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The efficiency of rumen microbial nitrogen and biomass synthesis of some indigenous range plants using ^{15}N-tracer technique

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ABSTRACT. This study was performed to evaluate, by the use of *in vitro* incubation technique with ruminal liquid and ^{15}N-tracer for 96 h, some perennial range plants (*Artemisia herba-alba* Asso, *Noaea mucronata* Forssk, *Lavandula angustifolia* Mill, *Astragalus spinosus* Forssk, *Capparis spinosa* L.) grown naturally on dry rangelands, in terms of rumen microbial nitrogen (M.N), microbial biomass (M.BM), true fermented organic matter (T.F.OM) and quantification of the efficiency of M.N and M.BM synthesis (M.N or M.BM / T.F.OM), and study the effect of polyethylene glycol (P.E.G) on the aforementioned parameters. *C. spinosa* had the highest ($p < 0.05$) values of T.F.OM, M.N and M.BM. Microbial N and M.BM values ranged from 0.57 to 0.82 mg g^{-1} DM and from 6.13 to 9.46 mg g^{-1} DM, respectively. There were no significant ($p > 0.05$) differences among plant species in terms of the efficiency of M.N and M.BM synthesis, and the average amounted to 0.282 g and 3.25 g 100 g^{-1} of truly fermented organic matter, respectively. M.BM and M.N values were negatively correlated with lignin but positively correlated with soluble nitrogen. P.E.G supplementation and the interaction between P.E.G treatment and plant species had no significant ($p > 0.05$) effect on the estimated parameters.

Keywords: microbial protein; nutrient; range plant; ruminant.

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Introduction

In arid and semi-arid regions, small ruminants experience from under feeding and malnutrition due to the shortage of feeds in most of the year, where drought and dry seasons exist. The most of the grazing animals in dry rangelands fed entirely on annual herbaceous plants that have shorting growing periods and lower yields and amounts of protein. Perennial shrub species are important sources of feeds for small ruminants in these regions during the dry seasons and provide vegetation with better nutritive value (Aregawi, Melaku, & Nigatu, 2008). These shrubs are tolerant to the rough environmental conditions and have possibility for assuaging some of the feed lacks and deficiencies assayed (Kamalak, Canbolat, Gurbuz, Ozay, & Ozkose, 2005; Hassen, Tessema, & Tolera, 2017). However, indigenous range plants grown in dry rangelands could be provide complementary feed sources and protection against wind erosion and have potential to provide sustainable feed production in degraded steppe areas. Leaves of trees and shrubs provide the required 'by pass' protein and improve the productivity of livestock (Preston, Leng, & Gomez, 2021).

The main source of protein and amino acids for ruminants comes from the microbial protein (M.P) synthesis in the rumen. Thus, the study of M.P synthesis and the efficiency of M.P synthesis are very necessarily for ruminants. Using ^{15}N-tracer technique for measuring microbial nitrogen *in vitro* enables to choice the forages dependence on the efficiency of microbial protein production synthesis. Several factors could be improved the M.P synthesis in the rumen such as increasing the level of digestible organic matter intake, lower rate of ruminal protein degradation, feeding a mixture of forage and concentrate instate of feeding only concentrate or forage, suitable nitrogen and carbohydrate sources and increasing the rumen outflow rate (Pathak, 2008).

Both tannins and phenols are harmful to normal protein degradation in the rumen. Some bushes and roughages could be contained anti-nutritional components which influence on the protein degradability in the rumen. The phenolic components in some bushes may connect to protein, thus making the protein un-digestible by rumen microbes. Polyethylene glycol (P.E.G) is able to form complexes with tannins and has been utilized to decrease the formation of protein-tannin compound (Makkar, Blümmel, & Becker, 1995;

Getachew, Makkar, & Becker, 2000). Using of P.E.G (2 g 100 g^{-1} DM) decreased cell wall constituents and total condensed tannins and increased the digestibility of olive leaves (García, Ruiz, Moumen, & Alcaide, 2004). However, incubated samples of olive pruning branches (Al-Masri, 2012) and some drought-tolerant range perennial shrubs (*Peganum harmala, Alhagi camelorum and Salsola vermiculata*) and herbaceous range plants (*Schismus arabicus, Erodium cacutarium and Poa sinaica*) (Al-Masri, 2011) with P.E.G and rumen fluid at a ratio of 2:1 P.E.G: substrate increased the values of digestible organic matter and metabolizable energy.

There is dearth information in the literature concerning the efficiency of microbial nitrogen and biomass synthesis of some perennial range species grown on dry rangelands and the interaction between P.E.G treatment and range plants, thus the studies on this interaction are necessary and valid to evaluate the quality of these specie and to investigate the efficiency of microbial nitrogen and biomass synthesis. Therefore, the aims of the current work were to evaluate, by the use of *in vitro* incubation technique with ruminal fluid and ^{15}N-tracer, some perennial range species grown naturally on dry rangelands in terms of microbial N and biomass production, true fermented organic matter and quantification of the efficiency of microbial nitrogen and biomass synthesis, and study the effect of P.E.G on the aforementioned parameters and the interaction between P.E.G treatment and plant species.

Materials and methods

Tested plant materials

Five perennial range species (*Astragalus spinosus* Forssk, Leguminosae; *Artemisia herba-alba* Asso, Asteraceae; *Capparis spinos* L., Capparaceae; *Lavandula angustifolia* Mill, Lamiaceae; *Noaea mucronata* Forssk, Chenopodiaceae), grown naturally on the south-eastern semi-desert of Syria (Gabajeb 35° 16' N, 39° 42' E and Al-Bishri 35° 22' N, 39° 46' E; 203 m above the sea), which receive a total annual precipitation of 100-120 mm, were collected from 8 different places of the field (about 2500 m^2). Plants were harvested at early bloom stage and hand-cut at 25 cm from ground level with 4 replicates (8 plants each) for each range species. The collected plants for each replicate of each species were mixed well, dried at 20-25°C for one week, ground to pass a 1 mm sieve and stored frozen at −20°C in sealed nylon bags for subsequent analysis and evaluation.

Parameters measured or estimated

The experimental samples (200 mg) were incubated in 100 mL glass syringes, standing upright in a water-bath, for 96 hours at 39°C with the 30 ml rumen fluid mixture and ^{15}N-labelled ammonium sulphate (> 90% ^{15}N), with or without added P.E.G (P.E.G 6000; Fluka Firm No. 81260) at a ratio of 2:1 P.E.G:substrate to estimate the biological activity of the tannins (Makkar et al., 1995) and microbial nitrogen (M.N) using ^{15}N-tracer technique (Abel, Coenen, & Immig, 1990; Blümmel, Makkar, & Becker, 1997). The rumen fluid was collected from three rumen-fistulated Awassi rams. Total nitrogen, as well as ^{15}N atom excess in the nitrogen pool of the sample and fluid mixture incubated for 96 h or in the fluid mixture alone (blank) were measured with an emission spectrometer (JASCO N-150, Japan Spectroscopic Com. Ltd, Tokyo, Japan). Details of methods of incubation, feeding of rumen-fistulated rams, collection of rumen fluid and determination have been described previously (Al-Masri, 2010; 2015).

Microbial biomass production (M.BM) and microbial nitrogen (M.N) were estimated as the following equations:

M.N (mg 200 mg^{-1} sample) = [1 − (%^{15}N atom excess in the N-pool of the sample and fluid mixture/ % ^{15}N atom excess in the fluid mixture)] * mg N in the sample.

M.BM (mg 200 mg^{-1} sample) = M.N/ 0.0864. The rumen microbes contain 8.64% nitrogen (Czerkawski, 1986).

The efficiency of M.N (E.M.N) or M.BM (E.M.BM) synthesis defined as M.N or M.BM to the true fermented organic matter (Hoover and Stokes, 1991; Al-Masri, 2003).

True fermented organic matter (T.F.OM) was determined in two stages by the method of Van Soest and Robertson (1985). After the incubation, the content of the whole syringe was refluxed for 1 h with the neutral detergent solution (NDS). The filter residues were dried, ashed and weighed. The T.F.OM was calculated as the weight of incubated substrate minus the weight of the residue after NDS treatment and the ash. Details of the method have been described previously (Al-Masri, 2003).

Statistical analyses

A 5 x 2 factorial-design was used in this experiment, with two fixed factors: (1) plant species (five species); (2) polyethylene glycol action (with P.E.G or without P.E.G). Results were subjected to analysis of variance

(ANOVA) using a Statview-IV program to test the effect of plant species and P.E.G treatment and the interaction. Means were separated using the Fisher's least significant difference test at the 95% confidence level.

Results and discussion

The changes in the values of true fermented organic matter (T.F.OM) of the experimental plant species due to P.E.G treatment are shown in Figure 1. T.F.OM was affected by plant species ($p < 0.0001$) but not affected by the P.E.G treatment ($p = 0.2407$). *C. spinosa* had the highest values of T.F.OM (309 g kg^{-1} DM) which was followed by *A. spinosus* and *L. angustifolia* (245 g kg^{-1} DM) and by *A. herba-alba* and *N. murconata* (201 g kg^{-1} DM). The true fermented OM of some unconventional feeds amounted to 279-849 g kg^{-1} DM (Al-Masri, 2003).

Nutritive components (especially lignin, crude protein and soluble nitrogen) could be negatively or positively influenced on the studied parameters. The experimental plant species were contained (g kg^{-1} DM): 103. 96.5, 104, 97.0, 229 crude protein (CP), 6.08, 6.40, 7.38, 7.50, 19.1 buffer soluble nitrogen (BS-N) and 164, 167, 113, 148, 51.8 lignin for *A. herba-alba*, *N. mucronata*, *L. angustifolia, A. spinosus, C. spinosa*, respectively (Al-Masri, 2013). The values of T.F.OM were positively correlated with CP fraction ($r = +0.85$; $p < 0.0001$) but negatively correlated with lignin ($r = -0.91$; $p < 0.0001$). Lower concentrations of cell wall constituents mean higher amounts of soluble carbohydrates which are accessible for fermentation (Getachew, Robinson, DePeters, & Taylor, 2004). Lignin can hinder the growth rate of the rumen microbes and reduce the activity of microbial enzymes in the rumen (McSweeney, Palmer, McNeill, & Krause, 2001).

The effects of plant species, P.E.G treatment and the interaction between P.E.G and species on the microbial nitrogen (M.N), microbial biomass (M.BM) and efficiency of M.N and M.BM synthesis are shown in Table 1. *C. spinosa* gave highest M.N and M.BM values ($p < 0.05$) in comparison with other species. There were no significant differences ($p > 0.05$) among *A. herba-alba* and *N. mucronata* concerning the values of M.N and M.BM (average 0.55 and 6.36 mg g^{-1} DM, respectively), and between *L. angustifolia* and *A. spinosus* (average 0.73 and 8.34 mg g^{-1} DM, respectively). The studied *C. spinosus* in this work, as a perennial range shrub, produced more M.N and M.BM (0.82 and 9.46 mg g^{-1} DM, respectively) than those (0.21 and 2.43 mg g^{-1} DM, respectively) reported by (Al-Masri, 2007) for *Poa sinaica* as a annual herbaceous range plant, and these could be related to the high CP content (229 g kg^{-1} DM) in *C. spinosus* compared to *P. sinaica* (81 g kg^{-1} DM).

The true fermented organic matter (T.F.OM) in the rumen provides the rumen microbes with an essential source of energy for their growth and improving the rumen microbial protein synthesis. The amount of M.N or M.BM production per 100 g T.F.OM reflects the efficiency of M.N or M.BM synthesis in the rumen. There were no significant ($p > 0.05$) changes in the values of the efficiency of M.N and M.BM synthesis between the plant species, and the average amounted to 0.282 g and 3.25 g 100 g^{-1} T.F.OM, respectively. The efficiency of M.N or M.BM synthesis for the experimental range plants was lower than those reported by Al-Masri (2003) for some unconventional feeds (0.7-2.9 g or 8-32 g 100 g^{-1} T.F.OM, respectively) and by Al-Masri (2015) (0.41-2.60 g or 4.66-29.6 g 100 g^{-1} effective degraded substrate; EDS, respectively) for some salt-tolerant tree species (*Tamarix aphylla*, *Acacia ampliceps*, *Casuarina equisetifolia*, *Parkinsonia aculeate*) estimated using ^{15}N-tracer technique. However, the efficiency of M.BM synthesis for the experimental range plants (3.25 g 100 g^{-1} T.F.OM) was lower than those (7.2 g 100 g^{-1} digestible OM) reported by Mullik, Poppi, and McLennan (2008) for green Pangola grass (*Digitaria erianthe* cv. Steudal) estimated using purine derivative excretion in urine. Pathak (2008) reported that the average efficiency of microbial crude protein (MCP) synthesis for forage based diets and forage-concentrate mix diets amounted to 13 and 17.6 g MCP 100 g^{-1} of truly digested OM in the rumen, respectively.

The efficiency of M.N or M.BM synthesis varied with the cutting length of the plant branches. The values of effective degradability of dry matter decline and cell-wall constituents increase with increasing the cutting length. Branches of olive tree cut at 25 cm length had higher efficiency of M.N and M.BM synthesis (0.23 and 2.66 g 100 g^{-1} EDS, respectively) than those cut at 100 cm length (0.14 and 1.59 g 100 g^{-1} EDS, respectively) (Al-Masri, 2016).

The results indicated that P.E.G treatment and the interaction between plant species and P.E.G had no significant ($p > 0.05$) effect on the values of M.N, M.BM and efficiency of M.N and M.BM synthesis. The absence of significant effect of P.E.G treatment concerning the estimated parameters of the experimental species could be related to their low contents of tannins.

Suitable amounts of soluble carbohydrate and soluble nitrogen with low amount of lignin in the fermented substrate could be lead to improve and increase the efficiency of microbial protein synthesis. However, synchronized

release of nitrogen, in the form of NH3-N, and energy from the fermented carbohydrate is very necessarily for effective utilization of the NH3-N in the rumen and improving the rumen microbial protein synthesis.

The high amounts of M.N and M.BM observed in *C. spinosa* could be related to their high content of buffer soluble nitrogen (BS-N, 19.1 g kg^{-1} DM) and low content of lignin (52 g kg^{-1} DM) in comparison with other experimental species, which have in average 6.84 g kg^{-1} DM of BS-N and 148 g kg^{-1} of lignin. The M.N and M.BM values of the experimental species were negatively correlated with lignin ($r = -0.986$; $p < 0.0001$ and $r = -0.992$; $p < 0.0001$, respectively) (Figure 2 and 3) but positively correlated with BS-N ($r = +0.886$; $p < 0.0001$ and $r = +0.909$; $p < 0.0001$, respectively) (Figure 4 and 5).

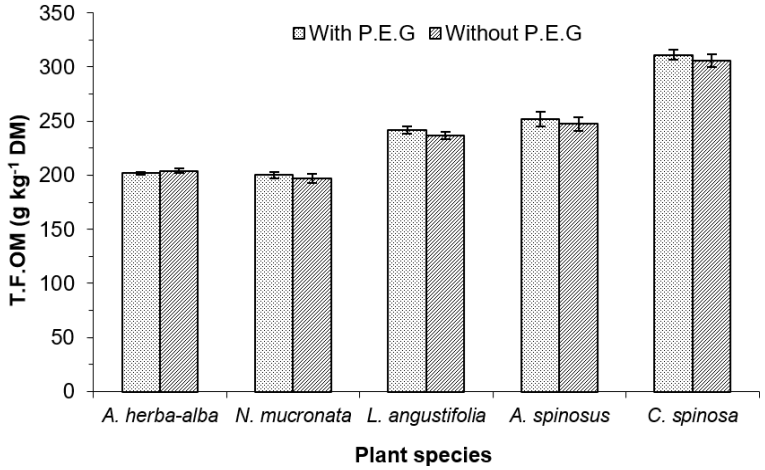

Figure 1. Changes in the fermented organic matter (T.F.OM) of plant species after incubation with or without polyethylene glycol (P.E.G).

Table 1. Changes in the microbial nitrogen (M.N), microbial biomass (M.BM) and efficiency of M.N or M.BM synthesis of the experimental range species.

	M.N	M.B.M	E.M.N	E.M.BM
	(mg g^{-1} DM)	(mg g^{-1} DM)	(mg g^{-1} T.F.OM)	(mg g^{-1} T.F.OM)
		Species (pooled)		
A. herba-alba	0.57^{b}	6.59^{b}	2.81^{a}	32.5^{a}
N. mucronata	0.53^{b}	6.13^{b}	2.67^{a}	30.9^{a}
L. angustifolia	0.77^{ab}	8.81^{ab}	3.22^{a}	36.9^{a}
A. spinosus	0.68^{ab}	7.87^{ab}	2.73^{a}	31.6^{a}
C. spinosa	0.82^{a}	9.46^{a}	2.66^{a}	30.8^{a}
SEM	0.04	0.49	0.18	2.16
		P.E.G treatment (pooled)		
+	0.67^{a}	7.90^{a}	2.80^{a}	32.9^{a}
-	0.67^{a}	7.75^{a}	2.82^{a}	32.6^{a}
SEM	0.06	0.69	0.26	3.09
		P -*value*		
Interaction	0.9995	0.9999	0.9992	0.9998

^{a,b}Means in the same columns with diverse superscript are different at $p < 0.05$. SEM, standard error of the means. P.E.G, polyethylene glycol ('+' with, '-' without); T.F.OM, true fermented organic matter; E.M.N, efficiency of microbial nitrogen synthesis; E.M.BM, efficiency of microbial biomass synthesis.

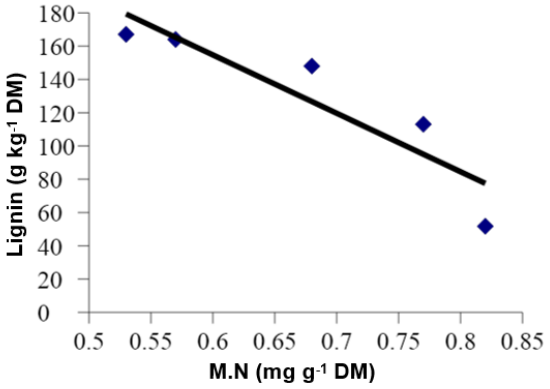

Figure 2. Relationship between microbial nitrogen (M.N) and lignin of the experimental plant species.

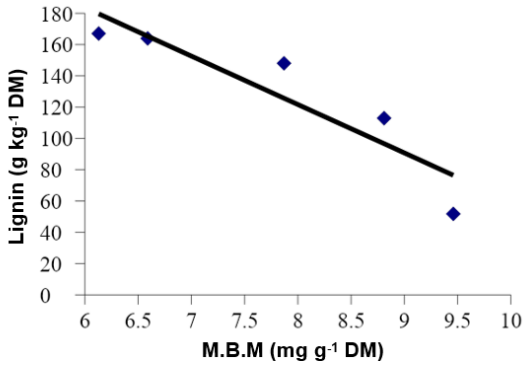

Figure 3. Relationship between microbial bio-mass (M.B.M) and lignin of the experimental plant species.

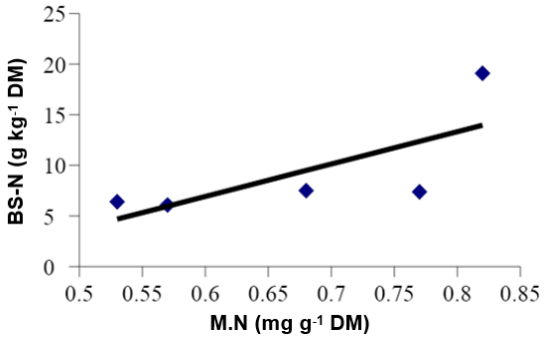

Figure 4. Relationship between microbial nitrogen (M.N) and buffer soluble nitrogen (BS-N) of the experimental plant species.

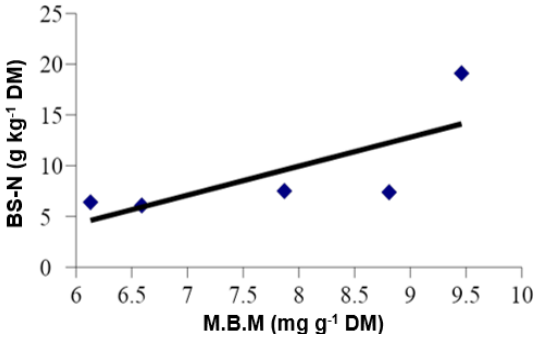

Figure 5. Relationship between microbial bio-mass (M.B.M) and buffer soluble nitrogen (BS-N) of the experimental plant species.

Conclusion

Taken together, the data suggested that *C. spinosa* had high values true fermented organic matter, microbial nitrogen (M.N) and microbial biomass production (M.BM). There were no significant differences between the experimental plant species in terms of the efficiency of M.N and M.BM synthesis. Polyethylene glycol (P.E.G) treatment and the interaction between plant species and P.E.G had no significant effect on the evaluated parameters.

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