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A RECOMMENDED SAFE AND POWERFUL INHIBITOR TO STORAGE FUNGI IN POULTRY RATIONS

(With 4 Tables & 1 Fig.)

By

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فعالية مركب السينا مالدهين والساليسالدهين فى منع نمو الفطريات
فى علائق الدواجن والتوصية بإستعمالهما

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تم فى هذا البحث عزل ٤٢ نوع من الفطور الملوثة لعلائق الدواجن فى مصر والتى تنتمى إلى ١٣ جنس . وتم مناقشة أهمية وجود الفطور الملوثة العليقة من الناحية الصحية والأقتصادية وفى محاولة للتوصل إلى بعض المركبات لتنشيط نمو الفطور فى العلائق لحماية الدواجن من المركبات السامة التى تفرزها بالإضافة إلى التغيرات التى تفسد مكونات العليقة وتسبب خسارة إقتصادية أمكن التوصل إلى مركبين عطريين هما السيناماالدهين والساليسالدهن كمنشط قوى يمنع نمو وتكاثر الفطور فى العلائق بتركيز ٣ جرام / طن عليقة للمركب الأول وتركيب ١٠ جرام / طن عليقة للمركب الثانى . وقد تم تغذية دجاج بياض على العلائق المعاملة بالمركب لإختبار سلامة هذه المركبات المخلوطة بالعليقة على صحيتها وإنتاج وأثبتت التجربة عدم أحداث تغيرات جوهرية على كريات الدم اوزون الدجاج وإنتاج البيض وقد إستخلصنا من نتائج هذا البحث أمكن لأول مرة الكشف عن هذين المركبين كمثبطات للفطور فى العلائق والتوصية بإستعمال أحد هذين المركبين / بالتركيز المشار إليه لما لذلك من أهمية صحية وإقتصادية كبيرة .

SUMMARY

Assay of the fungal flora of mixed poultry rations used in Egypt revealed 42 species belonging to 13 genera. The probable risk of such enormous contaminants to bird health as well as to feed were discussed.

Cinnamaldehyde and O-hydroxy benzaldehyde (Salicylaldehyde) were tested for its eficiency as fungistats in concentrate rations of poultry. Results revealed that these two aromatic carbonyl aldehydres are powerful inhibitors to storage fungi in such feeds at concentrations of 30 ug/kg ration for cinnamaldehyde compound and 100 ug/kg ration for salicylaldehyde compound.

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Treated rations were administered to LSL laying hens for 30 days experimental period. The results revealed that neither livebody weight, egg production nor blood erythrocytic, leukocytic and differential leukocytic count were significantly affected. It was concluded that these aromatic carbonyl adlehydes could be recommended as powerful and safe inhibitors to storage fungi in mixed concentrate rations.

INTRODUCTION

Stored feeds and other concentrate rations are subjected to molds which germinate, grow and elaborate their toxic metabolites when conditions of moisture, temperature and aeration are favorable (ABBAS, et al. 1984). It was demonstrated that some common species of fungi such as Aspergillus flavus and Fusarium moniliforme, increase the amount of secondary amines in contaminated food as dimethyl, diethylamine, methylbenzylamine after the decomposition of proteins of cerials thus promoting the formation of nitrosamines (SEN, et al. 1969). DMNA, DENA and MBNA are known as potent carcinogen causing the formation of liver cancer in many animals, and its relation to human esophageal cancer has been suggested by some workers (COOK., 1971). Moreover, mycotoxins-a toxic metabolites produced by toxigenic fungi has been recognized throughout the world as major problem in animal and poultry industry (HILL, et al. 1984).

OGUNDER (1987) reported that growth of toxigenic strains of Aspergilus on milled poultry feeds led to considerable decrease in the proteins, oil and crude fibre contents of the feed substrate, and increase in the free fatty acid fractions of the feeds. A determination of their extra-cellular enzyme profile showed the production of amylases, pectate lyase, cellulases, proteases, lipases,zyalanases, DNase and RNase. He showed that this activity was enhanced by the operating factors such as the temperature, moisture content, pH and the rich nutrients which readily support their growth and production.

The control of fungal activity in animal feed and ingredients is a subject that has attracted much attention in the past few years as a result of increasing awareness of the hazards presented by mycotoxins (STEWART, et al. 1977). It could be indicated that the easiest and most economically effecient way of controlling fungal activity is by the use of mold inhibitors. Pelleting poultry feed, although reduce mold counts, the pellets remain suseptible to reinfestation which could be delayed by the addition of fungistats to the pelleted feed (TABIB, et al. 1984).

A number of compounds have been tested or used with varying success in poultry feed to control fungal growth and possible mycoses and mycotoxicoses in poultry. It included gentian violet (CHEN and DAY, 1974), crystal violet, copper sulfate (STEWART, et al. 1977), short- chain fattyacids and their salts of which propionic acid is the most commonly used (PASTER, et al. 1985), isobutyric acid (HERTING, et al. 1974) and sorbic acid (SOFOS, et al. 1985). However, it is a common complaint that antifungal agents

in nutrients do not appear to be consistently efficacious (DABI, 1986). Inhibition wiht different organic acids was infuenced by the ration concentrations of limestone, fat and proteinaceous material high in basic amino acids (DIXON and HAMILTON, 1981 b) and by the particle size of the inhibitor and the ingredient (DIXON and HAMILTON, 1981 a). MEGALLA, et al. (1986) investigated some compounds of aromatic carbonyl aldehydes as inhibitors to storage fungi in wheat & corn flour and bread. He showed that salicylaldehyde and cinnamaldehyde inhibited completely fungal growth in flour and bread without any adverse effect on the bakery processes.

The objective of this study was to investigate the fungal microflora of poultry rations used in Egypt as well as to investigate the fungistat effectiveness of cinnamaldehyde and salicylaldehyde compounds after being applied to poultry rations and demonstrate its health significance expressed in blood picture and productivity in laying hens after the administeration of treated rations. This study was of great interest in animal and poultry keeping and industry both from health and from economic point of views.

MATERIAL and METHODS

Sampling of rations and assay of fungus microflora:

Samples from 10 different mixed poultry feeds used as commercial rations of poultry in Egypt, were kept in clean polyethylene bags and stored in the refrigerator until examination. 5 grams of each sample were placed in Erlenmeyer flask containing 100ml sterile distilled water. Flasks containing the suspensions were then shaken on a mechanical shaker for 20 min. The dilution plate method was used for isolation of fungi. One ml of the desired dilution was transferred aseptically using sterile pipette into 3 sterile petridishes. About 15 ml of molten agar medium at about 40°C were added to each petridish. Glucose-Czapek's agar medium was used for isolation of mesophilic fungi. Rose-bengal (1:15000) and chloramphenicol (0.5 g/L) were added to the media as bacteriostatic agents (AL-DOORY., 1980). Plates were incubated at 28°C for 7-12 days and the resulted colonies were isolated and identified as showed by ABDELFATTAH, et al. (1982).

Testing the fungistatic activity of cinnamaldehyde and salicylaldehyde:

Each of the two compounds mas added to glucose-Czapek's agar medium in proportion of 3 ug cinnamaldehyde per 100 ml medium while salicylaldehyde mas mixed in proportion of 10 ug per 100 ml medium. The activity of each aromatic compound on the fungal microflora in rations was tested by using the dilution plate method which previously described in this paper for the isolation of fungi present in the mixed poultry feeds.

Treatment of rations:

The desired amount of stock solution was dissolved in ethylether and the desired concentration was sprayed on to smaller amount of ration and thenafter left to dry

in hot air oven at 80°C. The stock amount of ration was mixed thoroughly with bulk ration to attain the appropriate concentration of the compound in ration. Cinnamaldehyde was mixed with ration in concentration of 30 ug/kg ration while salicyladehyde (0-hydroxy benzaldehyde) in concentration of 100 ug/kg ration.

Experimental birds:

Laying Lohman Selected Leghorn birds of 14 months age were used in this experiment. Two groups, each of seven layers were kept under cage system as test birds and ten layers were kept under same management conditions as control. All hens were clinically healthy and producers at the begining of experiment. Each of test groups were received a compound in treated ration daily for 30 days experimental period, while control birds received untreated ration. All birds were kept under observation throughout the experimental period, and weighted at the end of the experiment. Egg production was also recorded.

Haematological assay:

Samples of blood were collected from each hen including test and control groups at the end of the experimental period. The total number of erethrocytes (TEC) as well as the total number of leukocytes per cubic millimeter blood were calculated using normal saline as a diluent employing the Neubour haematocytometer (COLES, 1980). Blood smears were prepared for the differential leukocytic count. The smears were dried and fixed with Giemsa stain (SCHALM, 1965).

Statistical analysis:

The sttistical analysis of variance of treated and control gorups was carried out according to STEEL and TORRIE (1960).

RESULTS and DISCUSSION

Assay on the fungal flora of mixed rations used for poultry revealed the isolation of 42 species of mesophilic fungi belonging to 13 genera (Table 1). These enormous species of fungi enhabiting the feeds of poultry demonstrated the risk which could be reflected upon the health of birds from metabolites (DALVI, 1986) and upon economics through deterioration of feeds (ORUNDER, 1987). These findings emphasis the urgent need of powerful, cheap and safe fungistats in feeds of livestock.

Cinnamaldehyde and salicylaldehyde in its effective concentrations used in the study were proved as powerful fungistats in rations used for poultry, which was in agreement with findings of MEGALLA, et al. (1986), in wheat and corn flour and bread.

Haematological examinations of treated hens revealed no significant changes in the parameters than control (Table 2, 3 and 4). Moreover, the mean live body weight of control group at the end of experimental period was 1.45 kg $\pm$ 150 g and test group received ration treated by cinnamaldehyde was 1.44 kg $\pm$ 160 g and test group received

ration treated with salicyaldehyde was 1.42 kg ± 170 g. Egg production in the 1st, 2nd, 3rd and 4th, week of the experimental period were 76%, 76%, 75% and 75% respectively for control group of hens. The two groups of hens received treated ration revealed no alteration in their egg productivity.

It was clear from the results that neither erethrocytic, leukocytic and differential leukocytic count or live body weight were significantly affected after receiving treated rations for long period of administeration. These findings could conclude the safety of such compounds used as fungal inhibitors inconcentrate rations and recommend its use for producers of concentrate rations to supress the activity of storage fungi and protect livestock health from harmful effects of fungal metabolites as well as the feeds from deterioration which lead to economic losses.

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Table (1)

Species of fungi revealed from examined ratios

Genus	Species
Aspergillus	A.flavus, A.niger, A.fumigatus, A.flavus var. calumnaris, A.ochraceus, A.flavipes, A.chevalieri, A.sydowi, A.tamarri, A.terreus, A.candidus, A.ustus, A.versicolor, A.pseudoglaucus, A.clavatus, A.penicilloides, A.subolivaceus, A.wentii, A.nidulans
Penicillium	P.chrysogenus, P.corylophilum, P.oxalicum, P.jenseni
Mucor	M.varians, M.silvaticus, M.circinelloides, M.fragilis, M.plumbeus, M.hiemalis
Scopulariopsis	S.brevicaulis, S.knoingii
Rhizopus	R.stolonifer, R.rhizopodiformis
Fusarium	F.moniliforme, F.solani
Syncephalastrum	S.racemosum
Neurospara	N.crassa
Paecilomyces	P.variotii
Cephaliophora	C.tropica
Botryotrichum	B.atrogriseum
Beuveria	Beuveria sp.
Isaria	Isaria sp.

Table (2)

Erythrocytic, leukocytic and differential leukocytic count per cubic millimeter blood of laying hens after receiving treated rations

Experimental birds		Parameters						
		Total Erythrocytes (10^6)	Total Leukocytes (10^3)	Lymph.	Het.	Eos.	Bas.	Mono.
Control	1	2.74	18	66	23	1.5	1	8.5
	2	2.68	19.2	63	25	2	1	9
	3	3.1	20.1	59	26	2	2	11
	4	2.8	19.7	61	22.5	4.5	2	10
	5	2.58	19.9	68	21	2.5	0.5	8
	6	2.77	19.1	66	22	2	1	9
	7	2.91	18.6	63	24	1.5	1	10.5
	8	2.86	19.1	60	24	1.5	2	12.5
	9	2.72	19.3	65	20	2	1	12
	10	2.83	18.8	68	21	2	1	8
Test (I)	1	2.62	18.3	64	22	3	1	10
	2	2.73	18.9	66	23	2	1.5	7.5
	3	3.1	19.1	65	25	2.5	1	6.5
	4	2.62	19	67	22	3	1	7
	5	2.8	18.7	68	22	2	1	7
	6	2.5	18.8	68	23	2	0.5	6.5
	7	2.66	17.9	66	24	1.5	1	7.5
Test (II)	1	3.12	17.8	64	22	2	1.5	10.5
	2	2.8	18.1	62	21	2	1.5	13.5
	3	2.95	19	60	23	1.5	2	13.5
	4	2.76	18.9	66	24	1	2	7
	5	3	18.1	63	25	1	2	9
	6	2.82	18.8	65	26	0.5	1.5	7
	7	2.76	18.7	65	24	1.5	1.5	8

Test (I): Laying hens reoeived ration the treated with cinnamaldehyde.
Test (II): Layinghens reoeived the ration treated with salicyladelhyde.

Table (3)

The average means of blood cells count (X ± S.E.) per cubic millimeter blood

Parameters	Overall mean	Control	Treatment (I)	Treatment (II)
Erythrocytes	2801571.43±32530	2799000±49690.40	2718571.43±59391.39	2887142.86±59391.39
Leukccytes	18779.05±110.46	19180± 168.73	18671.43± 201.67	18485.71± 201.67
Lymphocytes	64.59± 0.52	63.90± 0.79	66.23± 0.95	63.57± 0.95
Heterocytes	23.14± 0.35	22.85± 0.53	23.00± 0.63	23.57± 0.63
Eosinophils	1.93± 0.15	2.15± 0.23	2.29± 0.28	1.36± 0.27
Basophiles	1.32± 0.09	1.25± 0.13	1.00± 0.16	1.71± 0.16
Monocytes	9.02± 0.40	9.85± 0.62	7.43± 0.74	9.79± 0.74

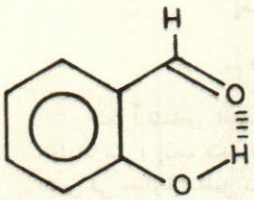
Test (I): Laying hens received ration the treated with cinnamaldehyde.

Test (II): Layinghens received the ration treated with salicyladehyde.

Table (4)

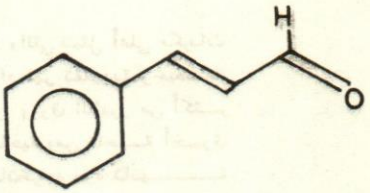
Least-square analysis of variance of different treatments

Source of variance (S.V.)	Degree of freedom (D.F.)	Mean squares (M.S.)						
		Erythrocytes	Leukocytes	Lymphocytes	Heterocytes	Eosinophiles	Basophiles	Monocytes
Between treatments	2	49771964285.714	1115363.095	15.979	1.125	1.824	0.926	14.223
Within treatments	21	24691360544.218	284707.483	6.288	2.797	0.515	0.169	3.794



O-Hydroxy benzaldehyde

(salicylaldehyde)



Cinnamaldehyde

Fig. (1)