

## CAMEL RUMINAL BACTERIA, THEIR COUNT AND ANTIBACTERIAL EFFECT IN COMPARISON TO OTHER RUMINANT ANIMALS.

Fawziah A. Abdulla \*

Adnan M. Al-Rodhan\*

\*Department of Microbiology, College of veterinary Medicine, University of Basrah ,Basrah,Iraq

\*\*Department of Microbiology, College of veterinary Medicine, University of Basrah ,Basrah,Iraq

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### ABSTRACT

The bacterial and fungal population in the rumen fluid was measured by using different cultures media and incubation temperature. The Psychrophil, Camel, higher mean of mesophils, psychrophils, coli form and fungi count was found in the rumenal fluid of sheep. While higher mean of *staphylococci* and *Escherichia coli* count was observed in the rumenal fluid of camel. Significant mean difference among microbial population in the rumenal fluid was observed between camel and sheep and between sheep and cow concerning the psychrophilic bacteria. Also significant mean difference was observed in *E.coli* mean count among camel, sheep and cow rumenal fluid microbial population. There was no significant difference in the mean count of mesophils, coliform, *staphylococci* and fungi.

A freshly isolated *E.coli* from rumenal fluid of camel had antibacterial activity against *Streptococcus spp* and *Staph aureus*.

### INTRODUCTION

Ruminant animals are able to use plant fiber primarily composed of cellulose and xylan as an energy source because of symbiotic relationship with microbes (bacteria, fungi and protozoa) in the rumen. Of the rumen microbes, bacteria and fungi produce a wide range of highly active plant cell-wall degrading enzymes and their contribution to fiber digestion is estimated to be 80% of total activity<sup>(1)</sup>. Although rumen fungi possess superior ability such as penetration of plant cell wall and solubilization of lignin, their contribution to fiber digestion might be low, due to small biomass (8% of total microbial mass)<sup>(2)</sup>. Ruminant bacteria play a particularly role in the biological degrading of dietary fiber because of their much larger biomass. Bacteria inhabiting the rumen have been classified into four groups depending on their environmental existence; free-living bacteria, bacteria associated with feed particles and bacteria associated with rumen epithelium<sup>(3,4)</sup>. Some ruminal bacteria produce lactic acid at a rapid rate, and this acid can cause pronounced declines in ruminal pH, founder, and in severer cases, even death of the animal. Ruminant nutritionists, farmers and ranchers have used ionophores and other antibiotics to modify ruminal fermentation and increase the efficiency of feed digestion. However, there has been an increased perception that antibiotics should not be routinely used as feed additives<sup>(5)</sup>. Some bacteria produce small peptides (bacteriocins) that inhibit gram-Positive bacteria, and the

bacterioin , nisin, had effects on ruminal fermentation that were similar to ionophore .A variety of ruminal bacteria produse bacteriocins , but the effect of these peptides on ruminal fermentation had not been examined <sup>(5)</sup>.

The aim of this study is to count rumenal bacteria in different animal species and determination of antimicrobial activity of rumenal bacteria against other bacteria.

## MATERIALS AND METHODS

### Samples:

A total of 45 rumenal flued were randomly obtained for the microbial analysis .Fiften samples were collected from each of slaughtered camels ,sheep and cow seen in Basrah slaughter house .

### Microbial analysis:

All media used were obtained from Oxoid limited, London .The following test were conducted according to method of mahmood<sup>(6)</sup> .Ruminal fluid diluted 1 to 100 in basal medum)was streaked on to nutrient agar ,a total aerobic plate count with incubation at 37C° for 48 hr for mesophiles (APC) and at 4C° for 10 days for psychrophiles (PPC). Total coliform (TC) and *Escherichia Coli* (*E.coli* )were determined by using MacConkey agar and eosine methylen blue (EMB) agar with the incubation at 37C° for 48hr and at 45.SC° for 48hr ,respectively. Positive MacConkey plates were used to calculate TC and EMB plates for *E. coli* counts .The isolates from EMB plates were tested for indol production , methyle red ,Voges –Proskaur reation and citrate utilization *Staphylococcus aureus* count on manitol salt a gar at 352 for 48hr .Typical *staph . aureus* colonies were counted and randomly picked up and inoculated into brain heart infusion (BHI) broth for 24hr at 35C° and subjected to coagulase test .Fungi ( molds and yeasts)were enumerated on saburud dextrose agar (SDA) and incubated at 22C ° for 5 days.

Plated in all cases were incubated in triplicates and the microbial count were expressed as mean colony forming units per gram(CFULG).

### Detection of antibacterial activity of rumenal bacteria:

The antibacterial activity against gram positive bacterial growth was determine according to method of Peres *et al*<sup>7</sup>.The colonies of *E coli* from rumenal fluid that grown on EMB were picked and transferred to broth, 0.1 ml of this broth was taken from the broth to the wells{0.5mm in diameter} on the plates of mullor hinton agar MHA seeded with approximately 106 cell/ml of each *Streptococcus spp*, *Staphylococcus aureus* standard strain and *Bacillus subtilis* which were obtained from microbiology laboratory of biology department/ college of science. The plates were re-incubated at 37c° for 24hr and each isolate was scrod for its ability to create a distinct zone of clearing (≥3mm) in the agar overlay

### Statistical analysis

The result were analysis by one-way ANOVA test using statistical package for the social sciences (SPSS) version 9.0.

All data were expressed as meant std .Error . Differences between data were compared by least significant deference<sup>8</sup>.

## RESULTS AND DISCUSSION

The microbial analysis data of camel, sheep and cow rumenal fluid used in the present study are summarized in table- 1 and 2 as mean cfu/ml.

In table-1 the highest mesophiles and psychrophiles count were observed in sheep rumenal fluid in compare to camel and cow rumenal fluid but the significant difference among them was observed in psychrophiles mean count.

Table-2 show the differences in the mean bacterial count of camel, sheep and cow rumenal fluid in relation to different types of cultural media used in the isolation of these bacteria. According to this table the results of bacterial cultural media were higher than the results of SDA medium which was used in the isolation of fungi. This result was in agreement with the result of Stewart *et al*<sup>(9)</sup>.who reported that the rumen microbial ecosystems comprises at least 30 predominant bacterial species at  $10^{(10)}$  to  $10^{(11)}$  cell/ml of rumenal fluid and five species of fungi ( $10^5$ cell/ml) .The mean of TC count on MacConkey agar was higher in rumenal fluid of sheep. The mean *E coli* count was higher in camel rumenal fluid when EMB medium was used in the isolation.The mean *Staph aureus* count on manitol salt agar was higher in camel rumenal fluid in compare to other animals used in this study. The significance difference was observed in the mean *E coli* count of rumenal fluid of camel, sheep and cow only and there is no significant difference in the TC and *Staph aureus* mean count.The higher mean of fungal count was observed in sheep rumenal fluid in comparison to other studied rumenal fluid but this difference is not significance ( Table - 2). The differences in mean bacterial count of the camel, sheep and cow rumenal fluid may be related to difference in the diet fed which influence the number and relative proportion of the different microbial species in the rumen.

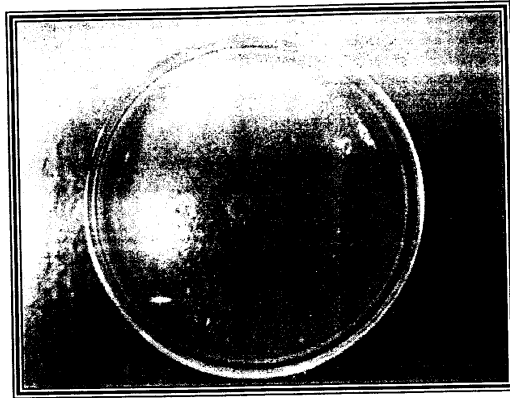
The antimicrobial activity of rumenal bacteria was observed in *E coli* isolates from camel rumenal fluid only .This activity was tested against gram positive bacteria like *Staph aureus* fig.1,Streptococcus spp fig.2 and *bacillus subtilus* fig.3.These results were in agreement with Other studies which reported that some rumenal bacteria produce bacteriocins, there were speculation that this compound had effects on rumenal fermentation

**Table (1):-** The bacterial isolates count of camel, sheep and cow ruminal fluid in relation to temperature of incubation

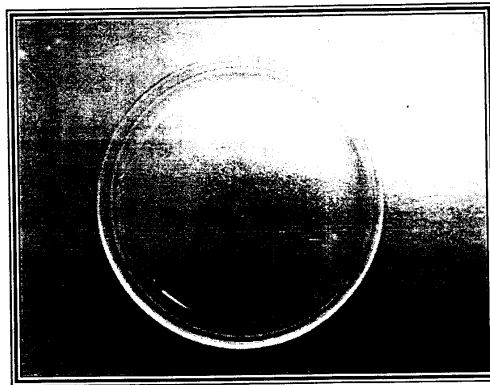
Microorganism	Mean $\pm$ SD		
	Camel	Sheep	Cow
Mesophiles	137.4667	162.8333	131.3333
	$\pm$	$\pm$	$\pm$
	68.4915	63.5612	66.2419
Psychrophiles	48.9667	97.2333	45.2667
	$\pm$	$\pm$	$\pm$
	62.2462	53.1561	35.6871

**Table (2):-** The difference in rumenal bacterial count in relation to different cultural media.

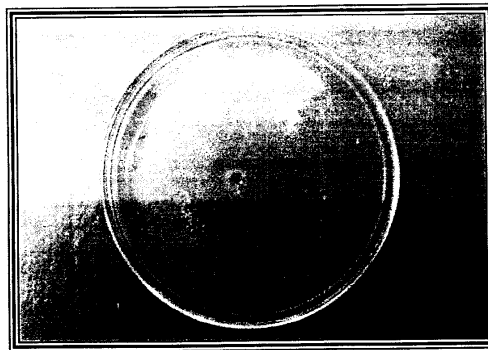
Medium Microorganism	Mean $\pm$ SD		
	Camel	Sheep	Cow
MacC. (TC)	65.4667	66.3000	63.0333
	$\pm$	$\pm$	$\pm$
	49.2954	29.9355	33.7551
Manitol (staph)	54.3667	37.5667	47.667
	$\pm$	$\pm$	$\pm$
	42.67967	24.1719	24.6030
EMB ( <i>E. coli</i> )	59.2000	27.6333	27.4000
	$\pm$	$\pm$	$\pm$
	42.4310	21.8199	215347
SDA (Fungi)	18.7000	28.5333	18.1933
	$\pm$	$\pm$	$\pm$
	15.8281	20.5707	11.0922



**Fig (1):-** The antibacterial activity of rumenal *E. coli* against *Staph. aureus*.



**Fig (2):-** The antibacterial activity of rumenal *E. coli* against *Streptococcus* spp.



**Fig (3):-** The antibacterial activity of rumenal *E. coli* against *Bacillus subtilis*

The bacterial and fungal population in the rumen was differed according to differences in the species of animals and different culture media and incubation temperature. Ruminant bacteria has antimicrobial effect against other bacteria.

### بكتريا كرش الجمال، أعدادها وتأثيرها المضاد للجراثيم مقارنة مع الحيوانات المجتررة الأخرى

فوزية علي عبد الله  
عدنان موسى الروضان  
فرع الاحياء المجهرية، كلية الطب البيطري، جامعة البصرة، البصرة، العراق

#### الخلاصة

تم حساب مجاميع الجراثيم والفطريات الموجودة في سائل الكرش باستخدام أوساط زرعية مختلفة وبدرجات حضان مختلفة. أن أعلى معدل لعدد الجراثيم المحبة لدرجات الحرارة المعتدلة والمحبة للبرودة والجراثيم القولونية والفطريات لوحظ في سائل كرش الأغنام بينما لوحظ أعلى معدل لعدد جراثيم المكورات العنقودية والشريكية القولونية في سائل كرش الجمال. ولوحظ فرق إحصائي معنوي في معدل عدد الجراثيم المحبة للبرودة بين الجمال والأغنام وبين الأغنام والأبقار وكذلك لوحظ فرق إحصائي معنوي في معدل عدد الشريكية القولونية الموجودة في سائل كرش الجمال والأغنام والأبقار. ولم يلاحظ فرق إحصائي معنوي في معدل عدد الجراثيم المحبة لدرجات الحرارة المعتدلة والجراثيم القولونية والمكورات العنقودية والفطريات. أن الشريكية القولونية المعزولة من سائل كرش الجمال أظهرت فعالية مضادة للجراثيم المكورات السحبية والمكورات العنقودية.

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