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MICROBIAL DEGRADATION OF PESTICIDES RESIDUES IN TOMATO HAULMS AND THEIR EFFECTS ON PERFORMANCE OF DAIRY COWS.

A.A. Hassan[1]; M.S. Khalel; [1] A.M. Shwerab[1]; M.H. Yacout[1]; B.E.E. Borhami[2] and H. Z. Ibrahim[3]

[1]Department of By-products Utilization - Animal Production Research Institute, Agriculture Research Center, Dokki, Giza, Egypt.

[2]Department of Animal Production, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria, Egypt.

[3]Department of Environmental Studies, Institute of Graduate Studies and Research, University of Alexandria, Egypt.

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SUMMARY

The study aimed to assess farmers' attitudes on pesticide toxicity and reducing pesticide residues in animal products and food crop products, and investigate the attitude changes of farmers on pesticide use. Eight crossbred Friesian cows were used in double 4 × 4 Latin squares design each period lasting 28 days. They fed according to their body weight (550 kg in average) and previous milk yield (10 – 12 kg/day average) on tomato haulms in fresh form (FTH), hay (HTH), hay treated with fungus, (*Trichoderma reesei*, THF) or silage (THS) in addition to concentrate feed mixture(CFM). Results showed that animals fed on the THF and THS rations had significantly higher ($P<0.05$) digestion coefficients, nutritive values and milk yield and composition than the other experimental rations. Total VFA's, rumen volume, total bacterial counts and microbial protein synthesis were significantly increased ($P<0.05$) in THF and THS rations than the other experimental rations. Animals fed THF showed more soluble, degradable, less un-degradable fractions and more effective degradability. Significant differences were observed between groups for all blood constituents. Also, milk yield, FCM, fat and protein yield production were significant increased ($P<0.05$). Pesticide residues were detected in FTH, HTH and milk produced by cows fed these rations. Tomato haulms treated with fungi and in silage form had low pesticides residues content. The pesticides residues and total PCBs of the milk of THF and THS rations showed the lowest values of pesticides residues compared with FTH and HTH rations. Serum glucose, cholesterol, urea, creatinine concentrations, serum aspartate aminotransferase and alanine aminotransferase were significantly decreased ($P<0.05$) in animals fed THF and THS rations than the other experimental rations. On the other hand, serum total protein and albumin, were significantly increased ($P<0.05$) in THF and THS rations. So, it could be recommended that tomato haulms be used after treating it with the fungus as first priority in ruminant feeds or as silage as second priority.

***Keywords**: tomato haulms, milk yield, degradability, pesticides residues, fungus treatment, silage.*

INTRODUCTION

Vegetables and dairy milk are important commodities in Egypt. However, agrochemicals are used intensively and excessively in the production system. Therefore, pesticide residues and contamination commonly occur in agricultural products and environments. Million tons of pesticides were annually applied in modern agriculture in order to increase productivity through controlling insects, fungi, bacteria, viruses as well as grasses grown in between the economical crops (Liu and Xiong, 2001). However, less than 5% of these products are estimated to reach the target organisms. One of the most important problems with the use of pesticides is their possible persistence in the environment and therefore, its possible incorporation into the food chain whereas it affects ecosystem and all human beings (Liu and Xiong, 2001).

Major problems are caused from the contamination of food by pesticide residues, and pollution of environmental ecosystems. Presently, indoor use of pesticides for pest control is widespread in Egypt. No accurate information of the types and amounts of Egyptian household pesticide use, or numbers of contamination incidents is available. Generally, use of indoor pesticides is inadequately managed. The results of a survey of Egyptian farmers' attitudes toward pesticides and their usage behavior garnered new insights as to how pesticides should be better controlled and regulated in Egypt (Mansour, 2008).

The use of pesticides has been known to have environmental impacts through its residues and contamination. Contaminated agricultural materials such as soils, water and sediments are suspected as the source of pesticide contamination in agricultural and animal products (Ntow, 2003). Organochlorines (OCs) are known as persistent accumulated compounds in the environment since they are non-degradable (Matsumura, 1976), which eventually becomes a common residue detected in food crops such as corn, cabbages, rice, tomatoes and soybean (Soejitno, 2002). Animal products such as eggs, meat and milk have also been reported to contain pesticide residues in Indonesia (Indraningsih, 1998), Egypt (Ibrahim *et al.* 1994), and Kenya (Kahunyo *et al.* 2001). In Egypt, extensive use of agrochemicals has led to public health and environmental problems (Yassin *et al.*, 2002). In Kenya, Maitho (1992) reported that OCs (DDT, dieldrin, aldrin and lindane) were detected in 22 out of 25 samples of fat tissues. While, DDT has been once detected in human milk in Indonesia and was regarded as the highest level of DDT residues ever being reported in the world (Burke *et al.* 1998 and Shaw *et al.*, 2000).

Pesticides and certain industrial chemicals such as polychlorinated biphenyls (PCB's) could also affect human health and none target animals. Pesticides poisoning commonly occurred in animal after consuming contaminated feed whereas it showed clinical signs such as hyperemia, eyes exudation, hyper salivation, diarrhea, dyspnoea, and finally death (Indraningsih, 1998). Several cases of toxicity or death described above may result from excessive use or misuse of pesticides at farm level, where precaution measure such as wearing protective gears are poorly followed.

Monitoring of pesticide residues in Egyptian tomatoes and its products was studied, where in average it contains hexachlorobenzene (HCB), lindane, dieldrin, heptachlor epoxide and DDT derivatives at levels 0.009, 0.003, 0.006, 0.008 and 0.083 (mg/kg), respectively (Abou-Arab, 1999). On the other hand, the levels of dimethoate, profenofos

and pirimiphos-methyl were 0.461, 0.206 and 0.114 (mg/kg), respectively (Abou-Arab, 1999). The distribution patterns of pesticide residues within the cuticular and sub-cuticular tissues in tomatoes were also studied. The skin samples were found to contain the highest levels of HCB, lindane, dieldrin and DDT derivatives. However, washing with water and/or detergent solution was found to be necessary to decrease the intake of pesticide residues. Freezing, as well as juicing and peeling, were necessary to remove pesticide residues in the skin. Cooking of tomatoes (including processing tomato to paste) helped to eliminate most pesticide residues from contaminated tomatoes (Abou-Arab, 1999).

The reactions that destroy pesticides change most pesticides residues in the environment to inactive, less toxic, and harmless compounds. However, degradation is detrimental when a pesticide is destroyed before the target pest has been controlled. There are three types of pesticide degradation, microbial, chemical, and photo degradation. Microbial degradation is the breakdown of pesticides by fungi, bacteria, and other microorganisms that use pesticides as a food source (DebMandal *et al.* 2008). Therefore, this study was carried out to evaluate two microorganisms (***Trichoderma*** *reesei* and lactic acid bacteria) and sun - dry treatments as detoxification of pesticides residues tools from plant in Nubaria area, Egypt.

MATERIALS AND METHODS

Four tomato haulms with capacity of 20 tons (5 ton each) were used to be fed as fresh, hay, hay treated with fungi (Trichoderma reesei) and silage. The Tomato haulms were collected from Nubaria area, Egypt; after harvesting, chopped (1 to 3 cm in length) and left to sun-dry for a period of 7-10 reaching a moisture content of 10-12%. The silage was prepared by filling successive layers of the chopped materials and heavily trod ten before adding the next layer; molasses was added at the rate of 3% at the ensiling time.

Eight crossbred Friesian cows in their third and fourth lactation seasons were used in double 4 × 4 Latin squares design with each period lasting 28 day (Steel and Torrie, 1980). Cows were paired according to body weight (550 kg average) and previous milk records (average 10 – 12 kg/day). Each diet was fed to two cows during a period of 28 days. The first 21 days were considered as preliminary period followed by a 7days collection period. Milk yield was recorded individually on two successive days, milk samples were collected twice daily for 7 days through the collection period from all cows according to Galatov (1994). Milk samples were chemically analyzed for total solid (TS), protein, fat and ash according to AOAC (1995) while lactose was calculated by difference.

Four experimental rations were composed of concentrate feed mixture (CFM) plus fresh tomato haulm (FTH) as control ration, CFM + hay tomato haulm (HTH), CFM + hay tomato haulm treated with fungi (Trichoderma reesei) (THF) and CFM + tomato haulm silage (THS).

The CFM was fed to supply the CP requirements according to NRC (2001), while, tomato haulm were allowed to be fed *ad libtum* in each group, the actual amount of tomato haulm was recorded. The CFM consisted of 35% yellow corn, 29% wheat bran, 12%

soybean meal (44% CP), 13% linseed meal, 7% molasses, 2% limestone, 1.5% common salt and 0.5% vitamins minerals premix.

For the digestibility trials three adult male Barki sheep weighing approximately 48.5 ± 2kg BW, were housed in metabolic cages for each treatment. Sheep were kept on the rations for a preliminary period of 21 days, and during the next 7 day, total feces and urine were collected. Sub samples (20%) of feces and urine were taken once daily and frozen until analyses.

Fecal samples were dried at 60°C for 72h. Feed and fecal samples were ground through 1mm screen on a Wiley mill grinder and the samples (50gm/sample/ treatment/sheep) were composed for analysis. The samples of feed and feces were analyzed for crude protein (CP), crude fiber (CF), ether extract (EE) and ash, while the urine sample was analyzed for nitrogen (N) according to AOAC (1995). Values of the total digestible nutrients (TDN) were calculated according to the classic formula of Maynard et al. (1978) on a dry matter basis (DM).

Samples of rumen liquor were taken at 0,1, 3 and 6 h post feeding from three fistulated adult Barki ewes sheep approximately 45.5 ± 0.5kg BW for each treatment, were analyzed immediately for pH using Orion 680 digital pH meter. Samples were strained through four layers of chesses cloth. For each sampling time, samples of rumen fluid were preserved for ammonia nitrogen (NH_3-N) determination by adding concentrated H2SO4 (3 drop per 5ml). The concentration of NH_3-N was determined by using magnesium oxide (MgO) as described by the AOAC (1995). Concentration of total volatile fatty acid (VFA's) was estimated by using steam methods (Warner, 1964). Total bacteria count was carried out according to Difco (1984). Rumen volume was determined by the calorimetric method using Cr-EDTA before and after 3 and 6 hrs of feeding according to El-Shazly *et al.* (1976). The microbial protein synthesized (g MP/day) in the rumen of sheep fed the experimental rations was calculated using the model equation by Borhami *et al.* (1992) as follow: g MP / day = mole VFA produced / day X 2 X 13.48 X 10.5 X 6.25 / 100

where one mole VFA yield about 2 mole ATP (Walker, 1965), one mole ATP produce 13.48 YATP (g DM microbial cell) Borhami *et al.* (1979), N % of dry microbial cell = 10.5 (Hungate, 1965).

The rate and extent of DM, OM and N loss using nylon bags for each of the roughages were evaluated using the three fistulated ewes. Bags (6 cm × 12 cm and 53 μm pore size) containing 5g of ground samples of each roughage were incubated in the ventral part of the rumen and were removed after 3, 6, 12, 24, 48 and 72 h. After removal from the rumen, they were washed in cold water with gentle squeezing until the water become clear. Zero time disappearance values were obtained by washing un-incubated bags in similar fashion (Ash, 1990). Bags were dried in oven at 60°C for 48h, and DM loss was recorded for each time. Nitrogen content was also determined.

In situ degradation data for DM, OM and CP were fitted to the equation of Ørskov and McDonald (1979): $P = a + b (1 - e - ct)$

Where P is a degradation rate at time t, a is the intercept representing the soluble fraction of DM, OM or CP (time 0), b is the portion of DM, OM or CP potentially degraded in the rumen, c is a rate constant of degradation of fraction b. The ruminally un-degraded fraction U = 100 – (a+b). The effective degradability (ED) for tested roughages

was estimated from the equation of Ørskov and McDonald (1979) as follow: ED = a + bc / (c +k) where, k is the out flow rate assumed to be 0.03/h under the feeding condition in the current study.

Cell wall was analyzed for neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) using Tecator Fibretic system. Hemicellulose and cellulose were determined by difference according to Van Soest (1982).

Blood samples were collected twice (the first one was taken before the beginning of the experiment and the other one at the end of the experimental period), from all cows. Blood samples were obtained from the jugular vein of the cows in the morning before access to feed and water. Serum was obtained by centrifugation of blood and was stored at – 20 °C until used for analysis. Glucose concentration was determined by the method of Trinder (1969). Serum cholesterol was determined using the colorimetric method of Stein (1986). Serum total protein (TP) was measured as described by the Biuret method according to Henry *et al.* (1974). Albumin (A) concentration was determined according to Doumas *et al.* (1977). Kidney function was evaluated by measuring blood urea using the colorimetric methods of Henry and Todd (1974) using commercial kits. Creatinine was measured using the colorimetric method according to Faulkner and King (1976). Liver function was assessed by measuring the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) by the method of Reitman and Frankel (1957).

Pesticide Residues Analysis:

Solvents and other reagents used (acetone, benzene, ethyl acetate, methylene chloride, n-hexane, florisil 60-100 mesh (pre-treated as in the method of Kadenczki *et al.* (1992); sodium hydroxide, stannous chloride, carbon disulfide, cupric acetate monohydrate, hydrochloric acid, ethanol, diethanol amine were analytical reagent grade. The analytical standards of the tested pesticides were kindly provided by Department of Environmental Studies, Institute of Graduate Studies and Research, University of Alexandria, Egypt. The selected analytical standards are: (a) - Chlorinated hydrocarbon insecticides: HCB, lindane, p,p'-DDD, p-p' DDE and p-p' DDT. (b)- Halogenated pyrothroids: Cypermethrin, lambda-cyhalothrin. (c)- Organophosphorus insecticides: Dimethoate, malathion.

A simple multi-residue method according to Kadenczki *et al.* (1992) was applied to extract several pesticides (chlorinated hydrocarbon, halogenated pyrothriod insecticides and organophosphate) from tomato haulms and milk. The principle of this method is based on having a homogeneous sample pulp adsorbed on the surface of activated florisil to obtain a free-flowing powder, which is extracted in a glass column with methylene chloride-acetone (9:1, v/v). The gas chromatograph (GC) used was HP-5890 Series II. Polychlorinated biphenyls (PCBs) were determined by gas chromatograph according to Willett and Hess (1975).

Statistical Analysis:

Means were calculated for all variables by cow within period. Data were analyzed using the MIXED procedure of SAS (SAS, 2000). Period and cow were considered random effects; diet and cannulation effects were considered fixed. Estimation method was restricted maximum likelihood and the degrees of freedom method was Kenward-Roger (SAS, 2000). Collected data of digestibility trail and rumen study were subjected to one way analysis of variance; statistical processes were carried out using the General Linear

Models adapted by SAS (2000). Significant differences among means were separated using LSD test according to Duncan (1955).

RESULTS AND DISCUSSION

Chemical analysis and composition:

Chemical analysis and cell wall constituents are presented in (Table 1). Tomato haulms treated with fungi showed higher values of CP and ash contents than the untreated and other treatments, while it recorded lower values of CF, NDF and ADF. The increase in CP from 7.83 to 18.69 % after treatment with fungi could be related to the addition of basal minerals media containing nitrogen salts and/or due to the release of water soluble sugar from polysaccharides which had led to faster growth of fungus and in turn resulted in higher CP content (Salem, 2003).

Table (1): Chemical analysis and cell wall constituents of the concentrate feed mixture (CFM) and tomato haulm fed to animals (on DM basis).

Item	CFM	FTH	HTH	THF	THS
DM	88.95	29.68	89.66	87.85	31.66
OM	93.32	91.07	91.03	88.21	91.01
CP	15.88	7.83	7.88	18.69	7.67
CF	6.72	42.75	43.59	32.83	40.69
EE	2.83	1.77	1.85	1.54	1.72
NFE	67.89	38.72	37.71	35.15	40.93
Ash	6.68	8.93	8.97	11.79	8.99
NDF	24.92	76.54	69.77	62.60	64.39
ADF	12.33	49.05	46.42	39.32	41.96
ADL	9.56	22.08	19.28	17.07	17.80
Hemicellulose	12.59	27.49	23.35	23.28	22.43
Cellulose	2.77	26.97	27.14	22.25	24.16

CFM: Concentrate fed mixture, FTH: Fresh tomato haulm, HTH: Hay tomato haulm, THF: Hay tomato haulm treated with fungi, THS: Tomato haulm silage.

The decrease in CF content could be a result of the cellulose enzymes secreted by the fungus whereas fungi among the microorganisms have been proved its capability in decomposing the agricultural by products (Fadel, 2001). The decreased in NFE and CF content may be due to the loss of soluble carbohydrates by the fungus resulted in reduced DM or the fungus could depend on carbohydrates including CF as carbon sources to grow up and convert them into microbial protein (El Ashry *et al.*, 2003). The decrease in fiber fraction as NDF, ADF, ADL, hemicelluloses and cellulose contents may be due to the microbial attack whereas they degraded the bonds between cellulose and other components during the incubation period (Mohamed, 2005).

Concentrations of pesticides residues and total PCBs (ppb) of the tomato haulms:

The Concentrations of pesticides residues and total PCBs (ppb) of the tomato haulms are presented in (Table 2). The tomato haulms treated with fungi and in silage form showed lower values of pesticides residues compared with the fresh and hay form. Brajesh and Allan (2006) reported that the biochemistry of organophosphorus compound degradation by most of the bacteria seems to be identical, in which a structurally similar enzyme (organophosphate hydrolase or phosphotriesterase) catalyzes the first step of the degradation. Catherine *et al.* (2002) found that organophosphorus hydrolase is a bacterial enzyme that has been shown to degrade a wide range of neurotoxic organophosphate nerve agents. However, the effectiveness of degradation varies dramatically, ranging from highly efficient with paraoxon to relatively slow with methyl parathion. Plants have evolved interactions and association with micoorganisms that can accelerate breakdown or transformation of certain pollutants in the plant root zones to products that no longer pose environmental hazards (Brimecombe *et al.* 2001). Sharaf *et al.* (2006) reported that understanding pesticide metabolism in plants and microorganisms is necessary for pesticide development, for safe and efficient use, as well as for developing pesticide bioremediation strategies for contaminated soil and water. Pesticide biotransformation may occur via multistep processes known as metabolism or co-metabolism. To increase the levels of degradation in soil, some researchers have inoculated polluted soils with various fungal species immobilized on different lignocellulosic supports (e.g., woodchips, corncobs, and wheat straw) (Andersson and Henrysson, 1996). These reports show the beneficial effect of immobilization on degradation of organic cyclic compounds. The white rot fungus (*P. chrysosporium*) immobilized in corncobs achieved 35% degradation of TNT after 18 d of treatment (Tudor *et al.* 1990), 62% degradation of 2, 4, 5-trichlorophenoxyacetic acid after 30 d of treatment (Ryan and Bumpus, 1989), 10% degradation of DDT after 60 d treatment (Bumpus *et al.*, 1988), and 23% degradation of chlordane after 60 d of treatment (Kennedy *et al.* 1990). Plant residues are a plentiful source of nutrients that favor fungal growth and soil colonization. Additionally, plant residues elicit production of adaptive ligninolytic enzymes (Castillo *et al.*, 2001).

Table (2): Concentrations of pesticides residues and total PCBs (ppb) of the tomato haulms fed to animals (on DM basis).

Item	FTH	HTH	THF	THS
Cypermethrin	1.34	0.66	0.03	0.07
Dimethoate	13.60	6.54	0.001	0.01
Malathion	18.50	10.93	0.02	0.08
HCB	0.033	0.019	N.D	N.D
Lindine	0.13	0.08	N.D	N.D
Total DDT	0.089	0.051	N.D	N.D
Total PCBs	2.69	1.81	0.06	0.1

FTH: Fresh tomato haulm, HTH: Hay tomato haulm, THF: Hay tomato haulm treated with fungi, THS: Tomato haulm silage, N.D: not detected.

Dry matter intake, digestibility, nutritive values and nitrogen utilization:

Dry matter intake, apparent digestion coefficients, feeding values and nitrogen utilization of TH fed to sheep were illustrated in Table (3). THF, HTH and THS rations had higher (P<0.05) DM intake than those fed FTH ration; the lower intake from FTH ration may be due to the effect of pesticides which may alter the bacterial population in the rumen (Lowrey *et al.* 1969). Prolonged exposure to low levels of pesticides can interfere with metabolic processes, hence altering normal utilization of nutrients by motility alterations or pathological lesions in the gastrointestinal tract (Shull and Cheeke, 1983). The hazardous effect of dimethoate and cypermethrin on feed intake could be due to its effect on the center nervous system (CNS), particularly the hypothalamus which contains centers governing feed consumption. Also, hyperglycemia which was observed in treated animals exposed to such insecticides (Hassan, 1997).

Table (3): Dry matter intake, digestion coefficients, nutritive values and nitrogen utilization for sheep fed the experimental rations (mean ± SE).

Item	Experimental rations CFM plus			
	FTH	HTH	THF	THS
DM intake (g/h/d):				
Tomato haulms intake, g	500.19 ± 18.91^{b}	572.37 ± 13.28^{a}	590.78 ± 13.01^{a}	562.89 ± 18.06^{a}
Total DMI, g	855.99 ± 18.91^{b}	928.13 ± 13.28^{a}	936.58 ± 13.01^{a}	918.69 ± 18.06^{a}
R : C ratio	58 : 42	62 : 38	63 : 37	61 : 39
Digestion coefficients (%):				
DM	55.81 ± 0.31^{c}	61.06 ± 0.08^{b}	66.51 ± 0.41^{a}	60.63 ± 0.46^{b}
OM	60.85 ± 0.41^{c}	65.10 ± 0.14^{b}	70.29 ± 0.27^{a}	65.46 ± 0.33^{b}
CP	49.70 ± 0.27^{d}	54.22 ± 0.42^{c}	63.23 ± 0.91^{a}	59.61 ± 0.34^{b}
CF	45.61 ± 0.37^{c}	53.42 ± 0.21^{b}	59.16 ± 0.73^{a}	57.04 ± 1.36^{a}
EE	61.83 ± 0.61^{c}	66.99 ± 1.10^{b}	71.84 ± 1.37^{a}	70.21 ± 1.80^{ab}
NFE	71.57 ± 0.80^{b}	74.41 ± 0.38^{a}	77.01 ± 0.47^{a}	70.88 ± 1.25^{b}
NDF	51.74 ± 1.67^{c}	58.29 ± 0.95^{b}	66.79 ± 0.46^{a}	61.66 ± 0.92^{ab}
ADF	42.84 ± 0.73^{c}	47.29 ±1.05^{b}	54.51± 0.83^{a}	49.58 ± 0.52^{ab}
ADL	9.34 ± 0.44^{c}	11.29 ± 0.72^{b}	14.39 ± 0.23^{a}	12.46 ± 0.29^{ab}
Nutritive values (%):				
TDN	57.70 ± 0.39^{c}	61.69 ± 0.12^{b}	64.38 ± 0.23^{a}	62.08 ± 0.24^{b}
DCP	5.56 ± 0.07^{c}	5.93 ± 0.07^{b}	6.87 ± 0.12^{a}	6.54 ± 0.11^{a}
Nitrogen utilization (g/h/d) :				
N-Intake (NI)	15.31 ± 0.23	16.26 ± 0.17	15.92 ± 0.16	15.58 ± 0.47
N-Absorbed (NA)	7.61 ± 0.08^{c}	8.81 ± 0.04^{b}	10.07 ± 0.08^{a}	9.28 ± 0.25^{b}
N-Retention (NR)	0.38 ± 0.02^{c}	1.28 ± 0.14^{b}	2.42 ± 0.25^{a}	2.05 ± 0.22^{a}
NR % of NI	2.48 ± 0.08^{c}	7.87 ± 0.96^{b}	15.22 ± 1.61^{a}	13.15 ± 1.04^{a}
NR % of NA	4.99 ± 0.19^{c}	14.53 ± 1.66^{b}	24.03± 2.71^{a}	22.09± 1.84^{a}

^{abcd} *Means within rows with different superscript are significantly differ (P<0.05).*

CFM: Concentrate fed mixture, FTH: Fresh tomato haulm, HTH: Hay tomato haulm. THF: Hay tomato haulm treated with fungi,THS: Tomato haulm silage.

Sheep fed THF ration showed higher (P<0.05) apparent digestibility of OM, CP, NDF and ADF than those fed other rations. These results were mainly related to the effect of pesticides altering the bacterial population in the rumen and resulted in lower digestibilities (Lowrey *et al.*, 1969). Abou Akkada *et al.* (1973) found that the *in vitro* cellulose fermentation was inhibited with the present of the insecticides DDT. The lower values of nutritive value were related to the less digestion of cellulose accompanied with the alteration of bacterial population (Milillio *et al.* 1993). These data suggested that several pesticides currently used could influence metabolic hormones, particularly thyroxin as a principal regulator of metabolism (Rawlings *et al.* 1998). Ball and Chhabra (1981) suggested that the failure of body weight gain of animals exposed to pesticides may be due to the malabsorption of nutrients from the gastrointestinal tract and impaired feed conversion efficiency. Treatment of tomato haulms with fungi resulted in more (P<0.05) digestibility of cell wall components compared with other treatment. The improvement in fiber fraction digestibility as a result of biological treatment may be due to the effect of cellulose enzyme of fungi, which may be responsible for the stepwise hydrolysis of cellulose to glucose (Nsereko *et al.* 2002).

Data of nitrogen balance in FTH was the lowest (P<0.05) value (0.38 g) while in THF and THS were the highest (2.42 and 2.05 g, respectively), this mean that treatments improved nitrogen balance. This was reflected in better (P<0.05) N-utilization of the ration fed to sheep.

Rumen fluid parameters:

Resulted of Table (4) indicated that rumen liquor *p*H values did not significantly differ among treatments. The NH_3-N concentrations were significantly (P<0.05) higher in THF and THS rations than other rations, which could be a result of proteolytic activity in the rumen (Ørskov, 1992). Yadov and Yadav (1988) noticed that increased ruminal NH_3-N concentrations may be due to the higher intake of nitrogen and higher CP digestibility. Highest value of VFA's concentrations were observed with THF ration as it was expected from the higher digestibility of CF. Diet contained fresh tomato haulms showed higher rate of outflow compared to the other rations, while the lowest (P< 0.05) rate was observed with diet contained tomato haulms treated with fungi. Abou Akkada *et al.* (1973) reported that addition of the pesticide DDT caused reduction in VFA's production and ruminal activity which was a result of the less microbial protein synthesis. So, the reduction led to the decrease of lamb performance provided with the tested pesticides. Kumar *et al.* (1997) found high concentration of TVFA's in the rumen fluid when biological-treated roughages were fed; they attributed such increase to the high fiber breakdown. Allam *et al.* (2006) reported that the VFA's concentration in rumen is governed by several factors such as DM digestibility, rate of absorption, rumen *p*H, transportation of the digesta from the rumen to other parts of the digestive tract and the microbial population in the rumen and their activities.

Total bacterial counts and microbial protein synthesis in the rumen of sheep fed experimental rations are presented in Table (4). There were significant (P<0.05) decline in bacterial counts and microbial protein synthesis in the rumen of sheep fed fresh and tomato hay, while those fed hay tomato haulms treated with fungi showed the highest values. However, microorganisms mostly used the fermentable sugars for protein

synthesis, whereas, the white rot fungi-exhibited promising ability for the decomposition of ligno-cellulose containing materials and for increasing the availability of carbohydrates and production of fungal protein (Iconomou *et al.* 1997). Most microbial degrade pesticides in the soil. Soil conditions such as moisture, temperature, aeration, *p*H, and the amount of organic matter could affect the rate of microbial degradation because of their direct influence on microbial growth and their activity (DebMandal *et al.* 2008). The metabolic fate of pesticides is dependent on abiotic environmental conditions, microbial community, plant species (or both), pesticide characteristics and biological and chemical reactions. Abiotic degradation is due to chemical and physical transformations of the pesticide by processes such as photolysis, hydrolysis, oxidation, reduction and rearrangements (Al-Qurainy and Abdel-Megeed, 2009).

Catabolism and detoxification metabolism occur when a soil microorganism uses the pesticide as a carbon and energy source (Digrak and Özçelik, 1998).

Table (4): Rumen liquor parameters, total bacteria counts and microbial nitrogen synthesis for sheep fed the experimental rations (mean ± SE).

Item	Experimental rations CFM plus			
	FTH	HTH	THF	THS
PH	5.94 ± 0.93	6.31 ± 0.62	6.06 ± 0.33	5.99 ± 0.58
NH_3-N (mg/100mlR.L)	10.57 ± 0.56^{c}	11.81 ± 0.24^{b}	15.33 ± 0.74^{a}	13.62 ± 0.23^{a}
Total VFA's (meq/100 ml R.L)	7.18 ± 0.34^{c}	9.87 ± 0.32^{b}	12.89 ± 0.15^{a}	11.37 ± 0.21^{a}
Rumen volumes (L)	2.81 ± 0.17^{c}	3.55 ± 0.25^{b}	4.05 ± 0.19^{a}	3.82 ± 0.31^{a}
Rates of outflow (% hr)	7.84 ± 0.65^{a}	6.48 ± 0.29^{b}	5.48 ± 0.56^{c}	6.89 ± 0.33^{ab}
Total bacteria counts ($X10^{3}$ cfu/ml)	1.04 ± 0.07^{d}	1.19 ± 0.11^{c}	1.49 ± 0.05^{a}	1.42 ± 0.08^{b}
Microbial nitrogen synthesis (g/h/d)	9.64 ± 1.67^{d}	17.88 ± 2.52^{c}	32.79 ± 2.48^{a}	28.52 ±1.98^{b}

^{abcd} *Means within rows with different superscripts are significantly different (P<0.05).*
CFM: Concentrate fed mixture.
FTH: Fresh tomato haulm.
HTH: Hay tomato haulm.
THF: Hay tomato haulm treated with fungi.
THS: Tomato haulm silage.

Degradation kinetics

Estimate ruminal degradation constants (a, b and c) fitted with rates of DM, OM and CP disappearance for tomato haulms are presented in Table (5). Predicted constants were lower (P<0.05) in HTH and FTH compared with the THF and THS for DM, OM and CP degradability. However, THF and THS had more soluble and degradable fractions (a and b), lower un-degradable fraction (u), and more effective degradability (ED) than in HTH and FTH rations. The great degradative effect of the rumen microorganisms helps the animal to tolerate considerable concentrations of the pesticides (Donald *et al.* 1964). Paintner (1971) suggested that the cellulolytic and hemicellulolytic bacteria are more sensitive to low pesticides concentrations than other types. Rumen microorganisms could play a great role in detoxification mechanism for some of the pesticides and herbicides to which ruminants may be exposed (Abou Akkada *et al.* 1973).

Table (5): Degradation kinetics of DM, OM and CP for experimental tomato haulms for sheep fed the experimental rations (mean ± SE).

Item	Experimental rations CFM plus			
	FTH	HTH	THF	THS
		DM		
a, %	20.65 ± 0.22^{b}	19.97 ± 0.54^{b}	26.54 ± 0.53^{a}	23.33 ± 0.34^{a}
b, %	27.01 ± 0.23^{b}	28.41 ± 0.12^{b}	35.93 ± 0.16^{a}	32.56 ± 0.21^{a}
a+b, %	47.66 ± 0.25^{c}	48.38 ± 0.22^{c}	62.47 ± 0.23^{a}	55.89 ± 0.42^{b}
c, %	0.057 ± 0.001	0.059 ± 0.001	0.058 ± 0.002	0.058 ± 0.001
U	52.34 ± 0.43^{a}	51.62 ± 0.16^{a}	37.53 ± 0.46^{c}	44.11 ± 0.37^{b}
EDDM, %	38.35 ± 0.29^{c}	38.80 ± 0.53^{c}	50.22 ± 0.33^{a}	44.79 ± 0.23^{b}
		OM		
a, %	21.98 ± 0.44^{b}	21.59 ± 0.32^{b}	25.99 ± 0.21^{a}	24.16 ± 0.22^{a}
b, %	30.54 ± 0.32^{b}	32.55 ± 0.48^{b}	36.46 ± 0.39^{a}	34.24 ± 0.21^{a}
a+b, %	52.52 ± 0.31^{c}	54.14 ± 0.32^{c}	62.45 ± 0.16^{a}	58.40 ± 0.22^{b}
c, %	0.046 ± 0.001^{b}	0.048 ± 0.001^{ab}	0.052 ± 0.002^{a}	0.050 ± 0.001^{a}
U	47.48 ± 0.12^{a}	45.86 ± 0.17^{a}	37.55 ± 0.21^{c}	41.60 ± 0.33^{b}
EDOM, %	40.46 ± 0.16^{c}	41.62 ± 0.24^{c}	49.11 ± 0.25^{a}	45.56 ± 0.31^{b}
		CP		
a, %	22.56 ± 0.32^{b}	22.50 ± 0.22^{b}	27.35 ± 0.24^{a}	25.38 ± 0.15^{a}
b, %	32.77 ± 0.21^{c}	35.88 ± 0.25^{b}	40.22 ± 0.27^{a}	38.89 ± 0.11^{a}
a+b, %	55.33 ± 0.18^{b}	58.38 ± 0.32^{b}	67.57 ± 0.12^{a}	64.27 ± 0.14^{a}
c, %	0.051 ± 0.001^{b}	0.050 ± 0.001^{b}	0.056 ± 0.002^{a}	0.054 ± 0.001^{a}
U	44.67 ± 0.22^{a}	41.62 ± 0.29^{a}	32.43 ± 0.24^{c}	35.73 ± 0.13^{b}
EDCP, %	43.19 ± 0.37^{c}	44.93 ± 0.21^{c}	53.54 ± 0.13^{a}	50.38 ± 0.11^{b}

abc Means between columns with different superscript are significantly differ (P<0.05).
CFM: Concentrate fed mixture.
FTH: Fresh tomato haulm.
HTH: Hay tomato haulm.
THF: Hay tomato haulm treated with fungi.
THS: Tomato haulm silage.
a = soluble fraction (%).
b = potentially degradable fraction (%).
c = rate of degradability (% h-1).
U = rumen undegradable fraction {100-(a+b)}.
ED = effective degradability (%).

Milk Yield and composition:

Data concerning milk yield and its composition are presented in Table (6). The milk yield and fat corrected milk (FCM) were significantly increased (P<0.05) for THF ration compared with the other rations. Improving the nutrients composition, digestion coefficients and the feeding values of THF was reflected on more percentage of FCM produced by cows fed THF ration. Milk fat and protein yield were also significantly increased (P<0.05). Total solid, solids not fat and ash were increased as well, but without

reaching the significant effect. Fat, protein and lactose content were not affected. The increase in milk yield may be due to one or more of the following reasons: 1) higher DMI and higher nutrients digestibility (Table 3). 2) slight increase of milk lactose, which had a positive correlation with milk yield. 3) the increase in rumen microflora activity (Table 4), which lead to improve feed efficiency and increase milk production. However, *Trichoderma reesei* could identify a complete set of cellulase enzyme required for the break-down of cellulose to glucose (Nevalainen *et al.* 1991).

Table (6): Milk yield and milk composition for lactating cows fed the experimental rations (mean ± SE).

Item	Experimental rations CFM plus			
	FTH	HTH	THF	THS
Milk yields, kg/d	10.22 ± 0.89^{b}	11.61 ± 0.65^{ab}	14.13 ± 0.85^{a}	13.21 ± 0.95^{a}
4 % FCM*	8.71 ± 0.40^{c}	10.68 ± 0.24^{b}	13.06 ± 0.78^{a}	11.73 ± 0.62^{ab}
Fat, kg/d	0.31 ± 0.01^{c}	0.40 ± 0.01^{b}	0.49 ± 0.03^{a}	0.43 ± 0.02^{b}
Protein, kg/d	0.36 ± 0.03^{c}	0.40 ± 0.02^{bc}	0.54 ± 0.02^{a}	0.47 ± 0.05^{ab}
Milk composition (%):				
Total solids	11.16 ± 0.25^{b}	12.07 ± 0.11^{a}	12.46 ± 0.14^{a}	11.98 ± 0.28^{a}
Solids not fat	8.06 ± 0.12^{b}	8.57 ± 0.25^{ab}	8.96 ± 0.12^{a}	8.71 ± 0.24^{a}
Fat	3.1 ± 0.25	3.5 ± 0.22	3.5 ± 0.12	3.27 ± 0.18
Protein	3.41 ± 0.16	3.47 ± 0.13	3.89 ± 0.21	3.56 ± 0.19
Lactose	3.68 ± 0.26	4.09 ± 0.29	3.94 ± 0.26	4.08 ± 0.39
Ash	0.97 ± 0.05^{b}	1.01 ± 0.04^{ab}	1.13 ± 0.03^{a}	1.07 ± 0.06^{ab}

^{abc} Means within rows with different superscript are significantly differ (P<0.05).
CFM: Concentrate fed mixture
FTH: Fresh tomato haulm
HTH: Hay tomato haulm
THF: Hay tomato haulm treated with fungi
THS: Tomato haulm silage
**4 % FCM was calculated as: 0.4 X milk yield (kg) + 15 X fat yield (kg); Gaines (1923).*

Concentrations of pesticides residues and total PCBs (µg/kg on fat basis) in milk:

The concentration of pesticides residue and total PCBs (µg/kg on fat basis) in cows' milk are presented in (Table 7). The pesticides residues and total PCBs in the milk of cows fed THF and THS rations showed low values of pesticides residues compared with other rations. DebMandal *et al.* (2008) reported that microbes (fungi, bacteria, and other microorganisms) could degrade or breakdown the pesticides whereas they used them as food source. Quintero *et al.* (2008) reported that white-rot fungi species have demonstrated a high capacity to degrade organic pollutants such as the insecticide lindane (y-HCH).

Blood biochemical and serum constituents:

Data of serum glucose and cholesterol are presented in (Table 8). Data showed that FTH and HTH rations caused a significant (P<0.05) increase in glucose and cholesterol levels than THF and THS rations. While THF ration had significant (P<0.05) decline in

glucose and cholesterol than FTH and HTH rations. The changes in carbohydrate metabolism induced by pesticides can be correlated with the effects of these chemicals on the activities of hepatic enzyme system which are intimately involved in glucose production, storage and metabolism and/or correlated with the endocrine activity of the pancreas (insulin activity).

Table (7): Concentrations of pesticides residues and total PCBs (µg/kg on fat basis) of the milk cows samples.

Item	Experimental rations CFM plus			
	FTH	HTH	THF	THS
Cypermethrin	0.26	0.003	N.D	N.D
Dimethoate	0.55	0.19	N.D	N.D
Malathion	0.83	0.067	N.D	N.D
HCB	0.003	N.D	N.D	N.D
Lindine	0.022	0.001	N.D	N.D
p.p' DDE	0.028	0.005	N.D	0.001
Total PCBs	0.51	0.1	N.D	0.03

CFM: Concentrate fed mixture
FTH: Fresh tomato haulm
HTH: Hay tomato haulm
THF: Hay tomato haulm treated with fungi
THS: Tomato haulm silage
N.D: not detected.

Table (8): Blood serum parameters for lactating cows fed the experimental ration (mean ± SE).

Item	Experimental rations CFM plus			
	FTH	HTH	THF	THS
Glucose mg/dl	125.62 ± 4.82^{a}	92.21 ± 2.44^{b}	81.23 ± 3.70^{c}	84.66 ± 2.96^{c}
Cholesterol mg/dl	209.55 ± 8.34^{a}	175.63 ± 3.98^{b}	92.34 ± 3.22^{c}	97.88 ± 2.77^{c}
TP g/dl	6.47 ± 0.22^{b}	6.84 ± 0.63^{b}	8.44 ± 0.41^{a}	8.05 ± 0.27^{a}
Albumin g/dl	3.13 ± 0.11^{c}	3.64 ± 0.18^{c}	5.58 ± 0.23^{a}	4.97 ± 0.13^{b}
Globulin g/dl	3.34 ± 0.09^{a}	3.20 ± 0.07^{a}	2.86 ± 0.13^{b}	3.08 ± 0.05^{ab}
Urea mg/dl	59.63 ± 3.22^{a}	51.44 ± 3.43^{a}	44.49 ± 2.52^{b}	41.56 ± 2.88^{b}
Creatinine, mg/dl	1.88 ± 0.05^{a}	1.62 ± 0.08^{b}	1.12 ± 0.01^{c}	1.02 ± 0.03^{c}
AST U/L	56.75 ± 3.63^{a}	48.25 ± 2.88^{b}	34.38 ± 2.54^{c}	31.75 ± 2.72^{c}
ALT U/L	23.11 ± 1.56^{a}	19.25 ± 1.13^{b}	14.45 ± 1.25^{c}	13.22 ± 1.43^{c}

[abc] Means within rows with different superscript are significantly differ (P<0.05).
CFM: Concentrate fed mixture
FTH: Fresh tomato haulm
HTH: Hay tomato haulm
THF: Hay tomato haulm treated with fungi
THS: Tomato haulm silage

Pesticides exposure could cause hyperglycemia which might be a result of glycogenolysis in muscle and liver causing a significant increase in blood glucose level. This disturbance in carbohydrate metabolism may be responsible for the toxic action of pesticides (Ferrando and Andreu-Moliner, 1991). Additionally, exposure of animals to pesticides may interfere with transport of glucose that crosses the gastrointestinal canal. Thus, daily administration of propoxur (carbamate pesticide) at 0.1 LD_{50} resulted in increasing transport and decreasing absorption of glucose in the small intestines (Raja *et al.* 1992). Carlson and Kolmodin-Hedman (1972) found that the increase level of serum cholesterol may be responsible for inducing atherosclerotic changes. They also reported that the accumulation of pesticides in liver was associated with the disturbance of lipid metabolism and an elevation in serum cholesterol. Wasserman and Wasserman (1970) reported high levels of liver cholesterol due to proliferation of smooth surface endoplasmic reticular induced by chlorinated pesticides. Data of serum total protein, albumin and globulin are presented in (Table 8). Data showed that FTH and HTH rations caused a significant ($P<0.05$) decline in the total protein and albumin, but globulin was significantly ($P<0.05$) increased compared with the THF and THS rations. The reduction of serum proteins, particularly albumin, in animals fed FTH treated with pesticides could be attributed to changes in protein and free amino acid metabolism and their synthesis in the liver (Rivarola and Blegno, 1991). Pesticides are capable of inhibiting RNA synthesis, breaking DNA strands and altering protein synthesis.(El-Sebae *et al.* 1988). The decrease in proteins may be due to their degradation and also due to possible utilization of lindane for metabolic purposes (Murthy and Priyamvada Devi 1982). The free amino acids (FAA) are found to play a vital role in synthesis of enzymes and hormones and their increase levels in liver and kidney indicate stepped up proteolysis, fixation of ammonia and keto acids resulting in amino acid formation (Kabeer Ahmed *et al.* 1978).

Feeding FTH, HTH, THF and THS rations on the kidney function parameters, showed that FTH and HTH rations caused a significant ($P<0.05$) increase in urea and creatinine than THF and THS rations. The increased in blood urea and creatinine concentrations revealed in the present study should be due to pesticides. Elevated blood urea is known to be correlated with an increase in protein catabolism in mammalian body or it could be resulted from more efficient conversion of ammonia to urea as a result of increased synthesis of enzyme involved in urea production (Rodwell, 1979). Additionally, the increased blood urea was closely correlated with histopathological changes in the kidney which were degenerative, and these changes caused disturbance in the transport system of biochemical constituents in the kidney (Janardhan *et al.* 1988). Bhatia *et al.* (1972) reported that urea content increased in tissues of mice treated with lindane. It is evident that excess of liver ammonia is converted to urea, which means that the liver tissue has accelerated urea synthesis to detoxify the excess of ammonia either produced by the liver or being transported from other tissues.

Data on AST and ALT of lactating cows fed FTH, HTH, THF and THS rations are presented in (Table 8). There is a significant variation among the effect of various feeding rations on AST and ALT. Data showed that FTH and HTH rations had a significant ($P<0.05$) increase in AST and ALT than THF and THS rations.

These results are in agreement with the results obtained by Rajinder *et al.* (1990) who showed the elevation of aminotransferases in blood has been used as an indicator of altered

permeability of plasma membrane and/or cellular damage. The increased activity of serum AST and ALT indicated liver damage and disruption of normal liver function (Shakoori *et al.* 1994). The increment of the activities of AST and ALT in plasma are mainly due to the leakage of a these enzymes from the hepatic cytosol into the blood stream (Navarro *et al.* 1993), which gives an indication on the hepatotoxic effect of lindane. Reduction of such activities in liver is mainly due to leakage of these enzymes from the liver into the blood stream as a result of lindane toxicity which leads to the liver damage.

CONCLUSION

Conclusively, it could be concluded that biological treatment with fungi or bacteria (silage) could be advisable in order to overcome the harmful effect of TH exposure to pesticide. However, more studies are needed in this respect.

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التحلل الميكروبى لبقايا المبيدات فى عرش الطماطم وتأثيرة على أداء الأبقار الحلابة

أيمن عبد المحسن حسن[1] ، محمد سمير محمود خليل[1] ، عمرو محمد حلمى شويرب[1] ، محمد حلمى ياقوت[1] ، برهامى عز العرب برهامى[2] و هشام ذكى إبراهيم[3]

[1]قسم بحوث إستخدام المخلفات - معهد بحوث الانتاج الحيوانى - مركزالبحوث الزراعية ، الدقى – جيزة - مصر.
[2]قسم الإنتاج الحيوانى - كلية الزراعة (الشاطبى) - جامعة الإسكندرية - مصر.
[3]قسم الدراسات البينية -معهد البحوث والدراسات العليا - جامعة الإسكندرية - مصر.

تهدف الدراسة مساعدة المزارعين للتخلص من سمية المبيدات وتقليل بقاياها فى المنتجات الحيوانيه ومنتجات المحاصيل. حيث غذيت 8 أبقار فريزيان خليط حلابة فى تصميم مربع لاتينى 4 x 4 ومدة كل فترة 28 يوما على عرش الطماطم الطازج ، أوعلى صورة دريس ، أو دريس معامل بفطر التريكوديرما ، أو على صورة سيلاج إضافة لمخلوط العلف المركز.

أظهرت النتائج أن الحيوانات المغذاة على العرش المعامل بالفطر أو على صورة سيلاج سجلت نتائج أعلى معنويا لمعاملات الهضم والقيمة الغذائية وإنتاجا للبن مع تحسن مكوناته مع قيم عالية معنويا لمجموع الأحماض الدهنية الطيارة وحجم الكرش والعد البكتيرى والبروتين الميكروبى ، إضافة الى أعلى ذائبية لمكونات العلائق مع قلة الأجزاء غير المتحللة وزيادة كفاءة التحلل وكمية الدهن وكمية بروتين اللبن وكمية اللبن المعدل لنسبة الدهن (4%) ، كما توجد فروق معنوية بين المجاميع فى مكونات الدم. لوحظ وجود بقايا المبيدات فى كل من العرش المأكول على الصورة الطازجة وعلى صورة دريس وفى اللبن المنتج من الأبقار المغذاة عليهما مع نقص نسب البقايا فى العرش المعامل بالفطر أو على صورة سيلاج ، مع ملاحظة انخفاض معنوى فى تركيزات سكر الدم والكوليسترول واليوريا والكرياتنين وبعض أنزيمات الكبد فى علائق العرش المعامل بالفطر وعلى صورة سيلاج بينما نسب البروتين الكلى والالبيومين كانت أعلى معنويا.

لذا يمكن التوصية بإستخدام عرش الطماطم فى تغذية المجترات بعد معاملته بالفطر كاختيار أول أو على صورة سيلاج كاختيار ثان.