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دراسة الفلورا الفطرية في عصارة كرشة الأغنام بعد تغذيتها ببقايا المحاصيل

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أمكن جمع وتعريف اثنين وثلاثون نوعا من الفطريات التي تنتمي الى خمسة عشر جنسا بالاضافة الى الفطريات ذات الخيوط العقيمة من عينات عصارة الكرشة المختلفة المستخلصة بعد تغذية الحيوانات على علائق مختلفة وكانت أكثر الانواع الفطرية انتشارا هي :
جيونزيكوم كاندديم ، واسبرجلس فلافس ، ينسيليام كربزوجينيم وفيوزاريوم مونيليفورم في خليط الكسب وقش القمح (١) ومصاصة القصب (٢) على التوالي ، بينما في حالة سيقان البرسيم المصرى (٣) كانت جيونزيكوم كاندديم ، اسكلاريوبسس بريفيكولاس ، وبنسيليام كريزوجينيم أما في حالة تغذية الحيوانات على سيقان الفول الأخضر (٤) كانت الاسبرجلس بنجر ، اسبرجلس فيوميجاتس ، فيوزاريوم اوكسس سيورم وميوكر راسسيموزاس ، بينما الفيوزاريوم أوكس سيوروم واسبرجلس نيجر ، وميوكر راسيموزساس كانت هي السائدة في حالة عروش البصل الخضراء (٥) .

ايضا تم الفحص الاكلينيكي لحيوانات التجربة وذلك لمشاهدة أى تغير فسيولوجي لها بعد اتباع النظم السابقة للتغذية وقد أظهرت الحيوانات التغيرات التالية :
المجموعة الأولى (١ ، ٣ ، ٤) من الحيوانات لم تظهر أى تغيرات فسيولوجية واضحة بينما أظهرت المجموعة الثانية (٢) بعض الخمول والفقد النسبي للشهية وايضا الضعف المتأخر لانقباض الكرشة ، أما المجموعة الثالثة (٥) فقد أظهرت الحيوانات بعض الخمول والترنح مع اسهال وسرعة في التنفس وظهور رائحة تشبه البصل .

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STUDIES ON FUNGAL FLORA OF RUMINAL JUICE OF SHEEP GAINED AFTER FED WITH CERTAIN FIELD STUBBLES

(With One Table)

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SUMMARY

Thirty two fungal species which belong to 15 genera in addition to fungi withsterile mycelium were collected and identified during this study. Generally the most frequent fungal species encountered from different samples tested were as follows:

Geotrichum candidum; and A. flavus, P. chrysogenum and F. moniliforme, and G. candidum, Scopulariopsis brevicaulis and P. chrysogenum; and A. niger, A. fumigatus, F. oxysporum, A. niger and M. racemosus in rumingal juice samples gained after fed with cotton seed cake mixture (I); bagasse (II); Egyptian clover's stems (III); green broad bean's stems (IV), and green onion's stems (V) respectively.

The clinical examination of the experimental animals was also carried out ot ensure the sound of physiological activity. Some groups (I, III and IV) ensure soundness of experimental animals but, depression, relative loss of appetite and weak delayed ruminal contractions was observed in group (II) whereas diarrhea, depression and staggring with accelerate breathing of onionic odour in group (IV).

INTRODUCTION

In Egypt, this present investigation to our knowledge is the first suggestion that the colonization of the rumen by microfungi has important inplication to the growth and health hazardous of ruminats from microbiological point of view.

On the other hand, HUNGATE et al. (1952), OLTJEN et al. (1962) and BREWER and TAYLOR (1969) stated that any feed stuff eaten by a ruminant were immediately subjected to microbial fermination in the rumen. It seems that the changes in the feed eventually causes significant changes in microbial activity which in turns may affect in the host. In this respects, CALDER et al. (1962) explained the extreme slow growth rate of sheep in summer and autumn due to the presence of fungi among the ruminal flora.

Recent studies revealed that most of fungal species frequently found in the air or growing on substrates use as food or feed were almost pathogenic and capable for producing toxic metabolites (mycotoxins) (AUSTWICK and VENN, 1961; AUSTWICK and EL-PHICK, 1964; RAPER and FENNELL, 1965, WOGAN, 1965; JUNGERMAN and SCHWARTZMAN, 1972; WILSON, 1973; HESSELTINE, 1974; EMMONS et al., 1977; YLIMAKI et al., 1979; EL-KADY and MOUBASHER, 1981; EL-KADY and EL-MARAGHY 1982 a & b and several others).

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METHODS

To accumplish this study, different diets were offered to 5 groups of 48 balady ewes (3-5 years old). Each diet was given for 30 days, with 15 days interval between each diet. Of these, three diets: bagasse (II), green broad bean's stems (IV) and green onion's stems (V) represent field stubbless, the other two diets used were commercial concentrat ration and wheat straw mixture (I) in autumn and Egyptian clover's stems (III) in summer. This study was extended from September 1979 to May 1980.

The ruminal juice samples were taken 5 hours post feeding, three times at 10 days interval. The stomach tube apperatus essentially as described by ABDEL-SALAM (1981) was employed. About 300 - 500 ml of ruminal juice was collected for each sample. Immediately, the whole ruminal sample was placed in water bath at 30°C during the period of examination. About 50 ml was drawn out under complete clean precautions into a sterile container and kept in the refrigerator for fungal examination. Also the clinical examination of the animals before and during each experiment was observed.

For estimation of ruminal juice fungi, the dilution plate technique as described by JOHANSON et al. (1959) was employed.

One ml of the ruminal juice sample was transfered to 20 ml steriliwed test tube containing 9 ml sterile distilled water and then the tube was shaken for 5 minutes. One lm of this suspension (1/10) was drawn (while in motion) into a sterile 1 ml pipette and transfered into 9 ml sterile water blank container to give a dilution of 1/100 which was found to be approperiate in most cases giving about 25 colonies per plate.

2. One ml of desired dilution was then transfered immediately into sterile Petri-dishes (5 replicates were used for each sample). About 15 ml of Czapek's agar medium were poured into each Petri-dish. Also rose-bengal at the rate of 1 : 30.000/L and streptomycin (30 ug/ml) were added to each plates just before solidification of agar as bacteriostatic agents (MARTIN, 1960).

3. The plates were incubated at 28°C for 7 days and the resulting colonies were counted and identified. Single colonies may be transferred to slant tubes or plates of Czapek's agar medium for further study.

RESULTS and DISCUSSION

The results presented in Table (1) revealed that the highest fungal population was encountered in ruminal juice gained after fed with green onion's stems (78200 colonies calculated per/ml runinal juice) followed by bagasse (Wheat straw) and cotton seed cake mixture (63640 and 53540 colonies respectively). The least fungal population was estimated in case of Egyptian colver's stems (32120 colonies) and green broad bean's stems (32800 colonies).

Thirty two species which belong to 15 gnera in addition to fungi with sterile mycelium were isolated and identified during present study. The broadest spectra of fungi (15 genera and 32 species) was recorded in case of Egyptian clover's stem's (least fungal count). The least spectrum (6 genera and 13 species) was estimated in case of green onion's stems (highest fungal population). The other samples appeared relatively high fungal spectra (about 13 genera and 26 species) as shown in Table (1).

Aspergillus was the most abundant and frequent genus recovered in all ruminal juice samples tested. Its highest count was almost behined the high fungal population of the ruminal juice

influenced after fed with green broad-bean's stem (yielding 52.2% of total fungi); green onion's stem (37.9%) and bagasse (37.6%). The least count of Aspergillus was determined in case of Egyptian clover's stems (17.6% of total fungi).

From Aspergillus 12 species were collected and identified of which A. flavus and A. niger were the most common (12 cases each) in the ruminal juice samples obtained after fed with bagasse and green onion's stems which contributing 35.1 and 35.5% of total fungi respectively. In case of runinal juice influenced after fed on green broad bean's stems, A. niger (31.5%) and A. fumigatus (17.2%) were only the most prevalent species of genus Aspergillus. A. sydowi (8.4%), A. niger (8.4%), A. niger (6.8%) and A. versicolor (5.2%) and A. niger (7.5%), A. sydowi (5.5%) and A. terreus (1.3%) were collected in moderate frequences of occurrence (6 - 8 cases out of 12) from ruminal juice samples gained after fed with cotton seed cake mixture and Egyptian colver's stems respectively. The other remaining species of Aspergillus recorded in this investigation were isolated but in less frequencies of occurrence than the preceding ones (Table, 1).

The importance of Aspergillus spp. lies in that many of them are pathogenic species growing on substrates used as food and feed for man and animals and may produce mycotoxins which were appeared during digestion and act on one or more body systems. The diseases produced by this type of activity was known as asspergillotoxicosis (AUSTWICK and EL-PHICK, 1964; RAPER and FENNELL,1965; TRENK et al., 1971; JUNGERMAN and SCHWARTZMAN,1972; WILSON, 1973, EMMONS et al. 1977 and several others). BREWER and TAYLOR (1969) and LUNDT (1974) come in agreement with our results in which A. fumigatus is found in ruminal juice of female sheep. Its endotoxins may explain the haemorrhagic lesions or abortion produced in animals especially in cattle and sheep (JUNGERMAN and SCHWARTZMAN, 1972 and EMMONS et al. 1977). Furthermore Aspergillus which are most frequentely found on mouldy hay, straw and cereal grains are of the most commonly pathogenic e.g. A. nidulans, A. flavus, A. terreus A. sydowii and several other (RAPER and FENELL, 1965; TRENK et al., 1971 and HESSELTINE, 1974). On the other hand, pathological effect of A. ochraceus including acute nephrosis, hepatic degeneration and enteritis when animals fed with contaminated cereal grains with " Ochratoxins " had been reported by TRENK et al. (1971). A. niger was one of thsoelisted by WILSON and WILSON (1961) as present in mouldy feeding stuffs associated with owalate posioning. Also, RAPER and FENELL (1965) reported that the American and French workers stated that the isolates of A. clavatus obtained from mouldy feed pellets when grown on maize and wheat and fed to calves and dairy cows produced depression with himorrhage and congestion of the liver, inapetence and many animals were died after short time of the appearence of these symptoms.

The aflatoxins can be regarded as highly toxic, potent hepatocarcinogens and are mutagenic (WOGAN, 1965, ENOMOTO and SATIO, 1972; EL-ZAWAHRY et al. 1977 and several others). Howeve r aflatoxins are produced mainly by strains of A. flavus in cereals or other food and feed stuffs (AUSTWICK and EL-PHICK, 1964; RAPER and FENNELL, 1965; ENOMOTO and SATIO, 1972; HESSELTINE, 1974; MOUBASHER, et al. 1977 a & b and several others).

The other Aspergillus spp. recovered in the present study were mostly recorded by several workors as human and animal pathogenic, but doubt still exists of the ability of them to cause disease for the degestive system of ruminates.

Penicillium Chrysogenum was also isolated in high frequency of occurrence (12 cases) from the ruminal juice samples gained after fed with bagasse and Egyptian clover's stems (representing 30.0 and 14.9% of total fungi respectively). In jother ruminal juice samples, it was isolated either in moderate (6 - 8 cases) or low (3 - 5 cases out of 12) frequencies of occurrence but its count was sharply reduced with regard to the other ones (Table, 1).

AUSTWICK and EL-PHICK (1964), HESSELTINE (1974) and EMMONS et al. (1977) stated that many animals were died after eating feed naturally contaminated with Penicillium spp. which were responsible for toxicosis (Paxilline) outbreak. Congestion and haemorrhage especially in liver was prominant features of the disease.

Fusarium represented by three species of which F. oxysporum was themost frequent species in ruminal juice samples influenced after fed with green onion's and green brood bean's stems (cam first and second before and behind A. niger with regard to its count which accounting 54.5 and 22.4% of total fungi respectively). F. moniliforme only came third (21.6 %) behind A. flavus and P. chrysogenum in ruminal juice obtained from animals fed with bagasse (sugar cane). Its count in the other ruminal juice samples were either sharply reduced or completely absent (Table, 1).

On the other hand; Fusarium species is widely distributed in stored grains and feed stuffs and has shown to be involved in a number of serious outbreaks of mycotoxicoses among farm animals. Two types of mycotoxins are usually produced by the different species of Fusarium and these are an estrogenic metabolite called F-2 or Zearalenone and the other is the trichothecenes mycotoxins. The F-2 toxin was found in samples of hay that had been fed to dairy cattle. This hay caused an increase in the number of artificial insemination.

Most of the trichothecene are local irritant causes extensive hemorrhaging necrotic foci of the liver and intestines (KOSURI et al., 1970; JUNGERMAN and SCHWARTZMAN, 1972; HESSELTINE, 1974; EMMONS et al., 1977; YLIMAKI et al., 1979 and EL-KADY and EL-MARAGHY, 1982 a).

Geotrichum candidum (yeast-like fungus) came first with regard to its count in ruminal juice samples gained after fed with cotton seed cake (71.4% of total count) and Egyptian clover's stems (35.1%) before A. sydowi (8.4%) and S. brevicaulis (17.9%) respectively. Its count was sharply declined or completely absent from the other ruminal juice samples tested. However EMMONS et al. (1977) reported that Geotrichum had a low degree of verulance for animals.

Scopulariopsis was represented by two species, S. brevicaulis and S. candida. The former was the second most frequent species behind G. candidum and before P. chrysogenum in the ruminal samples influenced after fed with Egyptian clover's stems (17.9% of total fungi). Its count was sharply declined or completely disappeared from the plates of other ruminal juice samples.

ELLIS (1971) and EMMONS et al. (1977) stated that S. brevicaulis was considered as one of the most common and wide spread fungus isolated from air, animals and fodd stuffs.

Mucorales (Phycomycetes) which represented in our study by 3 genera and 6 species and the most frequent mucors were estimated in the ruminal juice samples gained after fed with green broad bean's (6.5%) and Egyptian clover's stem (6.3%) and these counts were mainly to the high population of Mucor racemosus (42.6% of total mucors) and Rhizopus stolonifer (34.2%) and M.racemosus (75.4%) and M.hiemalis (36.5%) respectively. These previous species in addition to M.pusillus M.circinelliodes and Syncephalastrum racemosum were also encountered in the other ruminal juice samples but in low frequent than the preceding ones or completely missed from the plates tested (Table 1).

Ulcerative lesions similar to those seen in gasteric and intestinal phycomycosis due to Rhizopus spp. and Mucor (M.circinelloides, M.circinelloides, M.pusillus and M. racemosus), had been observed by AUSTWICK and VENN (1961);MORQUER et al.(1965); CLARK (1968);JUNGERMAN

and SCHWARTZMAN (1972) and EMMONS et al. (1977).

Stachybotrys chartarum was mostly recovered from all ruminal juice samples but in relatively low fungal population ranged between 0.2 -1.5% of total fungi (Table 1).

Many workers reported that straw, hay, cotton and sugar cane roodts (used either as feed or bedding) contaminated with toxin producing strains of S.chartarum. Stachybotryotoxicoses is a mycotixicoses produced by S.atra (= S.chartarum), it usually effects horses and humans but the lesions can be produced in sheep, calves and swine (STANKOUSHEV et al., 1965; PVLOV et al. 1967; GOLDBLATT, 1969; SARKISOV et al., 1971; NIKOV et al. 1974; JONG and DAVIS, 1976; HINTIKKA, 1977 and EL-KADY and MOUBASHER, 1981). Symptoms include salivation, swelling and cracking of the lips, prolonged prothrombin time, leukopenia, diarrhea and death (FORGAES et al., 1958; and WOGAN, 1965). HINTIKKA (1977) reported that wild animals were usually able to avoid the ingestion of feed stuffs contaminated with S.chartarum, therefore there was no records of Stachybotryotoxicoses among them.

Chaetomium globosum, Botryotrichum piluliferum, Paecillomyces terricola, Cephalosporium acremonium, Myrothecium verrucaria and Gliocladium roseum in addition to fungi with sterile mycellium were also collected and identiffied from different ruminal juice samples but mostly less frequently than the preceding ones (Table 1).

Generally most of these species were considered as pathogens or capable for elaborating mycotoxins (WILSON, 1973 and JONG and DAVIS, 1976).

The clinical examination of experimental animals was also carried out to ensure the sound of physiological activities. The results reveal that ensure soundness of animals in groups 1, 111 and IV, but depression, relative loss of appetite and weak, delayed ruminal contractions was observed in sheep of group II. Diarrhea, depression and staggring with accelerate breathing of onionic odour in sheep of group V.

CALDER et al. (1962) and BREWER and TAYLOR (1969) attributed the extreme slow growth rate of sheep in summer and autumn due to the presence of fungi among the ruminal flora.

However the results must therefore be regarded as preliminarily data and further work in progress.

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Table (17: Percentage count (calculated to total count and number of cases of isolation out of 12 samples) of fungal genera and species revovered from ruminal juice samples aftrer fed with certain diet on Czapek's agar medium at 28°C.

Organisms	I		II		III		IV		V	
	%C	NCI &OR	%C	NCI &OR	%C	NCI &OR	%C	NCI &OR	%C	NCI &OR
Aspergillus	21.7	12H	37.7	12H	17.6	12H	52.2	12H	37.7	12H
A. niger Van Tieghem	6.8	8M	1.9	6M	7.5	6M	31.5	12H	35.6	12H
A. flavus Link ex Fr.	0.3	2R	35.1	12H	0.6	3L	0.9	6M	0.2	2R
A. fumigatus Fresenius	0.3	2R	-	-	0.5	4L	17.2	12H	0.7	5L
A. sydowi (Bain. & Sart.)Thom & Church	8.4	8M	0.1	1R	5.5	8M	-	-	0.1	1R
A. verricolor (Vuill.)Tirab.	5.2	6M	0.1	1R	0.3	2R	0.9	4L	-	-
A. terreus Thom	-	-	0.1	1R	1.3	6M	0.6	3L	1.0	4L
A. candidus Link ex Fr.	0.3	2R	0.1	1R	0.5	4L	0.4	3L	-	-
A. nidulans (Eid.) Winter	0.1	1R	0.1	1R	0.4	3L	-	-	0.1	1R
A. ochraceus Wilhelm	0.1	2R	0.1	1R	0.5	3L	0.4	3L	-	-
A. clavatus Desm.	0.1	1R	0.1	1R	0.2	1R	0.1	1R	-	-
A. ustus (Bain.) Thom & Church	0.1	1R	-	-	0.1	1R	0.1	1R	-	-
A. amstelodami Thom & Church	0.1	1R	-	-	0.2	2R	0.1	1R	-	-
Penicillium chrysogenum Thom	0.9	6M	30.0	12H	14.9	12H	1.6	6M	1.0	4L
Fusarium oxysporum Schlecht	1.9	6M	-	-	0.3	2R	22.4	10H	54.5	12H
F. moniliforme Scheldon	0.2	2R	21.6	12H	0.8	3L	-	-	-	-
F. solani (Mart.) Sacc.	0.1	1R	-	-	0.1	2R	1.0	3L	-	-
Geotrichum candidum Link	71.4	12H	0.7	5L	35.1	10H	0.5	4L	-	--
Scopulariopsis brevicaulis (Sacc.) Bain	1.4	6M	4.4	6M	17.9	9H	2.1	6M	-	-
S. candida (Gueg.)Vuill	-	-	-	-	1.6	4L	0.5	3L	-	-
Mucors	0.7	6M	3.6	8M	6.3	12H	14.3	12H	6.5	10H
Mucor hiemalis Wehmer	0.1	1R	0.3	2R	2.0	4L	1.2	4L	0.1	1R
M. circinelliodes Van Tieghem	0.1	1R	-	-	0.3	2L	-	-	-	-
M. racemosus Fresen.	0.1	1R	2.7	6M	1.8	6M	6.1	9H	4.9	9H
M. pusillus Lindt	-	-	0.2	2R	1.4	4L	2.1	6M	0.3	2R
Rhizopus stolonifer (Ehrenb. ex. Fr.)Lindt	0.3	2R	0.1	1R	0.5	3L	4.9	6M	1.2	6M
Synecephalastrum racemosum(Cohn.)Schroel	0.1	1R	0.3	2R	0.3	2R	-	-	-	-
Stachybotrys chartarum(Ehrenb.ex.Link) Hughes	0.1	2R	0.3	2R	0.9	4L	1.5	3L	0.2	2R
Chaetomium globosum Kunze ex Fr.	1.0	6M	0.4	3L	1.0	3L	1.1	4L	-	-
Botryotrichum piluliferum Sacc.&March.	0.1	2R	0.4	3L	0.9	5L	0.6	4L	-	-
Pacilomyces tericola (Mill.,Gidd.&Post) Com. Nov.	0.3	3L	0.4	3L	0.8	5L	1.1	5L	-	-
Acremonium strictum W. Gams	0.1	2R	0.3	3L	0.9	5L	0.4	3L	-	-
Myrothecium verrucaria (Alb. & Schw.)Dit ex Fr.	0.1	2R	-	-	0.4	2R	0.3	2R	-	-
Gliocladium roseum Bain Sterile m	-	-	0.1	1R	0.1	1R	-	-	-	-
Sterile mycelium	0.1	2R	0.1	2R	0.4	3L	0.4	3L	0.1	1R
Total fungi/ml ruminal juice	53540		63640		32120		32800		78200	
Fungal spectrum	14genera & 28 spp.		14 & 24		15 & 32		13 & 26		6 & 13	

Group I = Cotton seed cake and wheat straw mixture
II = Bagasse (Sugar-cane)
III = Egyptian clover's stem's
IV = Green broad bean's stems
V = Green onion's stems
NCI = number of cases of isolation.
%C = percentage count (per total count)
OR = occurrence remarks:
H = high occurrence, more than 9 cases out of 12 (75 -100%)
M = moderate occurrence, between 6-8 cases (50-74%)
L = low occurrence, between 3-5 cases (25-49%)
R = rare occurrence, less than 3 cases (less than 25%).