RUMINAL BACTERIA ISOLATE RESPONSES TO TANNIC ACID IN NORTHERN EGYPTIAN SHEEP, CATTLE AND BUFFALO

A.Z.M. Salem[1] and Y.M.Gohar[2]

[1]Department of Animal Production, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria, Egypt
[2]Department of Botany (Division of Microbiology), Faculty of Science (El-Shatby), Alexandria University, Alexandria, Egypt.

(Received 27/1//2007, accepted 14/3/2007)

SUMMARY

This study was conducted to investigate the differences among ruminant species to tolerate the negative effect of tannic acid (TA) under the northern Egyptian conditions. Rumen samples were collected, immediately after animal's slaughter, in the slaughter house of the Faculty of Agriculture, Alexandria University, during the dry season. Fifty-nine ruminal bacteria isolates, detected from two sheep, two cattle-imported, three cattle-female, one cattle-male, two buffalo-female, six buffalo-male, and four buffalo-calves, were 9, 5, 11, 2, 4, 20, and 8 isolates, respectively. Sensitivity of ruminal bacteria isolates to 0.63, 1.25, 2.50, 5.00 and 10.00 mg TA per milliliter was determined by the clearance zone (CZ) of Kirby-Bauer disc diffusion susceptibility test. Dramatically increase in the sensitivity of all bacterial isolates with increase the concentration of TA was observed. In cattle, one isolate was detected to tolerate up to 10 g TA/litter in comparison to all cattle isolates, while in adult buffalos (female and male), four isolates and others in buffalo-calves were also resistant for the highest dose of TA. Highest sensitivity (higher CZ value) was observed in sheep and cattle (male and female). Buffaloes (male, female, and calves) reported the lowest ($P<0.05$) sensitivity to TA concentrations than other animal groups. Within cattle groups, cattle-females were more resistance (low CZ; $P<0.05$) to TA than cattle-imported or cattle-male. No significant differences ($P>0.05$) were observed among buffalo groups (male, female, or calves). Twenty seven percent of the bacterial isolates in buffaloes were tolerate more than 1% TA, while in sheep, all bacterial isolates did not tolerate more than 0.125% TA. In cattle, only 4% of isolates were tolerated up to 1% TA. According to the variability among ruminant species, a significantly differences were observed between buffalos and sheep ($P\leq0.02$) as well as with cattle ($P\leq0.005$) in response to TA (based upon F-test value). No significant differences ($P>0.05$) were observed between sheep and cattle ($P\leq0.95$), while the buffalo-calves were not differ with all ruminant species. In Egypt, this appears to be the first study regarding isolation of TA-degrading bacteria from the rumen fluid of some ruminant species such buffaloes and cattle.

Keywords: buffalo, cattle, sheep, clearance zone, ruminal bacteria isolates, tannic acid.

INTRODUCTION

Tannins are secondary polyphenolic components of plant origin of two distinct types, hydrolysable and condensed tannins, and known primarily for their ability to bind and precipitate proteins and other macromolecules (Spencer, et al., 1988). Gastrointestinal bacteria that degrade or tolerate tannic acid-protein complexes (Brooker, et al., 1994) and hydrolysable tannins (Nelson et al., 1995; Goel et al., 2005) have been isolated from animals previously exposed to plants containing these compounds. Tannic acid, as hydrolysable tannins, is toxic either to the animals (Kumar, 1992; Odenyo et al., 1997) or to the rumen microorganisms (Field and Lettinga, 1987; Bae et al., 1993; Jones et al., 1994). Inhibitory effects of tannins have been shown to be due to reduction of enzyme activity, dysfunctioning of cell membrane and deprivation of substrate metal ions and minerals (Goal et al., 2005). The toxicity of phenolic compounds in the environment has fostered studies of bacteria that are able to tolerate and/or metabolize high concentrations of these compounds, particularly under anaerobic conditions (Brooker et al., 1994; Nelson et al., 1995; Goel et al., 2005).

Differences among ruminant species in their tolerance to hydrolysable tannins have not been studied. The present investigation was completed to assess differences among ruminal bacteria populations isolated from the rumen of Egyptian sheep, cattle and buffaloes, during the dry season, in their response to different concentrations of tannic acid.

MATERIALS AND METHODS

Experiments were conducted at the Department of Botany (Division of Microbiology), Faculty of Science (El-Shatby), University of Alexandria. Rumen samples, as donor to ruminal bacteria isolates, were collected from animal groups (sheep, cattle, buffaloes) slaughtered in the slaughter house of the Faculty of Agriculture, Alexandria University.

Animals' rumen samples and bacterial isolates:

In the slaughter house of the Faculty of Agriculture, Alexandria University, rumen samples (liquor and particles, approximately 100 ml/sample), were collected immediately after animal slaughter. The normal regime of feeding during the dry season under the northern Egyptian conditions for these animals (from small farmers) was assumed a small amount of concentrate, and large amount of roughages. The samples from each animal were mixed and used directly for the inoculation on the thioglycollate medium agar (Merck, 1982).

The different ruminant animals which used as a donor for ruminal bacteria isolates were:

Animal group	No. of animals used	No. of bacterial isolates
sheep	(Two)	(Nine)
cattle-imported "carantina"	(Two)	(Five)
hybrid cattle-female	(Three)	(Eleven)
hybrid cattle-male	(One)	(Two)
buffalo-female	(Two)	(Four)
buffalo-male	(Six)	(Twenty)
buffalo-calves	(Four)	(Eight)

Isolation of ruminal bacteria:

Thioglycollate broth culture was used to cultivate and isolate ruminal bacteria in accordance with the recommendation of the National Institute of Health (1946).

An inoculum, one ml of rumen liquor of each fresh rumen sample was manually inoculated on the surface of a petri dish containing thioglycollate medium agar. All plates were incubated at 39°C for 72 h. After incubation, colonies were picked up and streaked to confirm purity. All incubations were under anaerobic conditions. Weekly transfers were necessary for survival of cultures, and for long-term storage, cultures of each ruminal bacteria isolates were frozen in 20% glycerol and stored at -80°C in cryogenic plastic tubes.

Culture media:

Stock culture of ruminal bacteria isolates were grown in fresh anaerobically sterilized media with cystine hydrochloride as the reducing agent. Thioglycollate Broth culture media contained (mg/L); 500 L-cystine, 2500 sodium chloride dextrose, 5000 yeast extract (Oxoid L21), 15000 pancreatic digest of casein (Oxoid) and 500 sodium thioglycollate. The pH of the medium was adjusted to 6.8 prior to sterilfzation, and supplemented with sodium resazurin (1 mg) and agar-agar (750 mg) when it was used to test the tolerance of ruminal bacteria to tannic acid. After sterilization at 121°C for 20 min, it was transferred in 7-8 ml quantities to glass plates purged with oxygen-free CO_2. The plate were then inoculated and prepared to the treatment by the antimicrobial compound (tannic acid) at several concentrations.

Tannic acid sensitivity testing:

Sensitivity of the isolated ruminal bacteria from the rumen of sheep, cattle, and buffalo, to tannic acid was determined by Kirby-Bauer disc diffusion susceptibility test (Moolman and Wyk, 2004). Filter paper discs (Whatman No. 1, 5 mm diameter) were impregnated with 10 µl of the tannic acid solution (e.g. tannic acid was dissolved in dimethylsulfoxide) containing 0.63, 1.25, 2.50, 5.00 or 10.00 mg TA per milliliter of growth medium. Discs were applied to the surface of agar plates that were previously inoculated with standard amount of 48 h old cultures of tested ruminal bacteria isolates (1 ml of 10^5 CFU). Plates were incubated at 39°C and the diameter of clear inhibition zone (mm) was measured after 72 h. Control discs were impregnated with 10 µl of dimethylsulfoxide solution. Three plates were replicated for each isolate.

Processing of data and statistical analyses:

Differences between the sensitivity (basis upon inhibition zone diameter) of ruminal bacteria isolates of each animal group were statistically analyzed according to a two ways design (Steel and Torrie, 1980) using the GLM procedure of SAS, (1999). To determine the differences among the animal groups and the concentrations of tannic acid, tests of hypotheses used within animal group as the error term. The sources of differences were animal group, the linear effect of TA, the quadratic effect of TA concentrations,

and the interaction between treatment and animal group.

To determine the differences among ruminant species, it was combined the date of cattle-imported, cattle-female, and cattle-male to represent data of cattle specie. Data of buffalo was also represented from the combination of male- and female-buffalo data. Buffalo-calves data were ejected with assuming their opposed to be functional ruminants. Variability between each two ruminant species was calculated by F-Test using Excel program depending on their response to different tannic acid concentrations.

RESULTS AND DISCUSSION

The sensitivity of ruminal bacteria isolates was measured by forming the clear inhibition zone (CZ; Fig. 1) around the discs impregnated with 10 µl of each tannic acid concentration (mg ml) in petri dishes inoculated with activated culture of ruminal bacteria isolate after 72 h of incubation at 39°C. In Table 1, mean CZ values were significantly differences ($P<0.05$) between animal groups. Highest sensitivity (higher CZ value) was observed in sheep and cattle (male and female). Buffaloes (male, female,; and calves) reported the lowest ($P<0.05$) sensitivity to TA concentrations than other animal groups. Within cattle groups, cattle-females were more resistance (low CZ; $P<0.05$) to TA than cattle-imported or male. No significant differences ($P<0.05$) were observed among buffaloes groups (male, female, or calves). The interaction between animal groups was not significantly differences ($P>0.05$) because the large differences among bacterial populations of animal groups, whereas the linear interaction effect was reach to the level of significant ($P=0.027$) among TA concentrations (Fig. 3).

These results were confirmed in Fig. 2 and Fig. 3. Overall, significant differences were observed among all ruminant species (Fig. 2) in their response to TA concentrations. Sheep had highest CZ vales (13.62 mm) than cattle (8.76 mm; $P=0.015$) or buffalos (4.81 mm; $P<0.05$), and therefore, buffaloes appeared a higher tolerance (low CZ value) than other ruminant species. In Fig. 3, increase the concentration of TA showed sharply increase ($P= 0.027$) in the CZ around discs with all bacterial populations.

Table 2, showed the percentage of ruminal bacteria isolates that tolerate TA concentrations in the rumen of each animal group. The isolates from the rumen of buffaloes showed a higher ($P>0.05$) tolerance to each concentration of TA than other isolates. In sheep, ruminal bacteria isolates cannot tolerate more than 0.125% TA of growth medium. However, in buffaloes, large number (27%) of isolates tolerates the highest dose (1% TA). In cattle, only 4% of isolates were tolerate up to the highest concentration used of TA.

At the highest dose used of TA, the variability (based upon F-test) was significantly differ between ruminal bacteria populations of buffalos with rumen bacteria of sheep ($P=0.0201$) as

Table (1): Differences among animal groups[1] in their responses to different concentrations of tannic acid.

Animal group (AG)[1]	TA concentrations (g/L)					SEM	*P- value*	Mean CZ value[2]	Interaction significance (*P-value*)			
	0.63	1.25	2.50	5.00	10.00				AG	TA	TA x TA	TA x AG
Sheep	8.89^{A}	10.58^{A}	15.24^{A}	16.15	17.67^{ABC}	1.903	*0.127*	13.71^{A}	0.14	0.03	0.14	0.96
Cattle-imported	$7.73^{a\,B}$	$9.86^{ab\,A}$	$10.81^{ab\,AB}$	11.63^{ab}	$20.57^{a\,A}$	1.045	*0.043*	10.63^{B}				
Cattle-male	$4.05^{a\,AB}$	$6.70^{a\,AB}$	$12.83^{a\,AB}$	14.25^{b}	$19.37^{a\,AB}$	0.691	*0.001*	11.43^{AB}				
Cattle-female	$4.70^{a\,AB}$	$5.63^{a\,AB}$	$7.12^{bc\,BC}$	14.47^{b}	$10.99^{a\,ABCD}$	0.897	*<0.01*	7.47^{C}				
Buffalo-male	$1.54^{a\,AB}$	$3.08^{a\,B}$	$5.45^{a\,BC}$	7.94^{b}	$9.25^{a\,BCD}$	0.401	*<0.01*	5.45^{CD}				
Buffalo-female	$0.00^{a\,B}$	$0.00^{a\,B}$	$2.66^{bc\,C}$	5.43^{ab}	$8.00^{a\,CD}$	1.166	*0.028*	3.21^{D}				
Buffalo-calves	1.33^{AB}	1.39^{B}	3.15^{C}	4.16	5.53^{D}	1.626	*0.109*	3.12^{D}				
SEM	2.236	2.032	2.296	3.316	3.151							
P-value	*0.062*	*0.010*	*0.007*	*0.109*	*0.040*							

[1] The number of animals used as donor to ruminal bacteria isolate of each group as follow: sheep= two (9 isolate); cattle-imported= two (5 isolate); Cattle-female= three (11 isolate); cattle-male= one (2 isolate) and the replicate of each isolate were considered as the error term; buffalo-male= six (20 isolate); buffalo-female= two (4 isolate); buffalo-calves= four (8 isolate). n= 3 for each isolate.

[2]Mean value of clearance zones (CZ) of each animal group.

Means in the same column with different superscripts (A–D) differ ($P<0.05$) among animal groups within TA concentration.

Means in the same row with different letters (a-d) differ ($P<0.05$) among TA concentrations within animal groups.

Table (2): Percentage[1] of ruminal bacteria isolates of different ruminant animals that tolerate to each tannic acid concentration.

TA concentrations	Ruminant animals			SEM	*P- value*
	Sheep	Cattle[2]	Buffaloes[2]		
0.63	20.00	45.83 ^{A}	78.13 ^{A}	19.454	*0.095*
1.25	20.00	20.83 ^{B}	65.63 ^{A}	20.489	*0.083*
2.50	0.00	12.50 ^{B}	42.68 ^{B}	17.352	*0.130*
5.00	0.00	4.17 ^{B}	27.08 ^{B}	13.473	*0.178*
10.00	0.00	4.17 ^{B}	27.08 ^{B}	13.473	*0.178*
SEM	1.141	1.826	1.919	4.572[$]	0.836[$]
P- value	*0.500*	*0.005*	*<0.001*		

[1] Tolerance (%) =

$$\left[\frac{\text{number of ruminal bacteria isolates reported zero clearance zone}}{\text{total number of isolates of animal specie}}\right] \times 100$$

Values in the same column with different superscripts (A and B) differ (P<0.05) among TA concentrations within animal specie.

[$] SEM and P-value of the interaction between TA and animal specie.

[2]Data of cattle-imported, cattle-female and cattle-male, as will as the data of buffalo-male and female were combined for the lower number of each animal group.

Table (3): Variability[1] between ruminal bacteria populations of each ruminant animal's in response to each tannic acid concentration.

Animal group	TA concentration (g/L)				
	0.63	1.25	2.50	5.00	10.00
Sheep × Cattle	0.240	0.046	0.004	0.012	0.950
Sheep × Buffalo	0.004	0.005	0.001	0.009	0.020
Buffalo × Cattle	0.009	0.114	0.832	0.904	0.005
Sheep × Buffalo- calves	0.126	0.063	0.037	0.076	0.273
Cattle × Buffalo-calves	0.463	0.648	0.986	0.909	0.228
Buffalo × Buffalo-calves	0.237	0.498	0.862	0.967	0.477

[1]Caculated by F-TEST depending on clearance zone values of each two animal groups at each TA concentration.

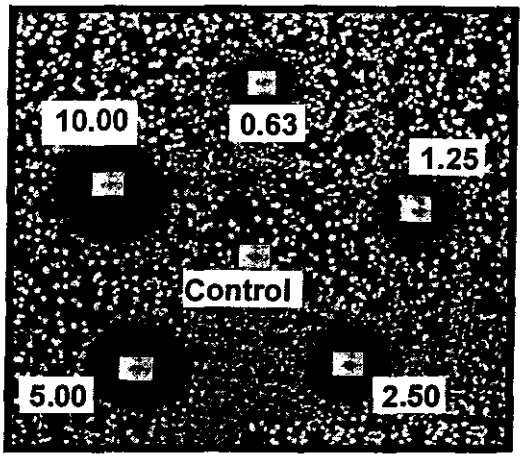

Figure (1): An example of forming the clear inhibition zone (CZ) around the discs impregnated with 10 µl of each tannic acid concentrations (mg/ml), measured by Kirby-Bauer disc diffusion assay, in petri dishes inoculated with activated culture of ruminal bacteria isolate after 72 h of incubation at 39 °C.

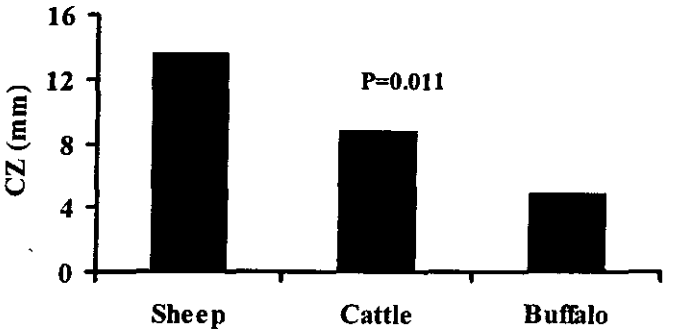

Figure (2): Differences among ruminant animal species in their sensitivity to variable concentration of tannic acid.

CZ: clear inhibition zone; data of cattle-imported, cattle-female and cattle-male, as will as the data of buffalo- male and female were combined for the lower number of each animal group.

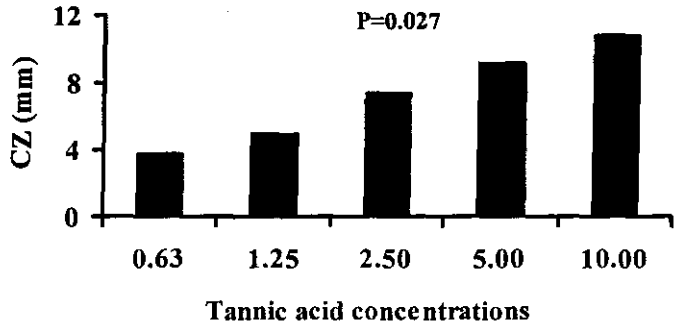

Figure (3): Liner effect of tannic acid concentrations (g/L of growth medium) on susceptibility of ruminal bacteria isolates from different animal groups.

CZ: clear inhibition zone.

well as with cattle (P=0.005). No significant differences were observed between sheep and cattle (P=0.95), while the buffalo-calves were not differ with all ruminant species (Table 3).

This is the first comparative study among some ruminant species (sheep, cattle and buffaloes) under the semi-arid conditions of northern Egypt in their response to gradual concentrations hydrolysable tannins (e.g. tannic acid, TA). This results demonstrates that a significant differences (P=0.011) between ruminal bacteria isolates from different ruminant species to TA, and reported a highly sensitivity in sheep than cattle and buffaloes (Fig. 2). The sensitivity of sheep and cattle to TA may be attributed with the induction of enzymes or metabolic transformation of the tannin molecule (O'Donovan and Brooker, 2001). Tannins are generally inhibited the growth of microorganisms by their complexion ability and reactive with the cell wall of bacteria and the extracellular enzymes secreted. Either interaction is likely to inhibit the transport of nutrients into the cell and retard the growth of the organism (McSweeny et al., 2001). This mechanism might be attributed to the toxic effect of tannic acid on the ruminal bacteria.

Buffaloes appeared to be the highest tolerant ruminant animal to TA, and sheep appeared to be more sensitive than other ruminant species. This finding suggested the different characteristics among ruminal bacteria populations of sheep, cattle and buffalo which reflected in the degradation or hydrolyzation of TA in the rumen. Degradation of tannins by ruminal microorganisms have been widely detected under different experimental conditions. Tsai and Jones (1975) isolated bovine ruminal *Streptococcus sp.* strains that capable to degrade phloroglucinol. Odenyo et al., (1999) reported the existence of tannin-degrading microorganisms in the ruminal fluid of different African ruminants. A strain of *Selenomonas ruminantium* that expresses tannin acylhydrolase activity (Skene and Brooker, 1995) and *Streptococcus gallolyticus* (*S. caprinus*) (Nelson et al., 1995, 1998; Sly et al., 1997) have been isolated from feral goat rumen samples, the latter being resistant to the concentrations of TA up to 7% (w/v) and to condensed tannins up to 4% (w/v). Several ruminal microorganisms that can degrade phenolic monomers have been isolated. *Eubacterium oxidoreducens* degrades gallate, phloroglucinol and pyrogallol to produce acetate and butyrate in the presence of hydrogen and formic acid (Krumholz and Bryant, 1986), and the biodegradation of gallotannins and ellagitannins was detected in some microorganism cells (Mingshu, 2006). Allison et al., (1990) detected some ruminal besteria that capable to degrade a toxic material such dihydroxypyridine produced from mimosine. Prior exposure of ruminants to some feedstuffs containing toxic components can promote the proliferation of rumen bacteria to be capable to tolerate and detoxify such compounds (Kumar, 1992; Odenyo et al., 1997).

In contrast, our results demonstrated that large number (27%) of buffaloes bacteria isolates were tolerate up to 1% (TA; w/v), but in cattle not more than 4% of isolates were resistance to 1% TA (Table 2). In sheep, all bacterial isolates

were more sensitive than other ruminant species and inhibited at concentration less than 0.125% of TA. Our results are in accordance with Goel et al., (2005) who isolated some ruminal bacteria could grow on agar plates supplemented with 1%TA using the clearance zone methods. Existence of TA-degrading microorganisms in the ruminal fluid of buffaloes may be reflecting their ability to tolerate the hard conditions of feeding more than sheep and cattle. Nelson et al., (1995) studied that a concentration of 30 g of hydrolysable tannins/L in a medium would be equivalent to 15% tannins in the diet of ruminant, but 10 g TA/L, in our experiment, would equivalent only 5% in the diet. This finding may be due to the unknown history of TA feeding of the animal species used.

The most likely explanation for these results, of appearing some isolates in buffaloes and cattle had an ability to tolerate TA up to 1%, was the capability of these ruminal bacteria to degrade hydrolysable tannins by producing enzymes such tannase, esterase or had a capability to cleaving the ester linkages in tannins-protein complex with the phenolic subunits (Skene and Brooker, 1995). Hydrolysable tannins are polymerized units of glucose esterified to gallic and hexahydroxydiphenic acid, and could be complex with protein by forming hydrogen bonds between the phenolic subunits of polymer and aliphatic and aromatic side chains (carbonyl groups of peptides) of protein. However, anaerobic bacteria that degrade hydrolysable tannins or hydrolysable tannins-protein complex have been isolated from the digesta of some animals by tannic acid as the model (Nemoto et al., 1995; Osawa et al., 1993). Sharply increase in TA concentrations could be activating the increase of bacterial extracellular matrix produced in medium (O'Donovan and Brooker, 2001) to reduce the negative effect of TA on bacterial cell. The secretion of extracellular polysacchrides (Brooker et al., 2000) could separate the microbial cell wall from reactive tannin, and formation of a thick glycocalyx or glycoprotein which has a high binding affinity for tannin (Nicholson et al., 1986; Chiquette et al., 1988).

Increase the concentration of TA in growth medium up to 1% detected a degradation of TA which could be hydrolysis of ester and depside bonds, yielding gallic acid, which can be decarboxylated by gallate decarboxylase to yield pyrogallol. This has been demonstrated in a number of bacterial systems (Krumholz and Bryant, 1986; Brune and Schink, 1992; O'Donovan and Brooker, 2001).

Moreover, Wanapat, (2001) found that buffalo animals had a capacity to tolerate the difficult environmental conditions than other ruminant animals, and attributed that to the characteristics of ruminal bacteria populations of buffaloes. In other studies, it has been reported that when cattle and buffalo were kept under similar conditions, buffalo utilize feedstuffs more efficiently with a digestibility of feed typically 2-3 percentages higher (Wanapat, 1989; Kennedy and Hogan, 1994; Wanapat et al., 1994). Salem (2005) observed that buffalo's rumen liquor showed a higher fermentation activity on *Acacia saligna* leaves than the liquor of sheep and cattle.

Higher variability (Table 3) between bacterial populations of sheep and cattle

with buffaloes may be due to the different characterization between the ruminal bacteria populations of the species in degrading TA. Sheep and cattle showed a much closed similarity in their responses to TA concentrations. These results suggested that the similarity in bacterial characterization between sheep and cattle and it could be use sheep as model of cattle for rumen liquor donor during the fermentation process. Salem, (2005) confirmed these finding during the *in vitro* fermentation of *Acacia saligna* leaves with rumen liquor of sheep, cattle, and buffalo.

CONCLUSION

This study paved the way for researchers in animal nutrition as to utilize the tree leaves and shrubs which containing toxic materials such as tannins in animal feeding. Surprising, the results obtained of the current study supported the tolerance of buffalo and their calves to the high concentration of tannins (up to 10 g of tannic acid per liter) due to existence of some tannic acid-degrading bacteria. This concentration of tannic acid would be equivalent to 5% tannin in the diet of ruminant. Such finding would open the way for raising adult buffaloes and their calves in the newly desert in Egypt in which a surplus of shrubs and tree are existing. In Egypt, this appears to be the first study regarding isolation of tannin-degrading ruminal bacteria from the rumen fluid of buffaloes and cattle.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Prof. Dr. Peter H. Robinson (Department of Animal Science, University of California, Davis, USA) for assistance, statistical analysis of data and helpful comments, advices and revision of the manuscript.

REFERENCES

Allison, M. J., J. A. Hammond, and R.J. Jones (1990). Detection of ruminal bacteria that degrade toxic dihydroxypyridine compounds produced from mimosine. Applied Environmental Microbiology, 58:590-594.

Bae, H. D., T. A. McAllister, L. J. Yanke, K. J. Cheng and A. D. Muir (1993). Effects of condensed tannins on enduglucanase activity and filter paper digestion by *Fibrobacter succinogenes* S85. Applied and Environmental Microbiology, 59:2132-2138.

Brooker, J. D., L. A. O'Donovan, I. Skene, K. Clarke, L. Blackall and P. Muslera (1994). *Streptococcus caprinus* sp. nov., a tannin-resistant ruminal bacterium from feral goats. Letters in Applied Microbiology, 18:313-318.

Brooker, J. D., L. A. O'Donovan, I. Skene and G. Sellick (2000). Mechanisms of tannin resistance and detoxification in the rumen. In: Tannins in Livestock and Human Nutrition. Brooker, J. D. (Ed.), pp. 117-122. ACIAR Proceeding No. 92, Australia.

Brune, A. and B. Schink (1992). Phloroglucinol pathway in the strictly anaerobic *Pelobacter acidigallici*: fermentation of

trihydroxybenzenes to acetate via triacetic acid. Arch. Microbiol. 157:417-424.

Chiquette, J., K. J. Cheng, J. W. Costerton and L. P. Milligan (1988). Effect of tannins on the digestibility of two isosynthetic strains of Birdsfoot trefoil (*Lotus corniculatus*) using *in vitro* and *in sacco* techniques. Canadian Journal of Animal Science, 68:751-760.

Field, J. A. and G. Lettinga (1987). The methanogenic toxicity and anaerobic degradability of a hydrolysable tannin. Water Research, 21:367-374.

Goel G., A. K. Puniya and K. Singh (2005). Tannic acid resistance in ruminal streptococcal isolates. J. Basic. Microbiol. 45(3):243-5.

Jones, G. A., T. A. McAllister, A. D. Muir and K. L. Chang (1994). Effects of sainfoin *(Onobrychis viciifolia* Scop.) condensed tannins on growth and proteolysis by four strains of ruminal bacteria. Applied and Environmental Microbiology, 60:1374-1378.

Kennedy, P. M. and J. P. Hogan (1994). Digesting and metabolism in buffaloes and cattle: are there consistent differences. In: Preceding of The 1st Asian Buffalo Association Congress (Eds. M.Wanapat and K.Sommart), Khon Kaen University, Khon Kaen, January 17-21, 1994, Thailand.

Krumholz, L. R. and M. P. Bryant (1986). *Eubacterium oxidoreducens* sp. nov. requiring H_2 or formate to degrade gallate, pyrogallol, phloroglucinol and quercetin. Arch. Microbiol. 14:8-14.

Kumar, R. S. (1992). Anti-nutritional factors, the potential risk of toxicity and method to alleviate them. In: Legume Trees and other Fodder Trees as Protein Sources for Livestock. Edited by A. Speedy and P. L. Pugliese. FAO Animal Production and Health, 102:145-160.

McSweeney, C. S., B. Palmer, R. Bunch and D. O. Krause (2001). Effect of the tropical forage Calliandra on microbial protein synthesis and ecology in the rumen. Applied and Environmental Microbiology, 90:78-88.

Merck, E. (1982). Thioglycolate broth. In: Handbook Culture Media Merck. Preparation for Microbiology, Cat. No. 8190. Pp. 161.

Mingshu, L., Y. Kai, H. Qiang and J. Dongying (2006). Biodegradation of gallotannins and ellagitannins. Journal of Basic Microbiology, 46 (1):68–84.

Moolman, G. J. J. and M.V. Wyk (2004). Kirby-Bauer disc diffusion susceptibility testing as a screening procedure for cephalosporin resistance in *Streptococcus pneumoniae* - correlation with the E-test. The Southern African Journal of Epidemiology and Infection, 19 (2):55-59.

National Institute of Health (1946). 2nd Rev., culture Media for sterility test.

Nelson, K. E., A. N. Pell, P. Schofield and S. Zinder (1995). Isolation and characterization of an anaerobic ruminal bacterium capable of degrading hydrolysable tannins.

Applied and Environmental Microbiology, 61:3293-3298.

Nelson, K. E., M. L. Thonney, T. K. Woolston, S. Zinder and A. N. Pell (1998). Phenotypic and phylogenetic characterisation of ruminal tannin-tolerant bacteria. Applied and Environmental Microbiology 64:3824-3830.

Nemoto, K., R. Osawa, K. Hirota, T. Ono and Y. Miyake (1995). An investigation of gram-negative tannin-protein degrading bacteria in fecal flora of various mammals. J. Vet. Med. Sci. 57:921-926.

Nicholson, R. L., L. G. Butler and T. N. Asquith (1986). Glycoproteins from *Collerotrichum graminicola* that bind phenols: Implications for survival and virulence of phytopathogenic fungi. Phytopathology, 76:1315-1318.

Odenyo, A. A., C. A. McSweeny, B. Palmer, D. Negassa and P. O. Osuji, (1999). in vitro screening of rumen fluid samples from indigenous African ruminants evidence for rumen fluid superior capacities to digest tannin-rich fodder. Aust. J. Agric. Res. 50:1147-1157.

Odenyo, A.A., P.O. Osuji and O. Karanfil (1997). Effect of multipurpose tree (MPT) supplements on ruminal ciliate protozoa. Animal Feed Science and Technology, 67:169-180.

O'Donovan, J. D. and J. D. Brooker (2001). Effect of hydrolysable and condensed tannins on growth, morphology and metabolism of *Streptococcus gallolyticus* (*S. caprinus*) and *Streptococcus bovis*. Microbiology, 147:1025–1033.

Osawa, R., T. P. Walsh, and S. J. Cork (1993). Metabolism of tannin-protein complex by facultatively anaerobic bacteria isolated from koala faces. Biodegradation, 4:91-99.

Salem, A. Z. M. (2005). Impact of season of harvest on *in vitro* gas production and dry matter degradability of *Acacia saligna* leaves with inoculum from three ruminant species. Animal Feed Science and Technology, 123-124P1:67–69.

SAS institute INC. (1999). SAS Companion for the Microsoft Windows Environment, Version 8. SAS Inc., Cary, NC, USA.

Skene, H. K. and J. D. Brooker (1995). Characterization for tannin acyl hydrolase activity in ruminant bacterium, *Selenomonas ruminantium*. Anaerobe, 1:321-327.

Sly, L. I., M. M., Cahill, R. Osawa and T. Fujisawa (1997). The tannin-degrading species *Streptococcus caprinus* and *Streptococcus gallolyticus* are subjective synonyms. Int. J. Syst. Bacteriol. 47:893–894.

Spencer, C. M., Y. Cal, R. Martin, S. H. Gaffney, P. N. Goulding, D. Magnolato, T. H. Lilley and E. Haslam (1988). Polyphenol complexation: Some thoughts and observations. Phytochemistry, 27:2397-2409.

Steel, R. G. D. and J. H. Torrie (1980). Principles and Procedures of Statistics. 2nd ed. McGraw-Hill

International.

Tsai, C. G. and G. A. Jones (1975). Isolation and identification of rumen bacteria capable of anaerobic phloroglucinol degradation. Can. J. Microbiol. 21:794-801

Wanapat, M. (1989). Comparative aspects of digestive physiology and nutrition in buffaloes and cattle. In: Proceeding Ruminant physiology and Nutrition in Asia (Eds. C.Devendra and E.Imaizumi) Jap. Soc. Zootech. Sci. Sendai, pp.27-43.

Wanapat, M. (2001). Swamp buffalo rumen ecology and its manipulation. Proceeding Buffalo Workshop December 2001. http;//www.mekarn.org/procbuf.

Wanapat, M., K. Sommart, C. Wachirapakorn, S. Uriyapongson and C. Wattanachant (1994). Recent advances in swamp buffalo nutrition and feeding. In: Preceding The 1st Asian Buffalo Association Congress (Eds. M. Wanapat and K. Sommart), Khon Kaen University, Khon Kaen, January 17-21, 1994, Thailand.

استجابة عزلات بكتيريا كرش الأغنام و الأبقار و الجاموس بشمال مصر لحمض التانييك

عبد الفتاح زيدان محمد سالم[1] – يسرى محمود جوهر[2]

[1] قسم الإنتاج الحيواني – كلية الزراعة (الشاطبى) – جامعة الاسكندرية – مصر
[2] قسم النبات (فرع الميكروبيولوجى) – كلية العلوم (الشاطبى) - جامعة الاسكندرية – مصر

أجريت هذه الدراسة بهدف معرفة الاختلافات بين بعض أنواع من المجترات (على أساس بكتيريا الكرش المعزولة) فى تحملها للتأثير السلبى لحمض التانييك. تم الحصول على سائل الكرش بعد الذبح مباشرة للحيوانات (٢ أغنام ، ٢ أبقار مستوردة "كارانتينة" ، ٣ اناث أبقار ، ١ ذكر بقرى ، ٢ اناث جاموسى ، ٦ ذكور جاموسى ، ٤ عجول جاموسى) فى مجزر كلية الزراعة بجامعة الاسكندرية خلال الموسم الجاف. وتم الحصول على ٥٩ عزلة بكتيريه (٩ ، ٥ ، ١١ ، ٢ ، ٤ ، ٢٠ ، ٨ عزلة بكتيرية من الحيوانات المجتره سابقة الذكر على التوالى). تم تقدير مدى حساسية هذه العزلات البكتيرية ل ٥ تركيزات تدريجيه من حمض التانييك (٠,٦٣ ، ١,٢٥ ، ٢,٥٠ ، ٥,٠٠ ، ١٠,٠٠ مليجرام/ ملليلتر فى بيئة النمو) و ذلك باستخدام طريقة Kirby-Bauer disc diffusion susceptibility test.

وكانت أهم النتائج المتحصل عليها كالتالى:

- لوحظ زيادة طردية فى حساسية كل العزلات للأنواع المختلفة من المجترات بزيادة تركيز حمض التانييك فى بيئة النمو.
- تم تحديد عزلة بكتيرية واحده فى الأبقار لها القدرة على تحمل كل تركيزات حمض التانييك حتى أعلى تركيز (١٠ جم/لتر) مقارنة بباقى العزلات البكتيرية بينما فى الجاموس (اناث وذكور) تم تحديد ٤ عزلات بكتيرية وايضا ٤ اخرى فى عجول الجاموس لهم القدرة على تحمل جميع تركيزات حمض التانييك فى بيئة النمو.
- بمقارنة أنواع الحيوانات المجترة بعضها ببعض لوحظ أن الأغنام كانت أكثر الحيوانات المجترة حساسية لوجود حمض التانييك من الأبقار و الجاموس. حيث لوحظ زيادة معنوية ($P<0.05$) فى متوسط قيمة ال Clearance inhibition zone بالمقارنة بباقى المجترات.
- وجد أن 27% من العزلات البكتيرية فى الجاموس كانت لها القدرة على مقاومة جميع تركيزات حمض التانييك حتى أعلى تركيز (١٠ جم/لتر) لكن فى الاغنام لم تتحمل جميع العزلات البكتيرية أكثر من ١,٢٥ جم/لتر من حمض التانييك فى بيئتها.
- وجد أن 4% فقط من العزلات البكتيرية فى الابقار كان لديها القدرة على مقاومة أعلى تركيز أستخدم فى هذة الدراسة من حمض التانييك فى البيئة (١٠ جم/لتر).
- لوحظ أن هناك اختلافات معنوية بين عشائر العزلات البكتيرية للأغنام مع الجاموس و لكن لم يلاحظ أى اختلافات معنوية بين الاغنام و الابقار فى الاستجابة أو التاثر بوجود حمض التانييك فى بيئة النمو.

تعتبر هذة الدراسة الاولى فى مصر من نوعها لدراسة المقارنة بين الأنواع المختلفة من المجترات فى مدى قدرتها فى تحمل أو مقاومة التاثير السلبى لحمض التانييك. وهذه الدراسة ربما تفتح المجال أمام الباحثين فى مجال تغذية الحيوان فى الاستفادة من أوراق الاشجار أو النباتات الصحراوية التى تحتوى على المثبطات مثل حمض التانييك فى تغذية الحيوان تحت الظروف الصحراوية المصرية. ويستخلص من هذه الدراسة أن الجاموس كان أكثر الحيوانات المجترة تحملا لحمض التانييك بالمقارنة بباقى الحيوانات المجترة موضوع الدراسة وهذا يرجع الى وجود بعض أنواع من البكتيريا لها القدرة على الاقلمة أو تكسير حمض التانييك فى الكرش.