Biotechnology in Animal Husbandry 36 (1), 1-16 , 2020 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636.087.8'636.38
https://doi.org/10.2298/BAH2001001M

EFFECT OF TREATED *CONOCARPUS ERECTUS L.* LEAVES WITH *KLEBSIELLA PNEUMONIAE* AND *ACINETOBACTER* AS TANNIN-DEGRADING BACTERIA ON DIGESTION ACTIVITY OF RUMEN MICROORGANISMS

Tahereh Mohammadabadi, Alireza Jolazadeh, Zeinab Ghezi

Department of Animal Science, Faculty of Animal Science and Food Technology, Agricultural Sciences and Natural Resources University of Khuzestan, P.O. Box 63517-73637, Mollasani, Ahvaz, Iran
Corresponding author: Tahereh Mohammadabadi, mohammadabadi@asnrukh.ac.ir
Original scientific paper

Abstract: The current research focuses on the effect of *Conocarpus erectus L.* leaves (CL) processed with two tannin-degrading bacteria *Klebsiella pneumoniae* (*K. p*) and *Acinetobacter (A. b)* on digestion activity and fermentation parameters of rumen bacteria and fungi in Arabian sheep. These isolates capable of utilizing tannic acid as sole carbon and energy source. Eight species of *Klebsiella pneumoniae* (A1, A2, A3, A4, A5, A7, A8, A9) follow by an *Acinobacter* sp. were used for biological treatment of CL for ten days. Then, digestion activity and some rumen fermentation parameters of ruminal bacteria and fungi in specific culture medium (SCM) were determined in Arabian sheep. Treated CL by bacterial isolates, reduced total tannin (TT) compared with unprocessed groups ($P < 0.05$). Dry matter disappearance (DMD) was affected by biological treatment for both rumen bacteria and fungi in different incubation times, except for 3 and 6 h of incubation in SCM of mixed rumen fungi, which indicated same pattern with untreated CON group ($P > 0.05$). The NDF disappearance (NDFD) and CP disappearance (CPD) in SCM of mixed rumen bacteria and fungi were increased during all incubation times ($P < 0.05$) due to biological prosses of CL compared to CON treatment. Biologically treated CL increased gas production from the fermentable fraction (b) pattern in both SCM of mixed rumen bacteria and fungi during all incubation times ($P < 0.05$), but, the gas production rate constant (c) was increased only at SCM of mixed rumen fungi ($P < 0.05$). Overall, the data indicated that bacterial inoculation of *K. p* and *A. b.* sp. could improve digestion activity and some fermentation parameters of rumen bacteria and fungi in Arabian sheep.

Key words: Conocarpus leaves, tannin degrading bacteria, biological processing, rumen microorganisms

Introduction

The role of fodder trees and shrubs in the diet of animals as good sources of proteins is important in countries like Iran where small land holdings and large ruminant densities cause main problem of feed availability from traditional green fodder like Lucerne to feed their animals (*Mohammadabadi and Jolazadeh, 2017*). In some arid and semi-arid countries, *Conocarpus erectus L.* leaves (CL) could be suitable for ruminants as a source of green fodders which used as an energy source for host animal and microbes in the rumen (*Al-Koaik et al., 2014*). It is widely distributed on shorelines in tropical and subtropical regions of the earth, and is popularly known as button mangrove (*Nascimento et al., 2016*). Phenolic compounds especially tannins are the major secondary metabolites of this species which often considered important factors limiting their use (*Abdel-Hameed et al., 2012*). The harmful consequences of the high percentage of tannin in the diet of ruminants can reduce feed intake, have a negative effect on the activity of rumen bacteria, reduce the efficiency of digestive enzymes in the livestock and also reduce the availability of nutrients, which ultimately reduce growth and production of these animals (*Garg et al., 1992; Frutos et al., 2004; Salinger et al., 1996*) .

Using suitable and practical processing methods for improving the nutritive value of feedstuff in ruminant nutrition have an important role in reducing production costs and improving production performance of ruminant animals. Various chemical and physical methods such as sodium bicarbonate, polyethyleneglycol, soaking in water, cooking or steaming, have been reported to reduce the amount of tannin and its harmful side effects (*Frutos et al., 2004*). Recently, bioprocessing has vast applies in livestock and poultry feed, using the enzymatic capabilities of microorganisms (*Motamedi et al., 2019; Bahaeddini et al., 2016*). It is a profitable method and can break down toxic compounds to innocuous products (*Azadi et al., 2014*). Biodegradation mainly utilizes microbes such as fungi and bacteria to improve the suitability of by-products for enzymatic hydrolysis (*Lotfi and Rouzbehan, 2012; Tahmourespour et al., 2016*). Condensed tannins in *Quercus incana* oak leaves were effectively degraded by *Sporotrichum pulverulentum* as a fungi specie (*Makkar et al., 1994*). Additionally, *Curiel et al. (2009)* reported that *Lactobacillus plantarum* is a safe microorganism that capable to produce tannase to degrade and eliminate tannin compounds.

Contrary to tannin antimicrobial properties, many microbes, especially bacteria can resist and develop different mechanisms for the tannin degradation in their habitats. So, Bacteria with the ability to grow in the presence of tannins as a sole source of carbon and energy are commonly considered tannin-degrading and

degradation like resistance is not limited by species or geographical barriers (*Tahmourespour et al., 2016*). Previously, many researchers reported that *Klebsiella pneumoniae* and *Acinetobacter* sp. were able to degrade tannic acids, they had to adapt in the presence of high concentrations of tannic acid. So, these isolates have a good potential for reduction of tannins antinutritional effects in animal feeds (*Tahmourespour et al., 2016; Arunachalam et al., 2003*). Little information (particularly involving bacteria) is available about the biological treating of taniniferous plants as ruminant feed. In the one case, *Motamedi et al. (2019)* reported that nutritive value of oak leaves was increased following to biological treatment with *Klebsiella pneumoniae*. Hence, this experiment was therefore planned to assess the chemical composition, digestion activity and fermentation parameters of CL leaf species following treatment with *K. p* and *A. b* as tannase producing bacterium isolated from deer rumen on digestion activity of sheep rumen microorganisms.

Materials and Methods

Sampling and chemical composition

Conocarpus leaves were harvested and cleaned to remove any foreign substances from Khuzestan province which is located southwest of Iran. Then, leaf samples were transported to the laboratory and oven-dried to a constant weight at 65 °C. The dried samples were then ground in a hammer mill with a 1-mm screen and stored in bags for later determination of chemical composition and *in vitro* incubations. The content of dry matter after drying in the oven, organic matter, Ash and crude protein of CL were 783, 828.5, 171.5 and 107 g/kg dry matter (DM), respectively.

Ground samples were analyzed in triplicate for DM, ash, crude protein (CP), according to *AOAC (1990).* The neutral detergent fiber (NDF) contents was analyzed without sodium sulfite, expressing regardless of residual ash according to *Van Soest et al., (1991).* Total tannins (TT) were conducted in three replicates as described by *Makkar (2000).*

Bacterial strains

Eight species of *Klebsiella pneumoniae* (*K. p*) follow by an *Acinobacter* sp. (*A. b*) were used for biological treatment of *Conocarpus* leaves. These bacteria were isolated from deer rumen located at the Dez National Park of Dezful (Khuzestan Province, Iran) with tannase production ability (45 U/ml) which could grow on liquid medium containing tannic acid as the sole source of carbon and energy and was identified based on 16S rRNA sequencing analysis (*Gheibipour, 2017*). Figure 1 presents the electrophoresis result of PCR product (1500 bp) of the isolates. These isolates included *Klebsiella pneumonia* (*K. p*) A1, A2, A3, A4, A5, A7, A8, A9 and an *Acinetobacter* A6 sp. (*A. b*).

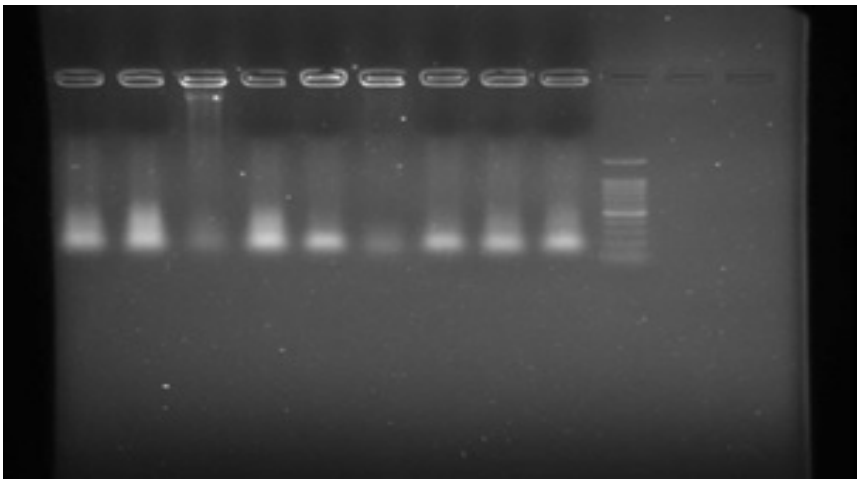

Figure 1. Amplification of 16S rRNA from i.e., A1- A9 isolates on agarose gel. M,1Kbp marker. right to left; i.e., A1- A9 (1500 bp).

Treating of leaves in liquid medium

The ground leaves were mixed with the *K. p* and *A. b*. For processing, Erlenmeyer flasks were filled with 500 ml of culture medium and 2.5% substrate (12.5 g per flask). Culture medium contained (per liter): 1.3 g $(NH_4)_4SO_4$, 1.0 g yeast extract, 0.37 g KH_2PO_4, 0.25 g $MgSO_4.7H_2O$, 0.07 g $CaCl_2.2H_2O$ and 0.02 g $FeCl_3$ (*Belur et al., 2010*). After sterilizing, corresponding flasks were inoculated with 3 ml of 24 h culture of *K. p* and *A. p* (10^7 CFU) in nutrient broth and were incubated in the shaking incubator for 10 days at 30 ^oC under aerobic conditions.

Treatments were 8 species of *Klebsiella pneumoniae* (A1, A2, A3, A4, A5, A7, A8, A9) and *Acinobacter* sp. were used for biological treatment of CL for ten days. Control was CL treated in liquid medium without bacteria. After 10 days of incubation, the contents of vials were centrifuged at 20,000×*g* for 20 min at 4 ^oC. Then were filtered and the residue collected, dried and were used for subsequent anlysis.

Rumen inoculum

Four individually housed healthy mature Arabian male sheep (body weight 50 ± 1.2 kg) were used as inoculum donor. The animals were fed alfalfa hay, wheat bran, barley grain, canola meal, mineral vitamin premix and salt twice daily, at 0900 and 1700, with free access to water. After a 14 days adaptation period to the diet, samples of ruminal fluid were collected from each sheep before morning feeding (0700) and maintained at 39 ^oC in an insulated flask during transport to the laboratory. At the laboratory, ruminal fluid was filtered through four layers of cheesecloth and maintained at 39 °C under continuous flushing with CO2.

Gas production and specific media

This experiment is part of a big study, that we performed these treatments in the other experiment by rumen fluid and in this part we specifically used rumen fungi and bacteria activity for these treatments.

Effect of treatments on digestion activity of ruminal bacteria and fungi in specific culture medium (SCM) were determined based on following methods. The bacteria SCM were performed according to *Mohammadabadi and Jolazadeh (2017).* Culture glasses containing 1 g experimental sample were autoclaved at 120 °C for 15 min. Rumen fluid centrifuged at 1000 rpm for 10 min and supernatant were added to specific culture medium of bacteria. The amounts of 36 ml of this solution as culture medium and 4 ml rumen fluid were inoculated into each culture glass. Then, the samples were cultured in incubator at 39 °C for 24, 48, and 72 h. At the end of each of the mentioned time, glasses were considered to determine the disappearance of DM, NDF and CP for each incubation time.

For fungi culture, experimental samples were cultured in specific culture of rumen fungi at 39 °C for 1, 3, and 6 days. At the end of each time, the disappearance of DM, NDF and CP was determined. Culture medium was transferred under anaerobic conditions into medium glasses and autoclaved. Rumen fluid centrifuged and supernatant were cultured in specific rumen anaerobic fungi culture.

In vitro gas production and related parameters (b and c) for two SCM were determined as described by *Blümmel et al. (1997)* in triplicate. The amount of gas produced per vial was recorded after 72 h of incubation for bacteria and 120 h (6 days) for fungi by using a digital pressure gauge (Model SDPG0015PG5, SenSym ICT, Honeywell Inc., Morris NJ) fitted with a 21 mm gauge needle. The content of each vial was centrifuged at 1500 rpm for 20 min and the residuals were collected and dried. Ruminal DMD was calculated by difference between weights of primary substrate from weight after incubation.

Statistical analysis

Data were analyzed based on a completely randomized design using to GLM option of (*SAS 2000*).

$Y_{ij} = \mu + T_i + e_{ij}$,

where Y_{ij} is the general observation, μ is the general mean, T_i is the effect of bacterial inoculation on the observed parameters, and e_{ij} is the standard error of term. Significance was declared at $P \leq 0.05$ and trends at $P < 0.10$ and $P > 0.05$ using Tukey's multiple comparison test.

Results

Treated CL with different species of *K. p* and an *A. b* sp. did not affect chemical composition, and bacterial inoculation did not change the DM, NDF or CP content of treated leaves (Table 1). However, after processing of CL by isolates, TT was decreased compared with unprocessed groups ($P < 0.05$).

Table 1. Chemical composition of treated *Conocarpus erectus L.* leaves with *Klebsiella pneumoniae* and *Acinetobacter* species (g/kg DM)

Treatment	Items				
	Dry matter	CP	Ash	NDF	TT (g/100 DM)
CON	783	107	171	313	5.82[a]
K. p A8	782	105	172	314	1.67[g]
A. b A6	775	107	132	328	1.01[h]
K. p A7	759	105	142	320	2.45[f]
K. p A1	762	103	137	302	3.29[c]
K. p A4	777	104	153	320	3.62[b]
K. p A5	778	102	169	302	2.89[e]
K. p A3	763	105	163	333	2.79[e]
K. p A2	759	106	165	333	5.64[a]
K. p A9	763	107	159	343	3.11[d]
SEM	43.3	6.1	15.9	19.5	0.11
P-value	0.200	0.742	0.153	0.711	0.003

[a, d] Means with different superscripts in the same column are different (P<0.05).
CON: CL treated in liquid medium without bacteria ; *K. p: K. pneumonia*; *A. b: Acinetobacter*;
CP, crude protein; NDF, neutral detergent fiber; TT, Total tannins.
SEM: Standard error of means

As shown in Table 2, DMD was improved by biological treatment for both rumen bacteria and fungi in SCM during different incubation times, except for 3 and 6 h after incubation in SCM of mixed rumen fungi, which all treatments indicate same pattern compared to the control diet ($P > 0.05$).

Table 2. Effect of treated *Conocarpus erectus L. leaves* with *Klebsiella pneumoniae* and *Acinetobacter* on dry matter disappearance by rumen bacteria and fungi inoculum

Incubation time	Treatment										SEM	*P*-value
	CON	*K. p* A8	*A.b* A6	*K. p* A7	*K. p* A1	*K. p* A4	*K. p* A5	*K. p* A3	*K. p* A2	*K. p* A9		
Dry matter disappearance in rumen bacteria inoculum (g/kg DM)												
24 h after incubation	344^{c}	447^{bc}	359^{c}	508^{ab}	382^{c}	490^{ab}	539^{a}	523^{a}	532^{a}	488^{ab}	33.3	0.028
48 h after incubation	417^{d}	433^{d}	467^{c}	554^{ab}	486^{c}	534^{ab}	544^{ab}	569^{a}	565^{a}	543^{ab}	10.9	0.045
72 h after incubation	302^{d}	379^{c}	347^{c}	413^{b}	365^{c}	412^{b}	509^{a}	434^{b}	503^{a}	403^{b}	8.0	0.044
Dry matter disappearance in rumen fungi inoculum (g/kg DM)												
1 h after incubation	273^{e}	387^{c}	323^{d}	448^{bc}	390^{c}	406^{bc}	545^{a}	492^{ab}	534^{a}	407^{bc}	21.7	0.010
3 h after incubation	307	394	355	490	393	454	624	501	564	411	23.1	0.154
6 h after incubation	254	314	319	442	364	364	578	466	533	378	19.6	0.500

[a, f] means within a row with different superscript letters are different ($P < 0.05$).
CON: CL treated in liquid medium without bacteria; *K. p: K. pneumonia*; *A. b: Acinetobacter*;
SEM: Standard error of means

Effect of biological treatment on NDFD and CPD by rumen bacteria and fungi are presented in Table 3 and 4, respectively. NDFD and CPD in SCM of mixed rumen bacteria and fungi were affected by experimental treatment during all incubation times ($P < 0.05$), and in the most case biological prosses could increase NDFD and CPD compared to CON treatment.

Table 3. Effect of treated *Conocarpus erectus L.* leaves with *Klebsiella pneumoniae* and *Acinetobacter* on NDF and CP disappearance by rumen bacteria inoculum (g/kg NDF and CP)

Treatment [a]	Incubation time					
	24 hours after incubation		48 hours after incubation		72 hours after incubation	
	NDF	CP	NDF	CP	NDF	CP
CON	205^{d}	199^{d}	232^{c}	206^{bc}	162^{e}	173^{bc}
K. p A8	274^{c}	282^{bc}	305^{c}	291^{bc}	253^{d}	251^{b}
*A.b*A6	244^{c}	245^{bc}	251^{c}	251^{bc}	203^{c}	194^{bc}
K. p A7	343^{b}	384^{a}	406^{b}	398^{b}	324^{c}	357^{b}
K. p A1	261^{c}	254^{bc}	268^{c}	285^{bc}	251^{d}	234^{bc}
K. p A4	298^{c}	348^{ab}	403^{b}	352^{b}	305^{c}	327^{b}
K. p A5	474^{a}	495^{a}	529^{a}	531^{a}	472.4^{a}	499^{a}
K. p A3	393^{b}	422^{a}	407^{b}	431^{a}	389^{b}	409^{a}
K. p A2	459^{a}	489^{a}	506^{a}	493^{a}	456^{a}	456^{a}
K. p A9	294^{c}	302^{bc}	402^{b}	309^{b}	271^{d}	287^{b}
SEM	26.9	29.8	24.0	34.5	22.0	45.4
P-value	0.025	0.057	0.011	0.045	0.023	0.030

[a, b] Means with different superscripts in the same column are different (P<0.05).

CON: CL treated in liquid medium without bacteria; *K. p: K. pneumonia*; *A. b: Acinetobacter*;
CP, crude protein; NDF, neutral detergent fiber;
SEM: Standard error of the mean

Table 4. Effect of treated *Conocarpus erectus L.* leaves with *Klebsiella pneumoniae* and *Acinetobacter* on NDF and CP disappearance by rumen fungi inoculum (g/kg NDF and CP)

Treatment	Incubation time					
	1 day after incubation		3 days after incubation		6 days after incubation	
	NDF	CP	NDF	CP	NDF	CP
CON	234^{f}	184^{c}	259^{dc}	225^{c}	231^{g}	194^{c}
K. p A8	303^{e}	233^{c}	357^{c}	240^{c}	298^{e}	195^{c}
Acin A6	300^{e}	197^{c}	304^{dc}	233^{c}	264^{ef}	195^{c}
K. p A7	385^{c}	252^{bc}	407^{b}	273^{abc}	354^{c}	251^{bc}
K. p A1	301^{e}	215^{c}	328^{c}	237^{c}	276^{e}	201^{c}
K. p A4	351^{d}	251^{bc}	400^{b}	267^{abc}	341^{c}	259^{bc}
K. p A5	551^{a}	424^{a}	584^{a}	454^{a}	486^{a}	403^{a}
K. p A3	431^{b}	347^{a}	431^{b}	354^{a}	392^{b}	308^{b}
K. p A2	432^{b}	365^{a}	457^{b}	386^{a}	414^{b}	373^{a}
K. p A9	342^{d}	251^{bc}	377^{c}	263^{abc}	323^{d}	246^{bc}
SEM	15.7	47.0	23.5	34.3	13.4	24.0
P-value	0.035	0.024	0.025	0.048	0.030	0.045

[a, c] Means with different superscripts in the same column are different (P<0.05).
CON: CL treated in liquid medium without bacteria*; K. p: K. pneumonia; A. b: Acinetobacter*
SEM: Standard error of the mean

Biologically treated CL increased gas production from the fermentable fraction (b) after 24, 72 and 144 h of incubation ($P < 0.05$), but had no effect on the gas production rate constant (c) in SCM of mixed rumen bacteria (Table 5), While, these values indicate increasing pattern in both SCM of mixed rumen bacteria and fungi during all incubation times ($P < 0.05$) (Table 6).

Table 5. Effect of treated *Conocarpus erectus L.* leaves with *Klebsiella pneumoniae* and *Acinetobacter* on *in vitro* rumen fermentation parameters by rumen bacteria inoculum

Item	Treatment [a]											
	CON	*K. p* A8	*A. b* A6	*K. p* A7	*K. p* A1	*K. p* A4	*K. p* A5	*K. p* A3	*K. p* A2	*K. p* A9	SEM	*P*-value
b, ml												
24 h after incubation	18.9^{c}	22.4^{bc}	20.5^{c}	24.8^{a}	20.2^{bc}	24.3^{a}	25.8^{a}	24.9^{a}	24.0^{a}	22.9^{bc}	1.22	0.022
48 h after incubation	19.7^{c}	20.6^{bc}	19.6^{c}	23.9^{a}	20.5^{bc}	22.9^{b}	24.4^{a}	23.8^{a}	23.7^{a}	22.2^{b}	1.90	0.040
72 h after incubation	15.2^{c}	20.6^{b}	18.2^{c}	23.1^{a}	19.9^{bc}	23.1^{a}	23.7^{a}	23.1^{a}	23.5^{a}	20.3^{b}	0.49	0.025
c, ml/h												
24 h after incubation	0.029	0.043	0.030	0.058	0.035	0.056	0.063	0.060	0.061	0.045	0.0192	0.290
48 h after incubation	0.023	0.033	0.025	0.049	0.028	0.038	0.061	0.059	0.060	0.035	0.0325	0.535
72 h after incubation	0.019	0.025	0.026	0.049	0.023	0.041	0.057	0.050	0.055	0.030	0.0255	0.140

[a] CON: CL treated in liquid medium without bacteria; *K. p: K. pneumonia*; *A. b: Acinetobacter*;
b: gas production from the fermentable fraction; c: the gas production rate constant;

[a, c] means within a row with different superscript letters are different ($P < 0.05$).
SEM: Standard error of the mean.

Table 6. Effect of treated *Conocarpus erectus L.* leaves with *Klebsiella pneumoniae* and *Acinetobacter* on *in vitro* rumen fermentation parameters by rumen fungi inoculum

Item	Treatment [a]											
	CON	*K. p* A8	*A. b* A6	*K. p* A7	*K. p* A1	*K. p* A4	*K. p* A5	*K. p* A3	*K. p* A2	*K. p* A9	SEM	*P*-value
b, ml												
1 day after incubation	9.15^{c}	16.8^{bc}	10.2^{c}	20.1^{ab}	15.2^{c}	19.8^{bc}	23.2^{a}	20.5^{ab}	22.7^{a}	18.9^{bc}	1.89	0.042
3 day after incubation	10.8^{c}	18.9^{bc}	15.6^{c}	23.1^{a}	18.9bc	20.7^{bc}	27.3^{a}	24.6^{a}	25.6^{a}	19.3^{bc}	1.15	0.035
6 day after incubation	6.31^{c}	15.5^{bc}	9.1^{bc}	18.2^{ab}	10.3^{c}	16.6^{bc}	21.0^{a}	18.5^{ab}	19.1^{a}	15.9^{bc}	1.50	0.021
c, ml/h												
1 day after incubation	0.043^{c}	0.055^{b}	0.045^{c}	0.060^{ab}	0.049^{c}	0.059^{b}	0.075^{a}	0.069^{a}	0.070^{a}	0.058^{b}	0.0169	0.025
3 day after incubation	0.045^{c}	0.059^{b}	0.048^{c}	0.065^{ab}	0.055^{c}	0.063^{ab}	0.076^{a}	0.070^{a}	0.075^{a}	0.060^{b}	0.0026	0.017
6 day after incubation	0.038^{c}	0.049^{c}	0.040^{c}	0.059^{b}	0.045^{c}	0.048^{a}	0.070^{a}	0.065^{ab}	0.069^{a}	0.050^{b}	0.0236	0.036

a CON: CL treated in liquid medium without bacteria; K. p: K. pneumonia; A. b: Acinetobacter;
b: gas production from the fermentable fraction; c: the gas production rate constant;

a, c means within a row with different superscript letters are different ($P < 0.05$).
SEM: standard error of means

Discussion

To the best of our knowledge, nutritive value of CL leaves has not been evaluated in experiments where treated by isolated bacteria. Bacterial inoculation did not change the DM, NDF or CP content of treated leaves when compared to the control (Table 1). The ineffectiveness of bacterial inoculation on the DM and NDF content of CL leaves is probably due to lack of microbial cellulase enzyme activity. Tannins form a complex with protein and lignocellulose, inhibit microbial enzyme and prevent microbial digestion, hence reducing fiber utilization by ruminants (*McSweeney et al., 2001*).

Decreasing TT content of leaves after bacterial inoculation was probably due to bacterial activity and secretion of tannase on substrates. Treatment of coffee pulp with *Bacillus* sp. improved its nutritive value by decreasing total phenol and TT content (*Ulloa Rojas et al., 2003*). Others have reported that that *Lactobacillus plantarum* were reduced phenolic compounds of pomegranate juice (*Mousavi et al., 2013*). *Rakesh et al. (2000)* reported that a 30% decrease in tannins of black

locust leaves after 30 days of incubation with tannase producer fungi caused a difference in digestibility and fermentation. Due to some differences between isolation conditions and the gastrointestinal tract of herbivores, enzymatic activity of isolates cannot indicate the true performance of microbes (*Kohl et al., 2015*). Also, the activity of tannase is induced in the presence of tannic acid that can be influenced by the season (*Dai et al., 2014*).

The observed higher DMD with biological treatment in SCM of mixed rumen bacteria and fungi, may have been due to the capacity of bacteria used to degrade anti nutritional factors present in the substrates and utilize their energy for growth and development. White-rot fungi (*Ceriporiopsis subvermispora* and *Cyathussteroreus*) as producer of tannin degrading enzymes have been reported to increase *in vitro* digestibility of *Sericea lespedeza* leaves by threefold (20–60%), but digestibility in the case of oak leaves was decreased. This could be due to different compositions of leaves, different white-rot fungi, and lower fermentation time used in studies with *S. lespedeza* leaves. An increase in fermentation time decreases cell solubility (consumed by the fungi for its growth) (*Makkar, 2003*).

Several researchers reported that *Klebsiella* strains capable of degrading phenolic compounds (*Motamedi et al., 2019; Pepi et al., 2013*). *Msimango (2018)* reported that *In vitro* dry matter degradability of *Acacia sieberiana* was affected positively by inocula from wild herbivores (giraffe, kudu, impala and consortia) in to goat microbial inoculum. *Tabacco et al. (2006)* showed that high tannin concentration in the diet caused reduction in microbial enzyme activities such as cellulase. These isolates probably enhanced pectinase and cellulase activity by changing pH and reduced toxic effects of tannins; and DM and OM degradation were improved. Researchers showed that the specific activity of tannin acyltransferase in the presence of *Selonomonas ruminantium* K2 was increased (*Babaei et al., 2015*).

Inhibitory effects and mechanisms of resistance to tannins in bacteria depend on the type and amount of tannins in the media (*Smith et al., 2005*). So, the observed maximum effects of biologically treated CL on NDFD and CPD is not unexpected, as the observed TT after processing indicate reduction pattern. In the present study, biological degradation of tannins by bacterial inoculation was led to decrease negative effect of tannin on the digestion activity of rumen microorganism which, previously reported by *Jayanegara et al. (2015)*. Tannin extracted from carob pod inhibited cellulolytic and proteolytic role of microbes in the artificial rumen technique (*Tagari et al., 1965*). Some researchers have reported reductions in NDF and CP digestibility in presence of tannin (*McAllister et al., 1994; Reed, 1995*). It is possible that improvement of the NDFD and CPD of CL leaves by bacteria processing with bacterial isolates due to the decrease of tannin content of *Conocarpus* might have increased digestibility and fermentation. Also, *Mosleh et al. (2014)* showed a reduction of the phenolic compounds by processing

of acorn with strains of *Streptococcus pneumonia* and *bovis* that increase digestibility and gas production.

Gas production from the fermentable fraction (b) were increased for both rumen bacteria and fungi in SCM for different incubation times, However, the c fractions were increased only at the SCM of fungi. These improvement in fermentation parameters of bacterial-treated leaves compared with the control may relate to better DMD, and CPD via decreasing TT in the former treatments (Table 1). The proper effect of some strains of *Klebsiella pneumoniae* on the fermentation parameters may be due to its higher enzymatic activity that can improve fermentation and digestibility. *McSweeney et al. (2001)* reported that bacterial strains of the same species can differ significantly in their tannin-degrading potential. *Elahi et al. (2012)* reported that the crude protein, fiber and phenolic compounds of the oak leaves were affected using the inoculum from two indigenous Iranian goats (i.e., Markhoz, Alam-out) which rich in tannin degrading bacteria, improve organic matter digestion and gas production. On the other hands, using the rumen fluid of Taleshi sheep fed tannin-rich diets, improved the organic matter digestion of the pistachio hulls (*Lotfi and Rouzbehan 2011*). In the similar research, *Motamedi et al. (2019)* reported that, GP and estimated parameters such as b and c were increased for *Q. infectoria* and only b, were increased for *Q. libani* following treatment with *K. pneumoniae*.

Another similar potential mechanism with NDFD and CPD values followed by breaking down the phenolic compounds of the substrates by bacterial inoculation, may occur for the gas production rate constant and thus increase nutrient digestibility. Several researchers reported that formation of tannin–macromolecule complexes which inhibit microbial enzymes and/or nutrient utilization by ruminal anaerobes (*McSweeney et al., 2001; Makkar, 2003*) could decrease cumulative gas production.

Conclusion

Biological processing of CL by *K. p* and *A. b* sp. can affect nutrients digestibility by bacteria and fungi of rumen, and processing with these bacteria decreased TT of CL. Therfore, it may be used as an alternative method for reduction tannin content of taniferus plants and prouned leaves of trees. But future studies should consider more, examining *in vitro* and *in vivo* effects of these isolates or other potent bacteria from different sources.

Uticaj listova *Conocarpus erectus* l. tretiranih sa *Klebsiella pneumoniae* i *Acinetobacter-om* kao bakterijama koje

razgrađuju tanin na digestivnu aktivnost mikroorganizama rumena

Tahereh Mohammadabadi, Alireza Jolazadeh, Zeinab Ghezi

Rezime

Trenutno istraživanje fokusira se na uticaj listova *Conocarpus erectus L*. (CL) tretiranih bakterijama koje razgrađuju tanin - *Klebsiella pneumoniae* (K. p) i *Acinetobacter* (A. b) na digestivne aktivnosti i fermentacione parametre bakterija i gljivica rumena arapskih ovaca. Ovi izolati mogu da koriste taninsku kiselinu kao jedini izvor ugljenika i energije. Osam vrsta *Klebsiella pneum*oniae (A1, A2, A3, A4, A5, A7, A8, A9) kao i *Acinobacter sp*. korišćeni su za biološki tretmane CL tokom perioda od deset dana. Zatim su u arapskim ovcama utvrđivane digestivna aktivnost, kao i neki parametri fermentacije rumena, bakterija i gljivica rumena, u specifičnom medijumu za kulturu (SCM). CL tretiran bakterijskim izolatima uticao je na smanjen ukupni tanin (TT) u poređenju sa netretiranim grupama (P <0,05). Na nestanak suve materije (DMD) uticao je biološki tretman i bakterija i gljivica rumena u različitim vremenima inkubacije, osim 3 i 6 h inkubacije u SCM mešovitih gljivica rumena, što je pokazalo isti obrazac sa neobrađenom CON grupom (P> 0,05). Nestanak NDF (NDFD) i nestanak CP (CPD) u SCM pomešanihh bakterija i gljivica rumena povećan je tokom svih vremena inkubacije (P <0,05) zbog bioloških procesa CL u poređenju sa tretmanom CON. Biološki tretirani CL povećao je proizvodnju gasa iz fermentabilne frakcije (b) obrasca u obe SCM pomešanih bakterija i gljivica rumena tokom svih inkubacionih vremena (P <0,05), ali konstantna stopa proizvodnje (c) povećana je samo kod SCM mešovitih gljivica rumen (P <0,05). Sveukupno, podaci su ukazivali da je bakterijska inokulacija *K. p* i *A. b. sp.* može poboljšati varenje i neke fermentacione parametre bakterija i gljivica rumena u arapskim ovcama.

Ključne reči: listovi konoplje, bakterije koje razgrađuju tanin, biološki tretman, mikroorganizmi rumena

Acknowledgements

The authors gratefully acknowledge the Agricultural Sciences and Natural Resources University of Khuzestan for their financial support.

References

ABDEL-HAMEED E.S.S., BAZAID S.A., SHOHAYEB M.M., EL-SHAYED M.M., El-WAKIL E.A. (2012): Phytochemical studies and evaluation of antioxidant, anticancer and antimicrobial properties of Conocarpus erectus L. growing in Taif, Saudi Arabia. European Journal of Medicinal Plants, 2(2), 93-112.

Al-KOAIK F., El-WAZIRY A.M., KHALIL A.I., METWALLY H., AL-MAHASNEH M.A. (2014): Evaluation of conocarpus (*Conocarpus erectus*) leaves and Bermuda grass (*Cynodon dactylon L*.) using chemical analysis and In vitro gas production technique. Bulgarian Journal of Agricultural Science, 20(4), 824-829.

AOAC. (1990): Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Washington, DC.

ARUNACHALAM M., MOHAN N., SUGADEV R., CHELLAPPAN P., MAHADEVAN A. (2003): Degradation of (+)-catechin by Acinetobacter calcoaceticus MTC 127. Biochimica et Biophysica Acta (BBA)-General Subjects, 1621(3), 261-265.

AZADI D., DIBAJ R., POURCHANGIZ M., NASER A., SHOJAEI H. (2014): Isolation and molecular identification of biodegrading Mycobacteria from water supplies of Iranian hospitals. Iranian Journal of Microbiology, 6, 240-245.

BABAEI Y., ROUZBEHAN Y., ALIPOUR D. (2015): Effect of rumen bacteria from sheep adapted to a tanninferous diet on in vitro fermentation parameters of pistachio hulls using bovine inoculum. Iranian Journal of Veterinary Research, 16, 357.

BAHAEDDINI R., KHAJAVI M., NAGHIHA R., PARSAEI S. (2016): Bioprocessing of acorn kernel with *Lactobacillus plantarum* to reduce its tannin. Journal of Research in the Ruminant, 28, 1-10. (In Farsi).

BELUR P.D., GOPAL M., NIRMALA K.R., BASAVARAJ N. (2010): Production of novel cell-associated tannase from newly isolated Serratia ficaria DTC. Journal of Microbiology and Biotechnology, 20(4), 732–736.

BLUMMEL M., MAKKAR H.P.S., BECKER K. (1997): In vitro gas production: a technique revisited. Journal of Animal Physiology and Animal Nutrition,77, 24-34.

CURIEL J.A., RODRIGUEZ H., ACEBRON I., MANCHENO J.M., RIVAS B.D.L., MUNOZ R. (2009): Production and physicochemical properties of recombinant Lactobacillus Plantarum tannase. Journal of Agricultural Food Chemistry, 57, 6224–6230.

DAI X., ZHANG B., WU X., JIANG L., ZOU Z., WANG A., WEI W., YANG S. (2014): Identification of tannin–degrading microorganisms in the gut of plateau pikas (Ochotona curzoniae) and root voles (Microtus oeconomus). Symbiosis, 63(1), 1-9.

ELAHI M.Y., ROUZBEHAN Y., REZAEE A. (2012): Effects of phenolic compounds in three oak species on in vitro gas production using inoculums of two

breeds of indigenous Iranian goats. Animal Feed Science and Technology, 176, 26-31.
FRUTOS P., HERVAS G., GIRALDEZ F.J., MANTECON A.R. (2004): Review; Tannins and ruminant nutrition. Spanish Journal of Agricultural Research, 2 (2), 191-202.
GARG S.K., MAKKAR H.P.S., NAGAL K.B., SHARMA S.K., WADHWA D.R., SINGH B. (1992): Oak (*Quercus incana*) leaf poisoning in cattle. Veterinary and Human Toxicology, 34, 161 – 408.
GHEIBIPOUR M. (2017): Isolation of the tannin-degrading bacteria from the gastrointestinal tract of deer and using for improving the nutritional value of tannin rich feeds. (unpublished M.Sc. thesis, Khuzestan Ramin Agriculture and Natural Resource University, Molasani, Iran)
JAYANEGARA A., GOEL G., MAKKAR H.P., BECKER K. (2015): Divergence between purified hydrolysable and condensed tannin effects on methane emission, rumen fermentation and microbial population in vitro. Animal Feed Science and Technology, 209, 60-68.
KOHL K.D., STENGEL A., DEARING M.D. (2016): Inoculation of tannin-degrading bacteria into novel hosts increases performance on tannin-rich diets. Environmental microbiology, 18(6), 1720-1729.
LOTFI R., ROUZBEHAN Y. (2011): The in vitro organic matter digestibility of pistachio hull using rumen fluid in Taleshi sheep. Iranian Journal of Animal Science, 42: 231-237 (abst).
MAKKAR H.P.S., SINGH B., KAMRA D.N. (1994): Biodegradation of tannins in oak (Quercus incana) leaves by Sporotrichum pulverulentum. Letters in Applied Microbiology, 18, 42–44.
MAKKAR H.P.S. (2000): Quantification of Tannins Tree and Shrub Foliage. A Labora-tory Manual for the FAO/IAEA Co-ordinated Research Project on Use of Nuclear and Related Techniques to Develop Simple Tannin Assays for Predicting and Improving the Safety and Efficiency of Feeding Ruminants on Tanniniferous Tree Foliage. Joint FAO/IAEA of Nuclear Techniques in Food and Agriculture. Animal Production and Health Sub-Programme, FAO/IAEA Working Document, IAEA, Vienna, Austria.
MAKKAR H.P.S. (2003): Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Ruminant Research, 49, 241-256.
MENKE K.H., STEINGASS H. (1988): Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. Animal Research and Development, 28, 7–55.
MCALLISTER T.A., BAE H.D., JONES G.A., CHENG K.J. (1994): Microbial attachment and feed digestion in the rumen. Journal of Animal Science, 72, 3004–3018.

MCSWEENEY C.S., PALMER B., McNEILL D.M., KRAUSE D.O. (2001): Microbial interactions with tannins: nutritional consequences for ruminants. Animal Feed Science and Technology, 91, 83-93.
MOHAMMADABADI T., JOLAZADEH A.R. (2017): Replacement of alfalfa hay (*Medicago sativa* L.) with subabul (*Leucaena leucocephala*) leaf meal in diets of Najdi goats: effect on digestion activity of rumen microorganisms. Tropical Animal Health and Production, 49(6), 1309-1316.
MOSLEH H., NAGHIHA A., KESHTKARAN A.N., KHAJAVI M. (2014): Isolation and identification of tannin-degrading bacteria from native sheep and goat feces in Kohgiloye and Boyer-Ahmad Province. International Journal of Advanced Biological and Biomedical Research, 2, 176-180.
MOTAMEDI H., AZIZI A., AHMADI M. (2019): Nutritive value of treated *Quercus infectoria* and *Quercus libani* leaves with the tannin-degrading bacterium *Klebsiella pneumoniae* for ruminant feeding *in vitro*. Journal of Applied Microbiology, 127(5), 1339-1348.
MOUSAVI Z., MOUSAVI S.M., RAZAVI S.H., HADINEJAD M., EMAM-DJOMEH Z., MIRZAPOUR M. (2013): Effect of fermentation of pomegranate juice by *Lactobacillus plantarum* and *Lactobacillus acidophilus* on the antioxidant activity and metabolism of sugars, organic acids and phenolic compounds. Food Biotechnology, 27: 1- 13.
MSIMANGO N.N.P. (2016): The effect of microbial consortia from wild herbivores on goat rumen fibrolytic activity and browse fermentation, 2nd International Conference on Livestock Nutrition, Brisbane, Australia 21-22.
NASCINMENTO D.K.D., SOUZA I.A., OLIVEIRA A.F.M., BARBOSA M.O., SANTANA M.A.N., JUNIOR D.F.P., LIRA E.C., VIEIRA J.R.C. (2016): Phytochemical screening and acute toxicity of aqueous extract of leaves of *Conocarpus erectus Linnaeus* in Swiss albino mice. Anais da Academia Brasileira de Ciências, 88(3): 1431-1437.
NRC. (2007): Nutrient Requirements of small ruminants: Sheep, Goats, Cervide, and New World Camelids. National Academy of Sciences, Washington, DC., USA, Pp. 362.
PEPI M., CAPPELLI S., HACHICHO N., PERRA G., RENZI M., TARABELLI A., ALTIERI R., ESPOSITO A., FOCARDI S.E., HEIPIEPER H.J. (2013): *Klebsiella* sp. strain C2A isolated from olive oil mill waste is able to tolerate and degrade tannic acid in very high concentrations. FEMS Microbiology Letters, 343, 105-112.
RAKESH D.D., BHAT T.K., SINGH B. (2000): Effect of fungal treatment on composition, tannin levels, and digestibility of black locust (*Robinia pseudoacacia*) leaves. The Journal of General and Applied Microbiology, 46(2), 99-103.
REED J.D. (1995): Nutritional toxicology of tannins and related polyphenols in forage legumes. Journal of Animal Science, 73, 1516-1528.

SAS (2002): SAS User′s Guide: Statistics. Ver 9.0. SAS Institute, Cary, NC, USA956.
SELINGER L.B., FORSBERG C.W., CHENG K.J. (1996): The Rumen: A unique source of enzymes for enhancing livestock production. Anaerobe, 2, 263–284.
SMITH A.H., ZOETEENDAL E., MACKIE R.I. (2005): Bacterial mechanisms to overcome inhibitory effects of dietary tannins. Microbial Ecology, 50, 197–205.
TABACCO E., BORREANI G., CROVETTO G.M., GALASSI G., COLOMBO D., CAVALLARIN L. (2006): Effect of chestnut tannin on fermentation quality, proteolysis, and protein rumen degradability of alfalfa silage. Journal of Dairy Science, 89(12), 4736-4746.
TAHMOURRESPOUR A., TABATABAEE N., KHALKHALI H., AMINI I. (2016): Tannic acid degradation by Klebsiella strains isolated from goat feces. Iranian Journal of Microbiology, 8(1), 14.
TAGARI H., HENIS Y., TAMIR M., VOLCANI R. (1965): Effect of carob pod extract on cellulolysis, proteolysis, deamination, and protein biosynthesis in an artificial rumen. Applied Microbiology, 13, 437–442.
ULLOA ROJAS J.B., VERRETH J.A.J., AMANO S., HUISMAN E.A. (2003): Biological treatments affect the chemical composition of coffee pulp. Bioresource Technology, 89, 267-274.
VAN SOEST P.V., ROBERTSON J., LEWIS B. (1991): Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74, 3583-3597.

Received 17 December 2019; accepted for publication 3 February 2020