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Voluntary Intake and Efficient Use of Metabolizable Energy in Sheep Fed Arboreal Forage

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ABSTRACT

Background: Animal production demands knowledge about energy needs and metabolism to enhance yields. **Aim.** To determine the effect of a totally mixed ration (TMR) containing different volumes of tree foliage (*Tithonia diversifolia*, *Morus alba*, *Leucaena leucocephala*) as an alternative to hybrid *Cenchrus purpureus* Cuba OM-22, for voluntary intake of dry matter and the efficiency of metabolizable energy use in sheep. **Methods:** A total of 24 whole weaned Pelibuey sheep (84±9.75 days of age), with an average live weight of 15.80±2.740 kg (7.92±1.00 kg $PV^{0.75}$), were divided into six groups, using a completely randomized design (CRD), for 120 days. The treatments consisted of several levels (20.40 %) of foliage from trees (Tithonia diversifolia, Morus alba, Leucaena leucocephala) with 80.60% hybrid Cenchrus purpureus Cuba OM-22, included in the experimental plant mixture of 50, 55, 65, and 60 days of age, respectively. An analysis of variance (ANOVA) was performed and the Duncan test was conducted to determine the differences of means. **Results:** The absolute dry matter intake was higher (1.31 vs 1.37 kg $d^{-1,}$ p< 0.0001), when 40% of white mulberry and Leucaena foliage was included in the diet, respectively. Though 40% of white mulberry represented a higher value than the protein: energy ratio (71 g $Mcal^{-1}$, p<0.0001). **Conclusions**: The inclusion of 40% white mulberry and Leucaena with 60% *Cenchrus purpureus* improved voluntary consumption. The efficiency in the utilization of metabolizable energy ingested for maintenance (km) was high, though it was low for growth (kg).

Key words: energy efficiency, energy metabolism, ovine nutrition, blood parameters (*Source*: *AGROVOC*)

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INTRODUCTION

The poor quality of tropical pastures limits their availability in terms of their high fiber and low protein levels (Pierrugues and Viera, 2021). However, the formulation of diets containing arboreal forage species in areas where gramineous availability is scarce, constitutes a major alternative for biomass production, along with the quality of its nutrients, high acceptance, and the possibility of meeting all the ruminant nutritional requirements of s (Núñez-Torres and Rodríguez-Barros, 2019)

Moreover, enhancing animal productivity demands knowledge about their nutritional needs, the efficiency of available feed resources, and energy metabolism, as the main nutrient shortcoming in animal productive performance in tropical conditions (Galvez-Luis *et al.*, 2020). In that sense, Piñeiro-Vázquez *et al.*, (2013) said that energy intake below the maintenance needs of adults may induce the mobilization of body fat reserves, which might lead to weight loss, and metabolic alterations associated with the activation of glycogenesis.

Likewise, the metabolicity (qm) of crude energy is the basis to determine the efficiency of metabolizable energy (ME) use, **(**AFRC, 1993). Besides, the amount of energy retained resulting from increases in the energy consumed, is quantified through k (efficiency) values (Magofke *et al.,* 2000), where the efficiency of metabolizable energy for maintenance is named km. When ME intake is greater than the ME needed for maintenance, k represents the efficiency retained in the ME of the ration for production (kg: weight increase or growth-fattening, kl: lactation, and kc: gestation).

Currently, there is little information available about the utilization of forage trees and shrubs as a considerable part of the diet, and their relation to involuntary ingestion of dry matter, particularly metabolizable energy, and its utilization in sheep efficiently.

Accordingly, this paper aimed to determine the effect of a totally mixed ration (TMR) containing different volumes of arboreal foliage (*Tithonia diversifolia, Morus alba, Leucaena leucocephala*) as an alternative to hybrid *Cenchrus purpureus* Cuba OM-22, for voluntary dry matter intake and the efficiency of metabolizable energy use in sheep.

MATERIALS AND METHODS

Location

The study was conducted at Villena Revolucion Forage and Pasture Station in Havana, at the Institute of Pastures and Forages (IIPF), the Ministry of Agriculture (MINAGRI).

Animals and design

A total of 24 whole weaned Pelibuey sheep (84±9.75 days of age) were included in the research, with an average live weight of 15.80±2.740 kg (7.92±1.00 kg $LW^{0.75}$), and distributed in six groups in pens, using a completely randomized design (CRD) for 120 days, with 20 days for adjusting to the experimental diet, and data collection.

Treatments

The treatments consisted of the inclusion of several levels (20.40 %) of foliage from trees (Tithonia diversifolia, Morus alba, Leucaena leucocephala) with 80.60% hybrid Cenchrus purpureus Cuba OM-22, in a completely mixed ration. The forage from *T. diversifolia*, *M. alba*, *L. leucocephala,* and *C. purpureus* were harvested manually from the biomass bank, and with 2 years of establishment (aged 50, 55, 65, and 60, respectively). The species were cultivated under dryland conditions in the absence of fertilizers. The TMRs with different forage types and proportions, depending on the treatments, were made at the beginning of the day (08.00 am), and supplied on one occasion (08:30), at a rate of 12% live weight, humid base, plus 15%, to ensure enough rejected material for later. It was removed from the trough twice (11:30 am and 16:30 pm) during the day; the animals could access water and mineral salts freely. Tables 1 and 2 show the chemical composition of forages and TMRs supplied to the sheep at different inclusion levels.

Table 1. Chemical composition of forages

Forages	DM %	CP, % (Nx6.25)	ME (Mcal) kg DM^{-1}	NDF, %	ADF, %	C, %	DMD , %	OMD, %	OMDDM, %
C. purpureus	90.3	10.0	2.28	74.5	31.9	9.9	64.0	66.2	59.6
T. diversifolia	91.4	19.2	2.45	47.8	42.2	18.4	73.5	76.2	68.6
M.alba	89.2	25.9	2.23	32.7	24.6	9.5	69.6	72.0	64.8
L.leucocephala	90.5	24.0	2.30	50.8	26.4	9.3	68.0	70.4	63.3

DM: dry matter; CP: crude protein; ME: metabolizable energy; NDF: neutral detergent fiber; ADF: acid detergent fiber; C: ashes; DMD: dry matter digestibility; OMD: organic matter digestibility; OMDDM: organic matter digestible in dry matter

Table 2. Inclusion levels and chemical composition of the totally mixed rations (TMR)

Forages	Arboreal inclusion level, HB%					
	20T: 80Cp	40T: 60Cp	20M: 80Cp	40M: 60Cp	20L: 80Cp	40L: 60Cp
C. purpureus	80	60	80	60	80	60
T. diversifolia	20	40	-	-	-	-
M.alba	-	-	20	40	-	-
L. leucocephala	-	-	-	-	20	40
	100	100	100	100	100	100
Chemical composition						
DM (%)	90.5	90.7	90.1	89.9	90.3	90.4
CP (Nx6.25,%	11.9	13.7	13.1	16.3	12.8	15.6
ME, Mcal $kgDM^{-1}$	2.32	2.36	2.29	2.30	2.29	2.30
OM, %	78.9	77.4	80.2	80.1	80.5	80.7
NDF, %	69.1	63.7	64.6	54.7	69.8	65.0

ADF, %	29.5	27.0	30.5	29.1	30.9	29.9
BE, kcal kg DM $^{-1}$	3.82	3.79	3.91	3.97	3.92	3.98

HB: humid base, inclusion levels with 20, 40 T: *T.diversifolia*; 20,40 M*: Morus alba*;
20, 40% *L. leucocephala,* Cp; C. purpureus hybrid Cuba OM-22

The voluntary intake of dry matter was calculated every four days in a row every month, and by estimating the weight difference between dry matter supplied and rejected per pen. A 5 kg±50 g dynamometer (SANSOM) was used, and the individual and average live weight variations were measured at the beginning and the end of the month, using a 50 kg ± 0.460 kg scale (HANSON-SMBUTA), when the TMR intake was adjusted. The pen area per treatment was 3.60 m^2, total experimental (21.6 m^2), with 0.72 m^2 total trough area, which ensured 0.30 m long trough facing the animals, drinking water containers (15 L buckets) for instant consumption, and salt trays (0.016 m^3).

Chemical analysis

The samples from the original foliage, the feeds, and the material rejected were dried in a forced air circulation stove at 60º C for 48, to reach constant weight for dry matter determinations. Then the material was milled (1 mm) and sent to the laboratory for chemical analysis, according to AOAC (2005), and fiber fractioning by Goering and Van Soest (1994). For dry matter digestibility estimations (DMD), and organic matter digestibility (OMD), the equations suggested by Minson (1982) and CSIRO (1990) were used. The metabolizable energy was determined from the digested organic matter (MODMS), according to AFRC (1995).

Likewise, the behavior of some physical variables of forages was included in the TMR, as described by Savón *et al.,* (2004). All the analyses were made at the Central Laboratory Unit (UCELAB), the Animal Science Institute (ICA). The metabolicity was estimated by the coefficient between the concentration of metabolizable energy and the contribution of crude energy to the rations (AFRC, 1995), according to the equation:

$$\mathbf{qm=\frac{ME}{CE}}$$

Where:

qm = the ration metabolicity

ME = metabolizable energy

CE = crude energy

Likewise, the efficiency in the utilization of metabolizable energy for maintenance (km), and growth (kg) were calculated using the following equations:

- *km*=0.350 x qm+0.503
- *kg*= 0.780 x qm+0.006

Statistical analysis

An analysis of variance (ANOVA) was performed and the Duncan test (1955) was conducted to determine the differences in means. Additionally, several Pearson correlations were performed (concentration of metabolizable energy in the ration: efficiency in the utilization of energy for maintenance and growth), and linear regressions (protein consumption of neutral detergent fiber, protein concentration in the diet; consumed metabolizable energy-protein ratio) in the variables studied, stating the statistical parameters and fit criteria of the equation, and the model (R^2, ±SE, p), respectively. All the data were processed using INFOSTAT (Di Rienzo *et al*., 2016).

RESULTS AND DISCUSSION

Table 3 shows the results of voluntary intake of the ration, and the ratio between different nutrients. Concerning the absolute dry matter intake (kg d^{-1}) and relative dry matter intake (% LW, g kg LW ^{075}) on a dry base, the treatments with the 40% inclusion of white mulberry and Leucaena in TMR differed from the rest, with the highest values for dry matter consumption and protein, in terms of live weight (LW), metabolizable energy consumption, and organic matter intake. Lower quantities may have influenced the productive response of the other treatments.

Table 3. Voluntary intake by sheep fed basic rations, with the growing inclusion of tree foliage.

Indicator	Arboreal inclusion, %HB					
	20T	40T	20M	40M	20L	40L
DMC, kg d^{-1}	1.23^{d}	0.95^{d}	0.98^{b}	1.31^{e}	0.99^{c}	1.37^{f}
DMC, %LW	4.4^{ab}	3.5^{a}	3.7^{a}	5.0^{b}	4.5^{ab}	5.2^{b}
DMC, g kgLW$^{0.75}$	146^{ab}	132^{a}	129^{a}	162^{b}	131^{a}	189^{c}
OMC, kg	0.97^{d}	0.73^{a}	0.78^{b}	1.05^{e}	0.80^{c}	1.11^{f}
OMC, g kgLW$^{0.75}$	115^{ab}	102^{a}	103^{a}	130^{b}	105^{a}	153^{c}
CPC, g d^{-1}	146^{d}	130^{c}	128^{b}	214^{e}	127^{a}	214^{f}
CPC kg LW^{-1}, g kg $^{-1}$	8.5^{a}	9.4^{a}	8.6^{a}	13.2^{c}	8.6^{a}	15.3^{d}
MEC, Mcal d^{-1}	2.86^{c}	2.24^{b}	2.24^{b}	3.01^{cd}	2.27^{b}	3.16^{d}
NDFC, g	0.85^{e}	0.60^{a}	0.63^{b}	0.72^{d}	0.69^{c}	0.89^{f}
NDFC, LW%	4.97^{a}	4.38^{a}	4.27^{a}	4.43^{a}	4.6^{a}	6.38^{b}
CPC: MEC, g $Mcal^{-1}$	51.1^{a}	58.0^{d}	57.4^{c}	70.9^{f}	55.9^{b}	67.8^{e}
Conc. ME Rat. Mcal kg DM^{-1}	2.32^{c}	2.36^{d}	2.29^{a}	2.30^{b}	2.29^{a}	2.30^{b}
Conc. CP rat. Mcal kg DM^{-1}	0.12^{a}	0.14^{c}	0.13^{b}	0.16^{d}	0.13^{b}	0.16^{d}
Cons. ME/req. ME %	73.11^{a}	67.62^{a}	61.88^{a}	75.17^{a}	64.96^{a}	99.57^{b}
DM supply selection indexes	1.1^{ab}	1.7^{bc}	1.2^{ab}	1.0^{a}	1.5^{abc}	2.0^{c}

CP supply selection indexes	1.1[a]	1.8[b]	1.1[a]	1.0[a]	1.4[ab]	1.9[b]

HB: humid base; *T.diversifolia*; M: *Morus alba*; L: *L. leucocephala*; DMC: dry matter consumption; CPC: crude protein consumption; MEC: metabolizable energy consumption; NDFC: neutral detergent fiber consumption; Conc. ME: Metabolizable energy concentration; Conc. CP: crude protein concentration; Req: Requirements according to NRC (2006).

Overall, the mean value (148.58±24.14 *vs* 118.45±20.08 g kg PV$^{0.75}$) of dry and organic matter intakes observed in this study, which were fit to the metabolic weight, showed higher values than the values (91.19 *vs* 80.84 g kg PV$^{0.75}$) reported by Rodríguez (2018), in studies where he included 16.5, 33.5, and 66,5 % moringa in whole sheep diets (Pelibuey), and the findings of Aguirre *et al.*, (2019) Sugarcane(89 *vs* 80 g kg PV $^{0.75}$) when fattening whole creole sheep fed fermented coffee pulp (30%) in an energy-protein supplement, along with sugarcane (the meal from leaf-free stems), maize, soybean, alfalfa meal, and mineral salts, supplied at a rate of 11 g kg LW^{-1} (dry base), and the findings of (82 *vs* 68 g kg PV $^{0.75}$) Parra (2022), when he supplied a diet made of air-dried kikuyo grass plus maize silage *ad libitum* to Pelibuey male sheep.

These results may have been influenced by the high level of selection of the feed supplied in TMR, in terms of dry matter consumption and protein. It appeared that the treatment with 40% white mulberry matched the unit (IS=1), which meant that the nutritional value and feed efficiency expected were similar, with high palatability, and maximization of fraction consumption.

The treatment with 40% white mulberry evidenced that the consumption of dry matter and the concentration of protein of TMR met the maintenance requirements and that some protein was allotted for sheep production, which could also show the synchronization that must have taken place between the protein sources and the carbon hydrates available to improve ruminal fermentation (Espinoza and Bionel, 2018; Galindo-Blanco et al., 2018), despite the fact that the metabolizable energy balance of the treatment met the energy demand in 75.17±8.45%, the NRC (2006) requirements.±8.45%.

The effect of energy unbalance should have been corrected by the ingestion of protein, and the formation of free amino acids from fermentation deamination in the rumen, which produces volatile fatty acids with a ramified chain that may have been used as a source of energy, and as growth factors for the bacteria in the rumen, thus improving protein synthesis (Sobrevilla, 2018), which along with low degradation of arboreal protein in the rumen, raised the protein flow (glycogenic amino acids) into the small intestine. Though the protein availability in this site depends on the protein proportion of the diet and the ingestion level (Arteaga, 2018), as must have occurred in this study, with 40% white mulberry in the feed, reaching a high protein concentration (25 g nitrogen kg DM^{-1}, releasing large amounts of soluble protein into the rumen thanks to the action of ruminal microorganisms (Chacón, 2018, and Núñez-Torres and Rodríguez-Barros, 2019).

Moreover, the results from the linear regression (a= -11.89 ±1.82 b_1=516.53± 11.59, R^2=98, ±SE=3.36, p<0.0001) showed that the protein concentration of the ration accounted for 98% of the variations observed in the CP: ME in the diet, an indicator used to evaluate the efficiency of feed utilization and to characterize the rate of weight gain in ruminants (Mayer *et al.*, 2018). However, the mean values found in this study were above (67.07±8.38 g $Mcal^{-1}$) the minimum requirements (22.99 g Mcal $^{-1}$), according to NRC (2006). The possible explanation for these findings must be associated with the high solubility of the diet protein in the rumen, the combination of the two nutrients, and their synchronization (Arteaga, 2018).

Furthermore, the high voluntary intake of dry matter observed was influenced (r= 0.84) by the elevated ingestion of NDF (Martínez *et al.*, 2022). It constituted 80.99±6.50% (68.29-87.62%) of ingested organic matter, where only 19.00±6.50% were soluble cellular constituents, a substrate available that could have been used as a source of energy during the digestion (Gutiérrez *et al.*, (2020a, 2020b). Additionally, the results of the positive linear regression model (a= 0.15 ±0.16, b_1=1.35± 0.21, R^2=72, ±EE=0.01, p< 0.0001), between dry matter and NDF consumption evidenced that from 1.35g of NDF consumed on, dry matter intake would be lower, a static moment that will depend on the feed's capacity to take space (physical filling effect). Then the emptying rate will diminish in the rumen, together with a reduction of digested feed replacement.

Likewise, Gutiérrez *et al.* (2018) reported that the availability of organic matter per metabolic weight unit is closely related to the digestibility of organic matter, as it simultaneously influences dry matter intake. In this study, this value was greater with the inclusion of 40% white mulberry in TMR. Rodríguez *et al.* (2019), stressed the significance of organic matter contribution to the feed, as well as the degradability and fermentation of the nitrogen in the rumen, when the protein available is accompanied by enough amounts of metabolizable energy to ensure the synchrony of the two sources.

Additionally, the low energy concentration (2.32 ±0.03, 2.29-2.36 variation Mcal kg DM^{-1}) commonly observed in the rations consumed, and the high consumption of dry matter (4.20±0.77, 2.70-5.68 % variation of LW) consumed by the sheep in terms of live weight was short-term regulated by physical, rather than metabolic factors (Aragadvay, 2020; Lorda and Pordomingo, 2020; Pérez Martell, 2021). It resulted from the capacity and volume of the rumen-reticulum, and the speed of feed digestion and absorption, along with the filling effect caused by the diet, rather than a chemical effect associated with the energy available in the ration consumed (Cantaro Segura, 2018). Meanwhile, the chemical or metabolic effect, according to Cangiano and Cangiano (1997) is characterized by the high concentration of energy (2.70-3.75 Mcal kg DM^{-1}) in the ration, and animals can ingest their feeds until they meet their requirements.

Besides, physical factors, such as DM solubility, liquid retention by the fiber material, the density of each forage plant, and their proportion in the feed, could be thought of as influencing nutrient absorption and the feed's digestion rate (Hoover and Stokes, 1991). In terms of solubility, the forage trees (white mulberry and tithonia) produced the highest values, though similar (33-35%)

to the findings of Verité and Demarquilly (1978) in tropical pastures, and with less liquid absorption capacity and density. It entails that both forage types must have shorter rumen retention time, and make the best use of the nutrients in the rumen, raising DM ingestion (Table 4).

Table 4. Behavior of some physical variables of forages included in the TMR

Variable	*Tithonia diversifolia*	*Morus alba*	*Leucaena leucocephala*	*Cenchrus purpureus*
DM solubility %	36.39	37.75	26.00	23.50
±SD	5.78	3.25	3.50	2.50
Water absorption capacity g/g	7.49	9.06	9.83	9.20
±SD	0.30	1.89	0.38	0.27
Density g/cm^3	0.15	0.22	0.28	0.18
±SD	0.01	0.05	0.11	0.02

SD: Standard deviation

Based on the previous, the high solubility of white mulberry may have been influenced by the degradation and fermentability of fibrous fractions, with their high volatile fatty acid concentration in the rumen (VFA, mostly acetate), which are absorbed by the rumen wall, and are used as a source of energy by the intestinal microbiota, having a synergistic effect with protein degradation (Della Rosa, 2018).

Furthermore, the regression response showed that the inclusion of 40% white mulberry in TMR produced consumption of 1.88 kg (a= 0.34 (±0.17), b_1=1.88 (±0.54), R^2=78, ±SE=0.02, p=0.0470) of the plthe ant by plant unit consumed, and 2.31 kg DM of white mulberry (a=0.45 (±0.19), b_1=2.31(±0.64), R^2=68, ±SE=0.02, p= 0.229l), by every kg of total dry matter ingested. Hence, an extrapolation of the regression line to the intersection with 0 (*inter-intercept coefficient: depending on the equation*), the demand of white mulberry in the TMR to ensure maintenance was 0.195 kg DM, accounting for 15% of the dry matter consumed. These results showed the additive effect of white mulberry forage in the TMR consumed by the sheep.

In this paper, the value of metabolicity (*qm*= 0.58 ± 0.04) of crude energy showed a high quantity of heat caused by fermentation and the activity in the rumen (AFRC, 1993). Likewise, these findings explain that, according to the numerical value, the diets used were similar to high-quality forages, when *qm* = > 0.50 (Lee, 2018), since it showed greater digestibility (Vázquez-Carrillo *et al.,* 2021) de la ration.

Additionally, the km value (0.71±0.01) was higher (0.66) than the values reported by Chay-Canul *et al*. (2016) for short-haired ovine breeds, when the forage in the diet was above 70%, as in that paper, where the ration was exclusively made of forages, though the authors noted that regardless of the diet and breed used, the average value was *km*=0.60.

However, the ME value achieved per kg was low (0.46±0.03), though the Pelibuey sheep are within the 0.38-0.48 range, according to Duarte *et al.*, (2012), which, according to the author, varies with age and weight, the younger the greater, due to the large deposition of fat in the body tissue. What is more, the km and kg values (r= 0.87 *vs* 0.95) were observed to be associated with higher energy concentration in the ration. Accordingly, in similar conditions, it is important to increase the energy values in the diet to meet the animals' requirements.

Actually, according to Roque *et al.*, (2020), the km and kg values achieved in this study were within the efficiency range of ME for the maintenance and growth of ruminant species.

CONCLUSIONS

The inclusion of 40% white mulberry and Leucaena with 60% *Cenchrus purpureus* (Cuban hybrid OM-22) in the mixed ration, improved dry matter intake. The metabolicity (qm) of crude energy and the efficiency in the utilization of metabolizable energy ingested for maintenance was high, though it was low for growth.

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Research conception and design: DGG, ARB; data analysis and interpretation: DGG, ARB; redaction of the manuscript: DGG, ARB.

CONFLICT OF INTEREST STATEMENT

The authors declare the existence of no conflicts of interests.