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Effect of Diarrheastat® and Enrosol-S® on rumen ecosystem in rams

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Abstract

The effect of two antimicrobials (Diarrheastat® and Enrosol-S®) on sheep rumen ecosystem (pH, viscosity, microbial activity and protozoal activity) was investigated in adult rams. The rams were randomly divided into two groups; each group included four rams dosaged orally one time daily for three successive days according to the manufacturer recommendations. Ruminal fluid was analyzed before dosage, after 24 hrs of the first, second and third doses, and after 3, 7 and 10 days after the last dose (3rd dose). No significant differences in ruminal fluid pH and viscosity with oral antimicrobial administration were noticed. Microbial activity tests used (methylene blue reduction test and floatation/sedimentation test) showed a significant reduction of microbial activity of rumen ($P<0.05$) without differences in staining characters of bacterial population. Protozoal activity of the rumen was influenced significantly ($P<0.05$) by oral antimicrobials with some differences between Diarrheastat® and Enrosol-S®. It was concluded from this study that dosing of Diarrheastat® and Enrosol-S® orally to rams one time daily for three successive days had an obvious effects on microbial and protozoal activity of the rumen.

Keywords: Ruminal fluid; Microbial activity; protozoal activity; Rumen microflora; Sheep.
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تأثير الـــDiarrheastat® و الـــEnrosol-S® في بيئة الكرش في الكباش

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فرع الطب الباطني والوقائي، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

دُرس تأثير اثنين من المضادات المايكروبية (Diarrheastat® و Enrosol-S®) في بيئة كرش الأغنام من خلال متابعة الأس الهيدروجيني، واللزوجة، والنشاط المايكروبي ونشاط الأوالي باستخدام كباش بالغة قُسّمت عشوائياً إلى مجموعتين متساويتين (أربعة كباش لكل مجموعة). جُرّعت كباش المجموعتين عن طريق الفم مرة واحدة يومياً لمدة ثلاثة أيام متعاقبة طبقاً لتعليمات الشركات المنتجة. حُلّل سائل الكرش قبل إعطاء الأدوية في المجموعتين، وبعد ٢٤ ساعة من إعطاء الجرع الأولى والثانية والثالثة، وبعد ٣ و ٧ و ١٠ أيام من إعطاء الجرعة الأخيرة (الثالثة). لم تلاحظ أية اختلافات معنوية في الأس الهيدروجيني ولزوجة سائل الكرش بعد إعطاء الأدوية في كلا المجموعتين مقارنة بمجموعة السيطرة. اتضح من الاختبارات التي تكشف النشاط المايكروبي للكرش (اختبار اختزال المثيلين الأزرق واختبار الطفو/الترسيب) انخفاض معنوي ($P<0.05$) في النشاط المايكروبي للكرش من دون اختلاف في خصائص تصبيغ الجراثيم في كلا المجموعتين مقارنة بمجموعة السيطرة. وتبيّن تأثر نشاط الأوالي تأثراً معنوياً ($P<0.05$) بعد إعطاء الأدوية في كلا المجموعتين مقارنة بمجموعة السيطرة. استنتج من الدراسة أن إعطاء الـــDiarrheastat® والـــEnrosol-S® للكباش عن طريق الفم مرة واحدة يومياً لمدة ثلاثة أيام متعاقبة يؤثر تأثيراً واضحاً في نشاط مايكروبات وأوالي الكرش.

Introduction

Ruminal ecosystem is a stable and highly diversified consisting of bacteria, ciliate protozoa, anaerobic fungi and bacteriophage; performing the function of bio-conversion of feed into volatile fatty acids which serve as a source of energy for the ruminants (1).

The ruminant's diet is the major influence on the nature of the rumen environment. Factors such as composition of the feed, the degree of physical processing and the presence of feed additives all affect the numbers, proportions and digestive activity of rumen microorganisms (2). Most predominant rumen bacterial species are susceptible to low concentrations to many antibiotics. Therefore, there has been interest in attempting to control the rumen fermentation by feeding these compounds, but usually for only a short time after the first supplementation to the diet (3). Many compounds, such as tylosin, monensin and flavomycin, used to alter rumen fermentation to improve efficiency of feed utilization (3-5).

On the other hand, (6) mentioned that oral administration of antimicrobials in ruminants is highly problematic which may cause damage to the ruminal microflora or have an undesirable selective effect. Oral administration of antimicrobials may cause a significant disruption to the ruminal flora, which may result in a syndrome of ruminal stasis, anorexia and depression (7). Moreover, Adams (8) denoted that chronic oral dosage with an antimicrobial agents can suppress microfloral activity, and thereby disturb carbohydrate digestion which is an essential function of fore-stomach. Therefore; Radostits *et al.* (7) recommended to re-establish the ruminal flora by cud transfer after the course of orally given antimicrobial agents.

There are few studies indicating the effects of anti-microbials on rumen, Gupta and Rai (9) referred to the effects of different antimicrobials on ruminal micro-flora and protozoal motility in their chronological order. Das (10) added that oral administration of tetracycline hydrochloride to healthy calves significantly reduced ruminal microflora. Natively, Phillip (11) indicated that administration of some antibiotics like oxytetracycline and sulfonamide for 3 successive days to each drug to buffaloes showed significant changes in ruminal pH and various microbial activity tests.

The aim of this study was to investigate the antimicrobial effects of Diarrheastat® and Enrosol-S® on the ruminal ecosystem in rams.

Materials and Methods

Adult Awassi rams bred in the Animals' House of College of Veterinary Medicine, University of Mosul were used. The rams were randomly divided into two groups, four rams each. The first group was dosaged Diarrheastat® (each ml contain: neomycin sulphate 1mg, sulfadimidine 2mg and sulfadiazine 3mg)/ Al-Faiha for Veterinary Industries, Syria. The second group was dosaged Enrosol-S® (each ml contain: enrofloxacin 100mg)/ Veterinary and Agricultural Products Mfg. Co. Ltd (VAPCO), Jordon. According to the manufacturer recommendations, the rams were dosaged orally one time daily for three successive days. Ruminal fluid were analyzed before dosage, after 24 hrs of the first, second and third doses, and after 3, 7 and 10 days after the last dose.

After restraining the rams properly, ruminal samples were collected by stomach tube with the aid of a vacuum pump, then filtered through a metal sieve and subjected for analyses (12).

Ruminal pH was measured immediately after collection using wide range pH paper (7) using pH value of 2-12, Macherey-Naged GmbH and Co., Germany.

Viscosity of the ruminal fluid was determined according to (13). Rumen samples were centrifuged for 1 hr at 5200 rpm to remove debris, protozoa, and most of the bacteria. Measurements of relative viscosity were made at 25ºC with an Ostwald viscosimeter, which is 'U' shape tube with two bulbs, two marks and capillary bore in one arm (Fig. 1). After the centrifugation, the rumen sample was drawn into the upper bulb of viscosimeter by suction, then allowed to flow down through the capillary into the lower bulb. Two marks (one above and one below the upper bulb) indicate a known volume. The time required for the level of the fluid to pass between these marks is proportional to the kinematic viscosity.

The time required for the fluid to pass between two marks, upper mark and lower mark, through a vertical capillary tube was determined. The time of flow of the fluid under test was compared with the time required for water, which is a known viscosity liquid. The viscosity of rumen fluid (millipascal-second "mPa.s") was determined using the following equation (14):

$$\eta_1 = \frac{p_1 t_1}{p_2 t_2} \eta_2$$

Where,
η_1= viscosity of unknown liquid
p_1= density of unknown liquid
p_2= density of known liquid
t_1= time of the unknown liquid
t_2= time of the known liquid
η_2= viscosity of known liquid

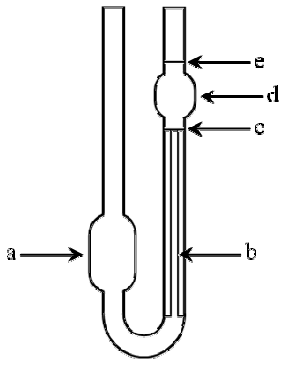

Fig. 1: A diagram of Ostwald viscosimeter. (a) lower bulb, (b) capillary bore, (c) lower mark, (d) upper bulb, (e) upper mark. (14).

According to Rosenberger (15), 20 ml of ruminal fluid was mixed with 1 ml of 0.03% methylene blue in a test tube and let to stand at room temperature. The time needed to decolorize the color and leaving a narrow ring of blue color at the top of tube was indicated.

For estimation the Floatation/sedimentation time, ruminal fluid sample was put in test tube and let to stand. The time needed for completion of sedimentation was indicated (15).

The motility of the protozoa were examined in a fresh film under low power magnification, and indicated as follow (15):

+++ highly motile and very crowded.
++ motile and crowded.
+ sluggish motility and low number.
0 no or sporadic alive infusoria.

Gram staining characters of the bacterial population of the rumen were observed. According to Guinn (16), Gram stain smears were made from ruminal fluid for direct microscopic examination to indicate the changes that occurred in microflora after dosages.

The data were analyzed statistically using the Statistical Package for the Social Sciences (SPSS). One-way analysis of variance (ANOVA) was used to detect the significant variation among treatments.

Results

Properties of ruminal fluid are presented in (Table 1). The results showed no significant differences in pH and viscosity of ruminal fluid after oral administration of Diarrheastat® and Enrosol-S® in comparison with pre-dosing.

The results indicated that there were a significant prolongation ($P<0.05$) in methylene blue reduction time after the second dose of Diarrheastat® and Enrosol-S®, and returned to normal ten days after the third dose (Table 2). The most prolongation in methylene blue reduction time was noticed after the 3rd dose (15.25 ± 2.06) in Diarrheastat® group, and (17.75 ± 1.60) in Enrosol-S® group in comparison with before dosages, (2.75 ± 0.25) and (2.50 ± 0.29), respectively (Table 2).

Floatation/sedimentation test had a significant differences ($P<0.05$) after oral dosing of Diarrheastat® and Enrosol-S®. There were a reduction in the time needed to forming of sediment after 2nd, 3rddose, and 3 days after the 3rd dose in both drugs (Table 2). The longest time of floatation/sedimentation test was found 3 days after 3rd dose, it was (19.75 ± 3.64), (18.00 ± 2.00) in comparison with post-dosage (6.50 ± 0.65), (6.75 ± 0.25) in Diarrheastat® group and Enrosol-S® group, respectively (Table 2).

The results indicated that the protozoal activity was affected with oral dosage of antimicrobials. Diarrhea-stat® had a rapid and prolonged inhibition action on protozoal activity than Enrosol-S® (Table 3).

The result showed no differences in Gram staining characters of bacterial population of the rumen after oral administration of Diarrheastat® and Enrosol-S® in comparison to pre-dosing.

Table 1: Properties of ruminal fluid pre- and post-dosages with Diarrheastat® and Enrosol-S®.

Dosages	Parameters			
	pH		Viscosity (mPa.s)	
	Diarrheastat®	Enrosol-S®	Diarrheastat®	Enrosol-S®
Pre-dosage	7.18 ± 0.118	7.18 ± 0.048	1.03 ± 0.028	1.02 ± 0.025
Post- 1st dose	7.35 ± 0.189	7.38 ± 0.085	1.04 ± 0.034	1.02 ± 0.031
Post- 2nd dose	7.40 ± 0.087	7.40 ± 0.091	1.03 ± 0.044	1.02 ± 0.015
Post- 3rd dose	7.48 ± 0.231	7.45 ± 0.087	1.02 ± 0.027	1.02 ± 0.025
3 days after 3rd dose	7.50 ± 0.041	7.40 ± 0.058	1.02 ± 0.033	1.02 ± 0.017
7 days after 3rd dose	7.38 ± 0.239	7.20 ± 0.108	1.02 ± 0.021	1.02 ± 0.008
10 days after 3rd dose	7.18 ± 0.063	7.13 ± 0.075	1.02 ± 0.020	1.01 ± 0.022

values are means ± standard error of mean.

Table 2: Activity of microflora of ruminal fluid pre- and post-dosages Diarrheastat® and Enrosol-S®.

Dosages	Parameters			
	Methylene blue reduction (minutes)		Floatation/sedimentation (minutes)	
	Diarrheastat®	Enrosol-S®	Diarrheastat®	Enrosol-S®
Pre-dosage	2.75 ± 0.25	2.50 ± 0.29	6.50 ± 0.65	6.75 ± 0.25
Post- 1st dose	6.25 ± 0.85	5.25 ± 0.75	9.50 ± 0.96	11.25 ± 1.89
Post- 2nd dose	10.50 ± 1.89 *	9.50 ± 0.96 *	14.25 ± 2.02 *	15.25 ± 2.75 *
Post- 3rd dose	15.25 ± 2.06 *	17.75 ± 1.60 *	19.25 ± 2.84 *	16.50 ± 3.02 *
3 days after 3rd dose	14.00 ± 2.74 *	10.50 ± 1.04 *	19.75 ± 3.64 *	18.00 ± 2.00 *
7 days after 3rd dose	11.75 ± 0.95 *	9.25 ± 1.65 *	8.75 ± 0.48	8.00 ± 0.41
10 days after 3rd dose	3.25 ± 0.25	2.75 ± 0.25	6.25 ± 0.48	6.00 ± 0.41

values are means ± standard error of mean, * Significant differences vertically at ($P<0.05$).

Table 3: protozoal activity of rumen pre-and post-dosages Diarrheastat® and Enrosol-S®.

Dosages	Protozoal activity	
	Diarrheastat®	Enrosol-S®
Pre-dosage	+ + +	+ + +
Post- 1st dose	+ +	+ + +
Post- 2nd dose	+ +	+ +
Post- 3rd dose	+	+
3 days after 3rd dose	+	+ +
7 days after 3rd dose	+ +	+ +
10 days after 3rd dose	+ + +	+ + +

+++ highly motile and very crowded, ++ motile and crowded, + sluggish motility and low number.

Discussion

Various tests were used in this study to evaluate the effects of oral administration of different antimicrobials (Diarrheastat® and Enrosol-S®) on rumen ecosystem in sheep. The study investigated properties of ruminal fluid (pH and viscosity), activity of the microflora, protozoal activity and staining characters of bacterial population as a reflection of rumen ecosystem.

No significant differences in rumen pH with oral antimicrobial administration was noticed in this study; since the pH of ruminal fluid is mostly dependable on the nature of the diet and the time interval between the last feeding and taking a sample (7, 17).

Viscosity of the ruminal fluid showed no significant differences in this study. This could be due to the type of diet which could affect ruminal viscosity (17,18). Gutierrez *et al*. (13) mentioned that the Glucose-containing polysaccharides may a contributing factor in viscosity changes. The viscosity tends to be higher when concentrates are fed (17,19). On feeding a high carbohydrate diets, certain species of bacteria proliferate to large numbers resulting in production of insoluble slime causing marked increase in ruminal viscosity (7). Church (17) added that the viscosity was also affected by pH, reaching maxima between pH 5.5-5.8 and between 7.5 - 8.5.

Ruminal dysfunctions could be due to usage of antimicrobials, which suppress microflora activity of the rumen, and thereby disturb the digestion (8,20). This was noticed through the microbial activity tests used in the study (methylene blue reduction test and floatation/ sedimentation test) that showed a significant reduction of microbial activity of rumen. A significant pro-longation in methylene blue reduction time (reduction time increased) encountered after the second dose of Diarrheastat® and Enrosol-S® indicate to inactive ruminal microflora (12). Also, Floatation/ sedimentation test was significant after oral dosing of Diarrheastat® and Enrosol-S®, which referred to inactive ruminal microflora. These results of present study agreed with Phillip (11) who found significant changes in time needed for methylene blue stain reduction and sedimentation activity test in buffaloes after administration of antibiotics. Also, they agreed with Das (10) who mentioned that oral administration of tetracycline hydrochloride to healthy calves significantly reduced ruminal microflora. Furthermore, Hungate (21) reported the inhibitory effects of antibiotics on the rumen microorganisms through the pure cultures of rumen bacteria which were founded to be inhibited by penicillin, terramycin, aureomycin, streptomycin, chloromycetin, sulfidine and norsulfazole.

Protozoal activity of the rumen was influenced by antimicrobial drugs with some differences between Diarrheastat® and Enrosol-S® (Table 3). This result agreed with the reporting of Phillip (11). Similar results were noticed in case of indigestion (22). Absence of ruminal protozoa is a reliable indicator of an abnormal state of the rumen (7). Diarrheastat®, which contain sulfonamides, had a rapid and prolonged inhibition action on protozoal activity than Enrosol-S®; since sulfonamides activity against some protozoa (23).

Gram staining characters of bacterial population of the rumen after oral administration of Diarrheastat® and Enrosol-S® showed no differences; since the dense of rumen microbial population depends on the continuous supply of the digestible feeds included in the ration (21).

Effect of antimicrobials on rumen fluid were detected after a short period of administration of antimicrobials in this study, while Adams (8) and Gupta and Rai (9) indicated that chronic oral dosage with antimicrobial agents can suppress ruminal microflora activity. Therefore, other broad studies are needed to evaluate the differences between the short and long dosing effects on ruminal activity.

From this present study, it can be concluded that dosing of Diarrheastat® and Enrosol-S® orally to rams one time daily for three successive days had an obvious effects on microbial and protozoal activity of the rumen.

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