Journal of Animal and Poultry Production

Journal homepage & Available online at: www.jappmu.journals.ekb.eg

Identification of Micro-Organisms that Tolerant to Anti-Nutritional Factors in the Rumen of Camel

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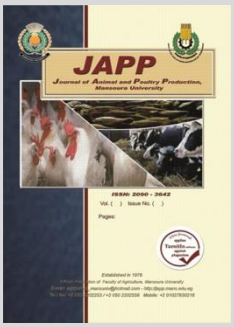

ABSTRACT

Fodder trees such as acacia are rich in antinutritional factors, mainly tannins, which constrain their utilization in animal feeding to fill the gap in feed resources. Rumen microbiota in the grazing and wild ruminant animals can detoxify plants' secondary metabolites. Therefore, understanding the interaction between plant and rumen microorganisms could improve the fodder plants utilization and reveal antimicrobial-resistant microbial isolates. This study was conducted to get insight into the bacterial colonization and degradation of non-extracted and extracted *Acacia saligna* in the rumen of three fistulated camels. The findings showed that acacia has a high content of crude protein, fiber, and tannins. Tannins extraction affected the chemical composition and rumen degradation of dry matter, crude protein, and neutral detergent fiber. Furthermore, rumen degradability was increased by prolonging incubation time from 6 to 72 h. The relative abundance of plant-attached bacteria in the camel rumen varied according to tannin extraction. The bacterial community was dominated by phylum Bacteroidetes and Firmicutes and the main bacterial genera were *Prevotella*, *RC9 gut group*, *Saccharofermentans*, *Butyrivibrio*, *Treponema* that were affected by tannin extraction. *Fibrobacteres* showed sensitivity to tannins and some genera such as *Alloprevotella*, *Selenomonas*, *Pyramidobacter* showed resistance to plant tannins, which highlight the camel rumen as an untapped source of tannin-resistant bacteria.

Keywords: Dromedary camel; rumen bacteria; acacia; tannins; next-generation sequencing.

INTRODUCTION

Grazing trees are promising solutions to supplement grazing land to overcome the feed deficiency and limited feeding resources in arid regions (McSweeney *et al*., 2002). Fodder trees, such as acacia, contain considerable amounts of protein, high dry matter, high digestible fiber, and soluble sugars (McSweeney *et al*., 2002; Goel and Makkar, 2012). Additionally, these plants tolerate drought stress and high salinity, which offers the potential to use them as fodder crops in different cultivation systems (El-Zaiat *et al*., 2020). Grazing plants in North Africa are dominated by acacia that is rich in tannins and other antinutrtional factors (Iqbal and Khan, 2001; McSweeney *et al*., 2002; El-Zaiat *et al*., 2020).

Acacia is widely used in animal feeding in tropical countries (Bhat *et al*., 2013). However, the availability of tannin and other antinutritional factors (AF) can seriously restrict the value of animal feeds by forming complexes with proteins and polysaccharides that generates astringent taste and reduce the palatability and animal feed intake (Sallam *et al*., 2009; Bhat *et al*., 2013). Plant tannins have an inhibitory effect on rumen microbiota and a toxic effect on the animal (McSweeney *et al*., 2002). Thus, the presence of plant toxins not only decline animal performance but also causes death (Bhat *et al*., 2013).

Certain ruminant animals can degrade these substances through rumen adaptation by regular grazing on tanniniferous plants or by the presence of tannin-binding proteins in the saliva of some ruminant species (McSweeney *et al*., 2002; Bhat *et al*., 2013). Dromedary camels were adapted to desert harsh conditions by different feeding and physiological mechanisms (Rabee *et al*., 2020). Camel can utilize poor-quality feeds such as thorny bushes, and toxic plant species that are avoided by other grazing animals (Iqbal and Khan, 2001). Therefore, camel is preferred animal in the tropical regions, wherever tanniniferous trees are available.

Rumen microbiota represent a complex metabolic network through which plant cell wall could be converted to microbial protein and organic acids that represent the main source of protein and energy for host animal (Hassan *et al*., 2020). Bacteria dominate the rumen microbial ecosystem and play a critical role in plant fermentation in the rumen (Mizrahi , 2013). Therefore, rumen bacteria have a significant effect on the animal efficiency (Hassan *et al*., 2020).

The rapid expansion of molecular techniques such as next-generation sequencing has enabled determining the changes in rumen microbiota under various treatment conditions, which offers the possibility to improve fiber digestibility of plant cell walls, and improving animal productivity (Rabee *et al*., 2020). Previous studies using high throughput total RNA sequencing showed that most of the rumen microbiota in camel rumen have fibrolytic activities and the bacterial community was predominated by phylum Bacteroidetes, Firmicutes, and Fibrobacteres (Rabee *et al*., 2020). High-concentration of plant tannins and other antintritional factors inhibit the growth, reproduction, and digestion enzymes of rumen microorganisms with different degrees (McSweeney *et al*., 2002; Derakhshani *et al*., 2016), which affect the rumen fermentation negatively. Previous studies reported that increasing dietary-tannin inhibited the

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DOI: 10.21608/jappmu.2022.114145.1025

activities of several microbial enzymes such as cellulase, urease, and protease (Makkar *et al*., 1988). Unlikely, some rumen microorganisms have different mechanisms for protecting the microbial cell wall or degrading and utilizing antinutritional substances (Min *et al*., 2005; Derakhshani *et al*., 2016; Loh *et al*., 2020). Subsequently, rumen bacteria contribute to the adaptability of ruminants to tannin-rich fodders (Smith *et al*., 2005).

Several chemical approaches have been developed for the deactivation or removal the antinutrtional factors from fodder plants to improve their nutritive value; however, these chemical treatments remove the soluble nutrients (Bhat *et al*., 2013). Also, Brouwer *et al*. (2019) reported that the extraction methods do not remove all tannin content from plants. Therefore, to develop proper detanninification strategies, it is important to carefully describe the nutritive value of extracted and non-extracted browsing plants. Soliva *et al*. (2005) reported a wide range of chemical composition and characteristics of ruminal fermentation for extracted and non–extracted moringa. On the other hand, plant extracts and compounds from natural plants were evaluated as alternatives to antibiotics to modulate the rumen microbial ecosystem and feed fermentation and to prevent bloat that could improve animal efficiency (Mergeduš *et al*., 2020).

Microbial colonization of ingested feed particles is the primary step in rumen fermentation; additionally, the structure of particles-attached microbes varies among forages types due to the difference in chemical composition, which influences the degradation of DM and other plant components (Liu *et al.* 2016). Microbial colonization to feed particles occurs shortly after ingestion and is highly affected by incubation times (Gharechahi *et al*., 2020). Therefore, understanding the interactions between rumen microbiota and extracted and non-extracted fodder plants is important to improve their utilization in animal feeding (Soliva *et al*., 2005; Elliott *et al*., 2018; Gharechahi *et al*., 2020). There is a lack of information regarding the diversity and composition of rumen bacteria colonizing tannin-rich fodder plants such as acacia. Therefore, this study used PCR-amplicon sequencing through Illumina Mi-Seq to assess the differences in the structure of colonizing-bacterial communities along with degradation of non-extracted and acetone-extracted acacia in the rumen of dromedary camels.

MATERIALS AND METHODS

Plant collection and tannins extraction

Acacia trees were grown in the grazing area in Maryout Research Station, Desert Research Center, Alexandria, Egypt. Samples of leaves and wet stems were collected from an ungrazed field. Then, they were oven-dried at 50 oc and ground to pass a 1 mm sieve. Phenolic compounds in the ground plant samples were extracted by 70% aqueous acetone (v/v) according to the protocol of Makkar (2003).

Animals and *In situ* rumen incubation

Three fistulated female dromedary camels were used in this study. The camels were kept in individual pens and were fed ad libtum on Barseem hay and allowed free drinking water. Four nylon bags (10 × 20 cm; pore size = 50 μm) containing 3g of extracted and non-extracted acacia were placed into the rumen of each fistulated camel before morning feeding. Two bags were returned from each camel's rumen after 6h, one bage was returned after 48 h, and one bage was returned after 72 h One bag (6 h) was rinsed with sterilized distilled water and transferred to a sterilized 50 ml tube and frozen for DNA extraction to identify the fiber-attached bacteria. Subsequently, other bags were rinsed with cold water until the water ran clear; then squeezed and dried at 60°C for 48 h to determine the disappearance of dry matter (DMD), neutral detergent fiber (NDFD), and crude protein (CPD).

Microbial cells recovery, DNA extraction, PCR amplification, and sequencing.

The microbial cells' dissociation from incubated extracted and non-extracted acacia was conducted using dissociation solution according to the protocol described by Pope *et al*. (2010). The dissociated microbial cell pellets were collected by centrifugation at 12,000 × g for 5 min and used in DNA extraction by i-genomic Stool DNA Extraction Mini Kit (iNtRON Biotechnology, Inc.). DNA quality was verified using agar gel electrophoresis. The V4-V5 region of 16S rDNA gene was amplified using primers 515F (5′-GTGYCAGCMGCCGCGGTAA-3′) and 926R (5′-CCGYCAATTYMTTTRAGTTT-3′) (Walter *et al*. (2015). PCR amplification was conducted in a thermal cycler under the following conditions: 94°C for 3 min; 35 cycles of 94°C for 45 s, 50°C for 60 s, and 72°C for 90s; and 72°C for 10 min. PCR-products purification and preparation for sequencing were conducted according to Comeau *et al*. (2017). The PCR-products were then sequenced using the Illumina Mi-Seq system in Integrated Microbiome Resource (Dalhousie University, Canada). All the generated paired-end (PE) Illumina raw sequence reads were processed in R (version 3.5.2) using DADA2 (version 1.11.3) pipeline as described by Callahan *et al*. (2016) and were compared using the SILVA reference database.

Chemical analysis

Dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF) were measured in extracted and non-extracted acacia before and after incubation in the rumen. DM was measured by drying the residual material for 48 h at 60 oc. ADF and NDF were determined by the method of Van Soest *et al*. (1991) without sodium sulfite. Crude protein (CP) was determined according to AOAC (1997). Total tannins were determined according to Makkar (2003a) using Folin-Ciocalteu method.

Statistical analysis

The effect of tannins extraction (E), incubation time (T) and T*E interactions on the differences in DMD, CPD, and NDFD of extracted and non-extracted acacia were studied using Mixed ANOVA. The effect of tannins extraction on the relative abundances of bacterial community colonizing extracted and non-extracted acacia in the rumen of camel was studied using indpendant T-test. The statistical analyses were performed using the SPSS v. 20.0 software package (SPSS, 1999).

RESULTS AND DISCUSSION

Effect of tannin extraction on chemical composition and *In situ* degradation

Understanding the colonization of rumen microorganisms to antinutritional-rich plants is crucial for utilizing grazing plants in animal feeding (McSweeney *et al*.,

2002; Elliott *et al*., 2018), and identifying tannin-resistant microorganisms that could be used as feed additives to improve grazing efficiency in susceptible animals (Smith *et al*., 2005; Derakhshani *et al*., 2016). The chemical compositions on DM basis of experimental plants are shown in Table 1.

Table 1.The chemical composition of extracted and non-extracted acacia.

Item	Acacia Non-Extracted	Acacia Extracted
Neutral detergent fiber (NDF)	53.5	55.77
Acid detergent fiber (ADF)	31.45	44.64
Crude protein (CP)	19.98	19.83

The results showed that acacia contains 6.01 % total tannins. This finding agrees with previous studies (Sallam *et al*., 2009: Soltan *et al*., 2012; El-Zaiat *et al*., 2020). Besides tannins and other phenolic compounds, acacia contains several antinutritional factors such as oxalate, saponins, cyanogenic glycoside, and fluoroacetate (McSweeney *et al*., 2002; Loh *et al*., 2020).

Moreover, tannin extraction affected the chemical composition as the NDF and ADF were increased by extraction. Brouwer *et al*. (2019) mentioned that the extraction not only removes tannins but also removes proteins, ash, soluble sugars, polysaccharides, and lipids, which affects the digestibility of plants' nutrients (DM, CP, and NDF) and bacterial colonization of extracted and non-extracted plants in the rumen (Elliott *et al*., 2018). The results revealed that DMD, CPD, and NDFD tended to increase ($P < 0.05$) with the prolonging the incubation time (Table 2). Furthermore, tannins extraction increased the CPD and NDFD significantly ($P<0.05$) at 6 and 48 h. These findings are in the line with previous studies (Min *et al*., 2003; Soltan *et al*., 2012). The decrease in the DMD at 72 h in extracted acacia could be attributed to higher NDF in extracted material (Gharechahi *et al*. (2020). Van Soest (1994) reported that the digestibility of high-cellulose forages in the rumen is constrained by the fermentation of plants' cell wall (NDF).

Table 2. The disappearance (%) of dry matter (DMD), crude protein (CPD), neutral detergent fiber (NDFD) of extracted and non-extracted acacia at 6, 48, and 72 hours.

	Extracted acacia					Non-extracted acacia					P value		
	6h	48h	72h	Mean	SE	6h	48h	72h	Mean	SE	E	T	E*T
DMD	28.5	40	43.3	37.2	2.7	22.4	36.3	48.3	35.7	3.7	0.536	0.0001	0.019
CPD	16.75	26.9	37.6	27	3	8.6	23.2	23.8	18.5	2.5	0.0001	0.0001	0.049
NDFD	24.45	33.9	45	34.4	3	20.8	27.8	29	25.9	1.3	0.0001	0.0001	0.0001

Analysis of bacterial community attached to the extracted and non-extracted acacia

The Illumine sequencing of the V4 region on the 16S rDNA in 6 samples revealed that bacterial community was affiliated to 15 bacterial phyla. Additionally, nine phyla were observed in all samples, including, Bacteroidetes, Cyanobacteria, Firmicutes, Kiritimatiellaeota, Planctomycetes, Proteobacteria, Spirochaetes, Synergistetes, and Tenericutes. Bacterial phyla that were not observed in raw or non-extracted acacia, including Actinobacteria, Fibrobacteria, Lentisphaerae, Patescibacteria, and Verrucomicrobia. In addition, phylum Chloroflexi was disappeared from extracted acacia (Table 3).

Table 3. The relative abundances (%) of bacterial phyla colonized extracted and non-extracted acacia in the rumen of camels.

Phylum	Non-Extracted acacia	Extracted acacia	Mean	SE	P value
Actinobacteria	0	0.079996	ND	ND	ND
Bacteroidetes	84.37	83.73	84.05	0.47795	0.56
Chloroflexi	0.076	0	ND	ND	ND
Cyanobacteria	0.6	0.21	0.4	0.13	0.13
Firmicutes	11.59	11.46	11.53	0.6	0.92
Fibrobacteria	0	0.10	ND	ND	ND
Kiritimatiellaeota	0.10	0.15	0.12	0.02	0.38
Lentisphaerae	0	0.069	ND	ND	ND
Patescibacteria	0	0.037	ND	ND	ND
Planctomycetes	0.59	1.06	0.82	0.3	0.49
Proteobacteria	1.4	0.39	0.90	0.23	0.001
Spirochaetes	0.68	0.9118	0.7982	0.26503	0.716
Synergistetes	0.2208	0.1962	0.2085	0.02604	0.688
Tenericutes	0.2356	1.5963	0.9159	0.33653	0.013
Verrucomicrobia	0	0.067177	ND	ND	ND

ND=Non-determined

Tannins extraction affected the relative abundance of some bacterial phyla such as Proteobacteria and Tenericutes ($P < 0.05$). The bacterial community that has colonized extracted and non-extracted acacia was dominated by phylum Bacteroidetes (84.05%) and Firmicutes (11.53%), which agrees with similar studies conducted on hay and straw (Liu *et al*., 2016), and *Lotus corniculatus* that have high-tannin content (Elliott *et al*., 2018). Fibrobacteria is the main cellulolytic bacteria in the rumen (Nathani *et al*., 2015) and sensitive to tannins (Martin, 1992; Bae *et al*., 1993), which demonstrate the presence of the Fibrobacteria and other phyla only in extracted acacia. Moreover, Gharechahi *et al*. (2015) found a positive association between NDF and Fibrobacteria.

Phylum Bacteroidetes dominated the bacterial community and showed higher relative abundance in non-extracted acacia without significant difference (Table 3). A similar result was obtained by Gharechahi *et al*. (2015) who studied the solid - attached microbiota in the rumen of grazing camels. The members of this phylum ferment a wide range of substrates, including cellulose, pectin, and soluble polysaccharides, and unclassified Bacteroidetes are more specialized in lignocellulose degradation (Mackenzie *et al*., 2015). This phylum was dominated by the family Prevotellaceae, Rikenellaceae, Muribaculaceae, and S11_gut_group (Table 4). Moreover, family Prevotellaceae was dominated by the genus *Prevotella* and *Prevotella* that were higher in non-extracted acacia without significant difference (Table 4). Genus *Prevotella* is fibrolytic bacteria that ferment several substrates, including cellulose, hemicellulose, pectin, proteins, and peptides (Liu *et al*., 2016). The higher abundance of this genus in non-extracted acacia indicates that its abundance is highly associated with the availability of specific growth substrates and highlights its resistance to plant tannins (Min *et al*., 2005). Genus

Alloprevotella showed adaptability to condensed tannin, which might explain the presence of this genus in non-extracted acacia (Salami *et al*., 2018; Mannelli *et al*., 2019). Genus RC9 gut group, that dominated family Rikenellaceae, had higher representation in non-extracted acacia (Table 4), which indicates to the ability of this group to withstand plant-tannins. Our explanation is supported by Salami *et al*. (2018), who observed that the members of the family Rikenellaceae tolerated condensed tannins from *Uncaria gambir*. In addition, this genus is specialized in cellulose fermentation (Bian *et al*., 2013; Mackenzie *et al*., 2015).

Table 4. The relative abundances (%) of dominant bacterial genera colonized extracted and non-extracted acacia in the rumen of camels.

	Non-Extracted acacia	Extracted acacia	Mean	SE	P value
Phylum: Bacteroidetes					
Family: Prevotellaceae	45.3	39.25	42.3	2.39	0.2
• Genus: Prevotella_1	31.5	25.9	28.7	1.9	0.1
• Genus: Prevotella_7	2.3	0.5	1.4	0.54	0.09
• Genus: Alloprevotella	0.349913	0	ND	ND	ND
• Genus: Prevotellaceae_UCG-004		0.1	ND	ND	ND
Family Rikenellaceae	8.5	6.3	7.4	1	0.3
• RC9_gut_group	8.3	6	7.2	1	0.28
Family: Muribaculaceae	6.6	21.1	13.8	3.5	0.009
Family: BS11_gut_group	0.4	0.3	0.3	0.09	0.6
Phylum: Firmicutes					
Family: Lachnospiraceae	2.7	3.3	3	0.36	0.45
• Genus: Acetitomaculum		0.6			
• Genus: Butyrivibrio_2	0.59	0.54	0.57	0.36	0.6
• Genus: Moryella	0.22	0	ND	ND	ND
Family: Ruminococcaceae	3.8	6.6	5.2	0.8	0.06
• Genus: Saccharofermentans	0.24	0.35	0.3	0.07	0.5
• Genus: Ruminococcus_1	0.27	0	ND	ND	ND
• Genus: Ruminiclostridium_6		0.25	ND	ND	ND
• Genus: Ruminococcus_2	0.25	0.43	0.34	0.07	0.28
• Genus: Caproiciproducens		0.1	ND	ND	ND
Family: Veillonellaceae	4.76	0.66	2.7	1.1	0.06
• Genus: Selenomonas_1	3.4	0	ND	ND	ND
• Genus: Selenomonas	0.66	0	ND	ND	ND
Family_XIII	0.27	0.37	0.32	0.02	0.007
• Genus: Mogibacterium		0.08	ND	ND	ND
• Genus: Clostridium		0.28	ND	ND	ND
• Genus: Romboutsia		0.08	ND	ND	ND
Phylum: Proteobacteria					
• Genus Succinivibrionaceae_UCG-002	0.65	0.16	0.4	0.1	0.03
• Genus Desulfovibrio	0.2	0.13	0.17	0.02	0.04
Phylum: Spirochaetes					
• Genus: Treponema_2	0.6	0.75	0.67	0.27	0.77
• Genus: Sphaerochaeta	0.15	0	ND	ND	ND
Phylum: Synergistetes					
• Genus: Fretibacterium	0.19	0.18	0.19	0.02	0.8
• Genus: Pyramidobacter	0.13	0	ND	ND	ND

ND=Non-determined

The members of phylum Firmicutes were dominated by four families Lachnospiraceae, Ruminococcaceae, Veillonellaceae, and Family_XIII, that were greater in extracted acacia except for Veillonellaceae that showed higher relative abundance in non-extracted acacia (Table 4). Family Lachnospiraceae was classified mainly to genus *Butyrivibrio*; furthermore, genus *Acetitomaculum* found only in extracted acacia and genus *Moryella* was observed only in non-extracted acacia. Family, Ruminococcaceae showed greater relative abundance in extracted acacia and was affiliated mainly to genus *Saccharofermentans* and *Ruminococcus_2*. Family Veillonellaceae was classified mainly to genus *Selenomonas* that was found only in non-extracted acacia. Members of Family_XIII were found only in extracted acacia. Genus *Butyrivibrio* and *Ruminococcus* are polysaccharides-degrading bacteria (Liu *et al*., 2016; Gharechahi *et al*., 2020). Additionally, *Butyrivibrio* has proteolytic activities that expains the higher representation in non-extracted acacia (Liu *et al*., 2016). Also, *Butyrivibrio* has the ability to degrade tannin, explaining the higher abundance of this genus in non-extracted acacia (Smith *et al*., 2005; Bhat *et al*., 2013; Derakhshani *et al*., 2016). The study of McSweeney *et al*. (2001) mentioned that supplementation of an animal diet with calliandra that is rich in tannin declined the population of *Fibrobacteres* and *Ruminococcus*, which support our finding. Genus *Selenomonas* that dominated family Veillonellaceae can utilize tannin as a carbon source, which illustrates the presence of this genus in non-extracted acacia (Smith *et al*., 2005).

Phylum Spirochaetes was dominated by genus *Treponema* with more representation in extracted acacia. Phylum Synergistetes was assigned into *Fretibacterium* and *Pyramidobacter*. *Pyramidobacter* was found in non-extracted acacia only. *Fretibacterium* has the adaptability to phenolic compounds that might explain the prevalence of this genus in non-extracted acacia (Evans and Martin, 2000; Yu *et al*., 2020). Additionally, genus *Pyramidobacter* detoxifies toxic compounds found in many plant species including acacia. In addition, this genus has a potential role in cellulose degradation, highlighting the presence of this phyla in non-extracted acacia (Pan *et al*., 2017; Leong *et al*., 2017; Loh *et al*., 2020).

Our results highlight acacia as appropriate fodder plants in animal feeding, which agrees with El-Zaiat *et al*., (2020). However, using acacia in animal feeding requires different chemical treatments and feeding methods to reduce the negative effect of tannins (Bhat *et al*., 2013). Additionally, animals should be adapted to the feeding on acacia and transferring rumen content of adapted animals to other sensitive animals is an effective technique to reduce the toxicity of tannins (Odenyo *et al*., 1997; McSweeney *et al*., 2002). In addition, Odenyo *et al*. (1997) reported that introducing acacia to sheep's diet at a low level (100 g/head/day) did not show negative results. Our results show that camel contains several bacterial genera that resist plant toxic compounds. Thus, camel rumen could be a source of bacterial isolates that could be transferred to other sensitive ruminants to protect them against plant toxins (Odenyo *et al*., 1997; McSweeney *et al*., 2002). Investigation of secondary metabolites in fodder plants and their interaction with rumen microbiota could improve their nutritive value besides these compounds have different biological activities as antimicrobial, and anti-inflammatory (Benhammoua *et al*., 2009).

CONCLUSION

The composition and tannins content of fodder plants such as acacia are the main drivers of microbial colonization in animal rumen. Different chemical treatments and feeding methods must be applied with acacia to be used in the animal feeding. This study expalins the adaptability of camels to plant toxins; therefore, camel rumen could be a source of detoxifier bacteria with different applications.

ACKNOWELDGMENT

The authours are grateful to staff of Maryout Research Station for their support during the experiment.

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تعريف الكائنات الحية الدقيقة المتحملة للمركبات المضادة للتغذية في كرش الإبل

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الاشجار العلفيه مثل الاكاسيا غنيه بمضادات التغذيه مثل التانينات والتى تعوق الاستفاده من هذه الاشجار فى تغذية الحيوان لسد الفجوه فى المصادر العلفيه. ميكروبات الكرش فى المجترات الرعويه او البريه لديها قدره على تكسير السموم النباتيه. لذلك فأن فهم العلاقه التفاعليه بين النبات وميكروبات الكرش يمكن ان يؤدى الى زيادة الاستفاده من النباتات العلفيه كما يمكن ان يؤدى الى اكتشاف سلالات ميكروبيه لديها القدره على مقاومة المضادات الميكروبيه. هذه الدراسه تم تنفيذها لفهم الاستعمار البكتيرى والهضم للاكاسيا المستخلصه والغير مستخلصه فى كرش ثلاث نوق فسيولا. أظهرت الدراسه ان الاكاسيا تحتوى على نسبه عاليه من البروتين والالياف والتانين كما ان نزع او استخلاص التانين كان له تأثير على التركيب الكيماوى وكذلك هضم الماده الجافه والبروتين ومستخلص الالياف المتعادل. كما ان الهضم فى الكرش زاد بزياده وقت التحضين. اظهرت النتائج ان نسب وجود البكتريا الملتصقه بالاكاسيا تاثرت بالاستخلاص . كانت العشائر البكتيريه السائده هى Bacteroidetes and Firmicutes كما ان الاجناس البكتيريه السائده هى *Prevotella, RC9_gut_group, Saccharofermentans, Butyrivibrio, Treponema_2* والتى تأثرت بنزع التانين. *وجد ان بكتريا Fibrobacteres* ذات حساسيه للتانينات كما ان *Alloprevotella, Selenomonas_1, Pyramidobacter* لديها قدره على تحمل التانيات مما قد يلقى الضوء على على كرش الابل كمصدر للبكتريا المتحمله للتانينات.