritive value similar to that of lucerne hay. Berseem hay can be introduced at levels up to 45% in experimental diets for growing rabbits (Heuzé *et al.,* 2015). In the current study, including tree foliage at a level of 15% of DM did not affect DM intake. However, feed intake in rabbits fed *A. saligna* tended to be reduced by about 11% compared to Control. El-Gendy (1999) showed that feeding rabbits on diets containing different levels (up to a dietary inclusion of 30%) of *A. saligna* leaf meal from 5 to 13 wk of age did not affect feed intake. The low preference of *A. saligna* by rabbits was probably due to the higher concentration of tannins. Tannins may cause adverse effects reducing palatability, digestibility, nutrient availability and weight gain (Butler *et al.,* 1986) through their ability to bind with proteins and other materials, resulting in decreased intake (Al-Mamary *et al.,* 2001). Tannins and phenolic compounds are responsible for an astringent taste of feed that induces lower intake due to reduced palatability (Butler *et al.,* 1986). In this regard, Mashamaite *et al.* (2009) reported that 4% is the acceptable level of tannins in rabbit feeds without negative effects on intake and digestibility. Kamel and Brekaa (2005) reported that 60% of *A. saligna* in a diet reduced feed intake significantly and affected the feed conversion ratio negatively.

Digestibilities of DM, OM, NDF, ADF and CP were decreased with the AS diet compared with the other 3 diets, which might be due to the higher fibre, lignin and condensed tannin contents in the AS foliage in comparison with berseem hay and foliage from the other tree species. Organic matter and protein digestibility coefficients observed in the current study seem to be somehow lower than values reported in the literature. An apparent faecal digestibility of

amino acid N from conventional feeds would range from 0.64 to 0.80 (Villamide *et al.,* 2010), whereas the diets used in the current study had protein digestibilities ranging from 0.56 to 0.65. The main factors affecting protein digestion in rabbits are the chemical structure and properties (solubility) of proteins and the accessibility to enzyme activity (Villamide *et al.,* 2010). Therefore, protein digestibility is highly determined by the type of feed ingredient or source of dietary protein (Villamide *et al.,* 2010), and Lowe (2010) reported average coefficients of apparent protein digestibility of 0.80, 0.67, 0.55 for protein concentrates, cereals and forages, respectively. The fibrous ingredients (berseem hay and tree foliage) used in the current study have not been tested previously, and considering the high level of inclusion of these feedstuffs (up to 30%) it can be assumed that the apparent faecal protein digestibility coefficient are within the range of values that can be observed in rabbits. A wide range of fibre digestibility coefficients can be found in the literature, and the values observed in the current study are above the average NDF digestibility of 0.34, but within the range 0.03-0.71 reported by Gidenne *et al.* (2010a) in a review of numerous publications on fibre digestibility of different diets in rabbits. Lebas (1989) stated that although fibre digestion could be low in rabbits, digestibility coefficients of up to 0.55-0.70 have been reported. Fibre digestibility will depend on the fibre content and lignification of feed. Control, MO and LL diets contained less insoluble fibre and lignin and were more digestible than the AS diet. Djakalia *et al.* (2011) observed that including *M. oleifera* in the diet of rabbits at 3% in the basal feed increased protein and fibre digestibility. El-Gendy (1999) observed unaffected feed digestibility and N balance with feeding diets with up to 30% *A. saligna* leaf meal to rabbits. The adverse effect of *A. saligna* on digestibility could be ascribed to the inhibitory effect of tannin on microbial activity (Salem, 2005). High tannin contents with AS reduced N intake and N digestion by about 19% and reduced N retention by 37% compared to Control rabbits. Tannin structure, source, type (hydrolysable or condensed) and its level and activity in browse determine the variable effects on feed digestibility (Mueller-Harvey, 2006; Patra *et al.,* 2011). *A. saligna* contained greater total phenols (125.8 *vs.* 105.6 and 94.4 g/kg DM, respectively) and condensed tannins (78.3 *vs.* 29.6 and 17.5 g/kg DM, respectively) concentrations compared to *L. leucocephala* and *M. oleifera.* Anti-nutritional factors at high concentrations can inhibit enzymes and directly form complexes with nutrients, rendering them indigestible by proteolytic enzymes (Abeke *et al.,* 2003).

As a result, decreased feed digestibility and N utilisation were observed when rabbits were fed AS foliage. Conversely, the supplementation of rabbit diets with LL or MO foliage did not affect the digestion of commercial diets for growing rabbits compared to berseem hay.

Growth performance

Daily weight gain ranged between 13.2 to 22.7 g/d, less than the values reported for NZW rabbits in other countries. This may be related to many factors including genetics, environmental and husbandry conditions, feeding regime, housing, density and management, among others. The growth rates observed with the diets used in the current study are comparable to those reported by Kabir *et al.* (2014) in Nigeria and AbuHafsa *et al.* (2014) in Egypt, and in other studies using berseem hay in diets for growing rabbits (Heuzé *et al.,* 2015).

Daily weight gain was decreased by about 39% when rabbits were fed on AS compared to Control diet. Yousef (2005) concluded that up to 40% acacia leaves could be used in the diet of rabbits without adversely affecting their reproductive performance. The reduced feed intake and digestibility may be responsible for the decreased daily weight gain. Average daily gain in rabbits was similar in Control, MO and LL rabbits. Abubakar *et al.* (2011) reported that weaned rabbits can utilise varying levels of *M. oleifera* leaf meal at up to 45% level in diets without adverse effects on growth performance, carcass yield, organ and gut characteristics. Adedeji *et al.* (2013) reported that the inclusion of increasing levels of *L. leucocephala* leaf meal (from 5 to 15%) improved rabbit performance and daily weight gain compared with an unsupplemented control diet, although feed intake was decreased. Growth rates and feed conversion were similar in MO and Control rabbits. Rabbits fed on AS and LL showed worse feed conversion ratios than Control and MO animals. Improved efficiency of protein utilisation in *M. oleifera* fed rabbits might be due to the high protein and low fibre and lignin contents in the foliage of this plant species. Odetola *et al.* (2012) reported that *M. oleifera* leaves possess good dietary protein quality for optimal growth of rabbits. *L. leucocephala* contains considerable amounts of mimosine (a toxic, non-protein amino acid in *L. leucocephala*) which could cause growth depression and increased mortality (Cheeke and Shull, 1985). Sugur *et al.* (2001) reported that 20-25% *L. leucocephala* leaves inclusion in the rabbit's diet may have deleterious effect on mortality. Regardless of the differences observed among groups in mortality, they did not reach statistical significance.

Caecal fermentation

Fermentation in the caecum of rabbits depends on continuous feed intake ensuring the flow of digesta to the hindgut. Although digesta can be retained in the caecum for a longer time than in the small intestine, after a fasting period (feed was withheld for 16 h before slaughter) the fermentative activity in the hindgut may decrease considerably, and the counts of some microbial communities (Lactobacilli) may be substantially reduced (Mountzouris *et al.,* 2009). Nevertheless, as the feed withdrawal time was the same for all the rabbits, this decline is not expected to affect the comparison among experimental treatments.

The caecum size and caecal fermentation parameters were within the ranges of values reported in a meta-analysis by García *et al.* (2002). There were differences among diets in caecal size, ammonia-N and the molar proportion of acetic acid, although these variations were not clearly related to the chemical composition of diets, in agreement with the results reported by García *et al.* (2002). The VFA produced by fermentation and absorbed in the hindgut constitute an important source of energy for the rabbit, providing up to 30 to 40% of the energy required for maintenance (Marty and Vernay, 1984). Concentrations of total VFA and the molar proportions of acetic, propionic and butyric acids are mainly affected by the NDF and lignin content of the diet (García *et al.,* 2002). The proportion of acetate increases and that of butyrate decreases when the dietary fibre is increased, whereas the proportion of propionic acid is correlated with the concentration of uronic acids in feed (García *et al.,* 2002; Gidenne *et al.,* 2010b).

Concentrations of ammonia-N varied among shrub species, and remained above the range (4.5-6 mmol/L) observed in rabbits fed a balanced diet and considered adequate for microbial protein synthesis (Villamide *et al.,* 2010). The metabolism of nitrogenous compounds in the rabbit hindgut is determined by the flow of nitrogen (undigested dietary N or N of endogenous origin) from the small intestine (Villamide *et al.,* 2010). Although within a narrow range, the caecal ammonia concentration was inversely related to the dietary digestible energy to digestible CP ratio (Villamide *et al.,* 2010).

Decreased total *E. coli* (by about 30 and 44%, respectively) and *Lactobacillus* spp. (by about 31 and 51%, respectively) counts were observed with MO diet compared to Control. However, microbial counts were not affected in the AS and LL diets compared to Control. Djakalia *et al.* (2011) reported that *M. oleifera* may have some antimicrobial activity and could be used to control pathogenic bacteria. *E. coli* are normal inhabitants of the intestinal tract of many animal species including rabbits, and can cause digestive disturbances that are often responsible for high morbidity and mortality of young rabbits after weaning, and consequently for important economic losses in rabbit farms (Licois, 2004).

To minimise the costs of rabbit production in hot climate countries, such as Egypt, low-priced alternative sources can partially replace berseem hay in diets of growing rabbits without deleterious effects and with better prospects of utilisation. *M. oleifera* and *Leucaena leucocephala* foliage can be considered as alternative sources of non-conventional feed, and their inclusion up to 15% in the diet resulted in growth performance, feed conversion efficiency, nutrient digestion, nitrogen utilisation and caecal fermentation similar to those achieved with more conventional protein-rich forages, such as berseem hay. Foliage from *M. oleifera* had a better potential as a feed for rabbits than that from *L. leucocephala.* Although foliage from *A. saliga* may also be used at 150 g/kg in the diets of growing rabbits; this level of inclusion may result in reduced feed digestibility and growth performance.

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