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MICROBIAL DEGRADATION OF PESTICIDE RESIDUES IN WATERMELON VINES AND ITS EFFECT ON PRODUCTIVE PERFORMANCE OF LACTATING GOATS

*Amal M. A. Fayed; A. A. Hassan; M. S. Khalel and Afaf H. Zedan
Animal Production Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.
*Corresponding author: AmalFayed70@yahoo.com

SUMMARY

This experimental work amid to investigate the effect of pesticide residues contaminate watermelon vines on productive performance of lactating goats and quality of their milk. The study based on the partial or total replacement of berseem hay (BH) by watermelon vines hay (WMVH) either untreated or biologically treated with fungus *(Trichoderma reesie*) in the rations of lactating goats. The test included a feeding trial and in vitro gas production estimation. Twenty-five lactating goats 2-4 years old and 30.58 ±1.23 kg body weight were assigned randomly to five groups (five each) using a randomized complete block design. The first group fed ration contained concentrate feed mixture (CFM) plus BH (1:1) and served as control (R1), while groups R2, R3, R4 and R5 were fed rations contained two levels 25 or 50% of watermelon vines hay either untreated (WMVH) or biological treated with fungus *Trichoderma reesie* (WMVF) (50 and 100 % from BH in the ration), respectively. Results showed that WMVF had higher values of CP and ash contents than those of untreated one but closer to CP percentage in berseem hay, (14.57 vs. 12.76%, CP), respectively. While the treated WMVF recorded lower values of CF, NDF and ADF than the untreated one. The treated watermelon vines with fungus showed lower values of pesticide residues compared with untreated one. The pesticide residues in milk of goats fed rations contained WMVH treated with fungus (R4 and R5) showed zero (not detectable) values compared with other rations having the untreated WMVH. Goats fed the R4 ration (50% CFM + 25% BH+25% WMVF) had comparable yields of actual milk, 4%-FCM, fat and protein, to those of control one (R1), while being significantly higher than the other tested groups. The milk constituent concentration mostly had similar trends to milk yield among the experimental treatments. Also, R4 ration recorded the best value of feed conversion and the best relative economic efficiency compared with the other experimental groups. Daily gain of kids from birth up to weaning was significantly ($P<0.05$) higher with R4 ration than kids fed the other tested ones, while its increase than control group was not significant. The NH_3-N concentrations were significantly ($P < 0.05$) higher with R4 than rations R3 and R5 but similar with R2 and control rations. Also, the highest values of TVFA's concentrations were observed with R4 compared with other tested rations but similar with control. Molar proportions of individual VFA were mostly affected significantly by some dietary treatments without clear trends among the experimental rations. The rations that formulated with treated WMVF (R4 and R5) had significantly ($P < 0.05$) lower values of CO_2 and CH_4 than those of R2 and R3 that formulated with the untreated WMVH. Serum glucose, cholesterol, triglyceride, urea, creatinine concentrations, serum aspartate aminotransferase and alanine aminotransferase were almost decreased significantly with animals fed R4 ration than other tested rations, while the values of R4 were similar to those of the control ration. Also, the serum total protein and albumin were similar between control and each of R4 and R5, but significantly higher than R2 and R3.

So, it could safely recommend introducing the WMVH treated with fungus to rations of lactating goats up to 25% (replaced 50% of berseem hay) to improve milk yield and composition without any adverse effect on productive performance of does, as well as the growth performance of their kids.

Keywords: watermelon vines; replacement; berseem hay; milk production, pesticides, fungus treatment, goats.

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INTRODUCTION

The feeding of some agricultural by-products that previously sprayed with pesticides to dairy animals is a common practice in most farming systems. The extensive use of pesticides, in particular with the vegetable crops, became a common practice to increase plants production. Meanwhile, under the limitation of cultivated forage lands to feed animal population, the livestock producers use the contaminated vegetable by-products for feeding their animals which consider as a normal feeding practices. **(Njiru, 1996)**.

Vegetables and dairy milk are important commodities in Egypt, while 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 have been annually applied in modern agriculture in order to increase productively through controlling insects, fungi, bacteria, viruses as well as the weeds that grown in the field of economic crops **(Liu and Xiong, 2001)**. One of the most important problems with the use of pesticides is their possible persistence in the environment and in turn possible incorporation into the food chain, which negatively affects ecosystem and all human beings **(Liu and Xiong, 2001**). The major problems are the contamination of food by pesticide and pollution of environmental ecosystems. The pesticides have been known to cause an environmental impact through its residues and contamination to agricultural water and sediments which suspected as the source of pesticides contamination in agriculture and animal products **(Ntow, 2003)**.

Organochlorines are known as persistent accumulated compounds in the environment since they are undegradable **(Matsumure, 1985**), which eventually become a common residue detected in food crops such as corn, cabbages, rice, tomatoes, watermelon and soybean **(Soejino, 2002)**. Animal products such as eggs, meat and milk have also been reported to contain pesticide residues in Egypt **(Ibrahim *et al.*, 1994**). In Egypt, extensive use of agrochemicals has led to public health and environmental problem **(Yassin *et al.*, 2002)**.

The reactions that destroy pesticides could be changing the most pesticide residues in the environment to inactive forms, less toxic and harmless compounds. There are many types of pesticide degradation such as 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 (**DebManadal *et al.*, 2008)**.

Biological treatments using some fungi (**Khorshed, 2000)** were tested to improve the nutritive value and digestibility of poor-quality roughages. **El-Ashry *et al.* (2003)** showed that enzymatic hydrolysis by fungi and biological conversion of cellulosic materials improves the nutritive value of residues especially crude protein and crude fiber. Biological treatment with fungi (*Trichoderma reesie*) was reported to be highly effective on reducing the level of pesticides. **Hassan *et al*. (2010)** showed that biological treatment with fungi or bacteria could be advisable in order to overcome the harmful effect of tomato haulms exposure to pesticide. Watermelon vine hay had good nutritive value, dry matter and protein degradability, as well as, it better utilized than both wheat straw and rice straw by ruminants **(Bassiouni, 2001)**. There are a few literatures on using watermelon vines in feeding ruminants; thereby we are in need for more studies on using this by-product in feeding farm animals. This study was carried out to evaluate the use of biological treatment by fungi (*Trichoderma reesie*) and sun-dry treatment for detoxification of pesticide residues from watermelon vines and also, the effect of partial or total replacement (50% or 100%) of berseem hay by watermelon vines either sun-dried or biologically treated with fungi in rations of lactating goats on their milk production, milk composition, rumen fermentation and blood parameters.

MATERIALS AND METHODS

This study was carried out at Noubria Experimental Station, Animal Production

Research Institute, Agriculture Research Center, Ministry of Agriculture, Egypt, to investigate the effect of partial or total replacement of berseem hay with WMV either untreated or biological treated with fungi (*Trichoderma reesie*), aiming detoxification of pesticides residues, on productive performance of lactating goats.

Biological treatments:

Fungi (Trichoderma reesie):

The fungus of *Trichoderma reesei* ATCC 28217 (RS) was obtained from the Microbiological Chemistry Center (MIRCEN), Faculty of Agric., - Ain–Shams University, Egypt. The organism was propagated and maintained on potato dextrose agar medium and the organism was grown and maintained on nutrient agar medium (**Difco Manual, 1984**). The optimal growth temperature for all organisms was 35±1^{0}C. For maintenance of microorganisms, agar slants of stock culture of microbial strains were kept in a refrigerator at 4^{0}C, and subculture was carried out every month. The purity of the cultures was regularly, microscopically tested.

Cultivation Procedures*:*

For preparation of fungi inoculum, *Trichoderma reesei* was first grown in a flask containing 500 ml of basal mineral medium with 1% glucose as described by **Hesseltine *et al.* (1966).** The flask was shaked for 72 hours in a water bath adjusted at 37^{0}C. Mycelium of the fungi was collected and broken into small hyphae bits using a warming blender. Inoculum, was used to inoculate a fermentor containing 50 liters of the sterilized medium (10% v/v) and adjusted at 37 ^{0}C for 72 hours. The fifty liters of fungal culture was transferred into 250 liters of a solution containing 2% molasses and 2 % urea (46.5%N). The above 250 liters were mixed well with about 250 Kg watermelon vines hay and left for 15 days as fermenting period. The moisture of materials was adjusted to approximately 70%. During the fermentation period, samples were taken biweekly to determine C/N ratio to evaluate success of the biological treatment, then samples of fermented watermelon vines were oven dried to constant weight at 60 ^{0}C overnight and ground for chemical analysis. After the fermentation period, the biologically treated watermelon vines hay was dried for 5 days, before formulating the tested rations. The concentration of antinutritional factors of watermelon vines was shown in Table (1) **(Amal Fayed *et al.*, 2019)**.

Table (1): Concentration (mg/100g) of antinutritional factors of watermelon vines.

Item	WMVH	WMVF
Total phénols	4.66	2.17
Total tannins	2.97	0.85
Saponins	2.78	1.01
Alkaloids	38.85	21.75
Flavonoids	1.06	0.88

WMVH: Watermelon vines hay (untreated watermelon vines). WMVF: Watermelon vines treated with fungi

Experimental design, animals and diets

Twenty-five lactating goats of 2-4 years old and 30.58 ±1.23 kg body weight were assigned randomly into five similar groups (five each) using a randomized complete block design. Animals fed berseem hay (BH) plus concentrate feed mixture (CFM) at the ratio 1:1 on DM basis (control) (R1) and two levels of replacement with untreated or treated WMV with fungi on the expense of BH (50% or 100%). The watermelon vines were collected from Noubaria area, harvested, chopped (1 to 3 cm in length) and left to sun-dry for a period of 7-10 days till reaching a moisture content of 10-12%.

Concentrate feed mixture was offered twice a day at approximately 7:00 am and 02: 00 pm, while BH and WMV were offered at 9:00 am and 4:00 pm. The feed allowances calculated according to **NRC (2007**) for goats. Animals were watered on fresh water at free choice. The experiment started from the last two months of pregnancy until the second month postpartum

(until weaning). The CFM used in this experiment was consisted of (%) 20 yellow corn, 19 soybean meal, 26 wheat bran, 25 barely, 6 molasses, 2 limestone, 1.5 salt and 0.5 mineral premix. Its chemical compositions (%, on DM basis) were 89.36, 93.46, 15.75, 6.68, 2.96, 68.07, 6.54, 36.85, 19.55, 6.57, 17.30 and 12.98 for DM, OM, CP, CF, EE, NFE, Ash, NDF, ADF, ADL, Hemi cellulose and cellulose, respectively.

After kidding does and kids were weighed directly after 15hr and at 15, 30, 45 and 60 days of age where kids weaned at 60 days of age. Representative milk samples were taken at 15, 30, 45 and 60 days post kidding from each doe at both milking time, twice daily at 7 am and 6 pm. Kids were isolated of their dams at 6 pm, till the next day morning at 7.00 am, where body weight of kids were recorded then they left for suckling their dams for 15 minutes and weighed again after sucking in order to measure the amount of suckled milk. The residual milk was hand milked and the total amount of morning milk was recorded. This procedure was repeated for the evening sucking at 6 pm. The amount of suckled plus the hand milked milk give the daily milk yield. Milk samples were taken and analyzed for total solids (TS), solid not fat (SNF), fat, protein (P) and ash % according to **Ling (1963)** and lactose was calculated by the difference.

Sampling and analysis of rumen liquor:

Rumen liquor samples were taken from three animals of each group at the last day of the experimental period using stomach tube at 0, 3 and 6 hrs after the morning meal. The rumen liquor was directly subjected to estimating the pH values, using Orian 680 digital pH meter. Samples were strained through three layers of cheese cloth for each sampling time, while ammonia nitrogen (NH_3-N) was determined using magnesium oxide (MgO) as described by **AOAC (2000)**. Total volatile fatty acid (TVFA'S) concentration was estimated using steam distillation methods **(Warner, 1964)** and microbial protein measured by sodium tangistate method according to **Shultz and Shultz (1970)**.

In vitro gas production measuring technique was used for assessing the digestion kinetics of both soluble and insoluble fractions of feedstuffs as described by **Menke and Steingass (1988)**. Samples (200 mg) of the air-dried feedstuffs were accurately quantitatively transferred to 50 ml calibrated glass syringe fitted with plungers. The rumen contents were kept in a water bath at 39ºC and saturated with CO_2 until inoculation took place. The buffer and inoculum (2:1 v/v) were mixed and kept in a water bath at 39ºC with CO_2 saturation **(Onodera and Henderson, 1980**). All laboratory handling of rumen fluid was carried out under a continuous flow of CO_2. Buffered rumen fluid (15ml) was pipetted into each syringe, containing the feed samples, and the syringes were immediately placed into the water bath at 39ºC. Syringes were incubated in vitro in water bath for 96 h and gently shaken every 2hr. The syringes continued incubation up to 96 h and gas production was recorded at 3, 6, 9, 12, 24, 72 and 96 h of incubation in vitro. Total gas values were corrected for blank incubation which contains only rumen fluid. The cumulative gas production (Y) at time (t) was fitted to the exponential model of **Ørskov and McDonald, (1979**).

Gas (t) = a+b × (1-exp^{-ct}). Where: a = the gas production from the soluble fraction (ml), b = the gas production from the insoluble fraction (ml), c = the gas production rate (ml/h) and t = incubation time (h).

The energy values were calculated from the amount of produced gas at 24 h of incubation with supplementary analyses of crude protein, ash and crude fat. (**Menke *et al.*, 1979**; **Menke and Steingass, 1988**). ME (MJ /Kg DM) = 1.06+ (0.157* GP at 24 h) + (0.084*CP) + (0.22* EE) –0.08* A, OMD (%) = 14.88+0.889*gas at 24h+0.45*CP+0.0651*A, NE (MCal/lb) = ((2.2+ (0.0272*GAS at 24h) + (0.057*CP) + (0.149*EE) /14.64, where: ME is the metabolizable energy, OMD is organic matter digestibility, GP is 24 h net gas production (ml/200 mg DM), A is ash (% of DM), NE is the net energy, and EE is ether extract or crude fat (% of DM). Short chain fatty acids (SCFA) were calculated according to **Getachew *et al.* (2005)**,

using the following equation: SCFA= (-0.00425+0.0222*GP at 24h)*100, where: GP is 24 h net gas production (ml/200 mg DM). Microbial protein was calculated as 19.3 g microbial nitrogen per kg OMD according to **Czerkawski (1986)**. Methane volume, carbon dioxide volume and the percentage of methane in the total gas were determined according to **(Fievez *et al*., 2005)**.

Blood samples

Samples collected at the end of collection period from the jugular vein of animals, allowed to flow into heparinized tubes, immediately centrifuged at 4000 rpm for 20 minutes to separate the serum and then stored at -20 °C for subsequent analysis. Blood serum was analyzed using special kits to determinate total protein as described by the Buiret method according to **Henry and Todd (1974)**, albumin determined according to **Doumas *et al.* (1971),** globulin calculated as the difference between total protein and albumin. Creatinine determined using the method of **Henry *et al*., 1974**, urea **(Fawcett and Soctt, 1961),** glucose **(Tinder, 1969)** cholesterol **(Allian *et al*., 1974**) and triglycerides (**Schalim *et al*., 1975)**. Alanine aminotransferase (ALT) (u/l) and aspartate aminotransferase (AST) (u/l) were measured according to **Reitman and Frankel (1957)**.

Pesticide Residues Analysis

A sample of multi-residues were implemented according to the method described by **Kadenczki *et al*. (1992)** which applied to extract several chlorinated hydrocarbon insecticides: HCB, Lindine and p, p' DDE; (b) Organophosphorus insecticides: Malathion; (c) Pyrethroids insecticides: Permethin- (d) Neonicotionoids insecticides: Acetamiprid, from watermelon vines and milk.

Statistical analysis

Data of growth statistically analyzed according to **SAS (2003**). The difference between means was tested by Duncan's Multiple Range Test **(Duncan, 1955)**. The model used is $Y_{ij} = \mu+T_i+e_{ij}$

Where: Y_{ij}= the observation on the 1th treatment.
μ= Overall mean.
T_i= Effect of the1th treatment.
e_{ij} = experimental error.

RESULTS AND DISCUSSION

Chemical analysis and cell wall constituents of berseem hay, watermelon vines hay either untreated or treated with fungi (% of DM basis).

The chemical analysis of berseem hay (BH), watermelon vines hay either untreated or treated with fungi are presented in Table (2). Watermelon vines treated with fungi showed higher values of CP and ash contents than untreated one but closer to CP percentage in berseem hay. The increase in CP from 8.63 to 14.57% after treatment with fungi could relate to the addition of basal minerals media containing nitrogen salts and or due to the release of water soluble sugar from polysaccharides which led to faster growth of fungus and in turn resulted in higher CP content. While the treated WMVF recorded lower values of CF, NDF and ADF than those of the untreated one, the decrease in CF content from 28.62, for untreated WMNH, to 24.66, for watermelon vines treated with fungi. These results are in agreement with those reported by **Hassan *et al*. (2010)** who showed that tomato haulm treated with fungi had higher values of CP, ash and lower values of CF, NDF and ADF than untreated one. **Fadel (2001)** reported that the decrease in CF content could be a result of the cellulose enzymes secreted by fungus whereas fungi is among the microorganisms proved capability to decompose the agricultural by products. Also, **El Ashry *et al*. (2003)** suggested that the decreased NEF and CF content might due to the loss of soluble carbohydrates by the fungus resulting in reducing DM or that fungus could depend on carbohydrates including CF as carbon sources to grow up and convert them into microbial protein. The decrease in fiber fraction as NDF, ADF, ADL, hemicelluloses and cellulose contents may

Table (2): Chemical analysis and cell wall constituents of berseem hay, watermelon vines hay either untreated or treated with fungi (% of DM basis).

Item	BH	WMVH	WMVF
DM	88.12	87.86	86.51
OM	92.67	91.45	89.08
CP	12.76	8.63	14.57
CF	24.81	28.62	24.66
EE	1.62	1.36	1.02
NFE	53.48	52.84	48.83
Ash	7.33	8.55	10.92
NDF	56.26	63.39	59.57
ADF	37.82	47.86	45.06
ADL	8.16	11.87	9.98
Hemi-cellulose	18.44	15.53	14.51
Cellulose	29.66	35.99	35.08

WMVH: Watermelon vines hay (untreated watermelon vines).
WMVF: Watermelon vines treated with fungi

be due to the microbial attack whereas they degraded the bonds between cellulose and other components during the incubation period and these results are in agreement with those reported by **Mohamed (2005)** and **Hassan *et al*. (2010)**.

Concentration of pesticide residues in watermelon vines:

The concentration of pesticide residues of watermelon vines either untreated or treated with *Trichoderma reesie* fungus are presented in Table (3). The treated watermelon vines with fungi showed lower values of pesticide residues compared with untreated one. Biological treatment with fungi (*Trichoderma reesie*) is reported to be highly effective in reducing the level of pesticides. **Hassan *et al*. (2010)** showed that biological treatment with fungi or bacteria could be advisable in order to overcome the harmful effect of tomato haulms exposure to pesticides. **Sharaf *et al*. (2006**) reported that understanding pesticide metabolism in plants and microorganisms is necessary for development of safe and efficient use of pestcides, as well as for developing pesticide bioremediation strategies for contaminated soil and water. Moreover, **Andersson and Henrysson (1996)** showed that pesticide biotransformation may occur via multistep processes through a certain metabolism or co-metabolism pathways in fungi. 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).

Table (3): Concentrations (mg/kg) of pesticide residues of watermelon vines (on DM basis).

Item	WMVH	WMVF
Permethin	0.86	0.17
Malathion	0.69	0.14
Acetamiprid	0.36	0.06
HCB*	0.16	0.02
Lindine**	0.23	0.06
p,p' DDE ***	0.11	0.01

WMVH: Untreated watermelon vines hay .WMVF: Watermelon vines treated with fungi.
** Hexachlorobenzene - ** gamma-hexachlorocyclohexan (γ-HCH)*
****Dichlorodiphenyldichloroethylene*

Milk yield and composition

Data concerning milk yield and composition of lactating goats fed the experimental rations are presented in Table (4). Milk yield and fat corrected milk (4%-FCM) were insignificantly increased (P<0.05) for R4

(50% CFM + 25% BH+25% WMV treated with fungi) compared with control ration (R1) while the other experimental groups R2, R3 and R5 were significantly decreased than control one (R1). Yields of milk fat and protein behaved the same trend. Concerning milk composition, goats fed R4 ration had significantly (P<0.05) higher contents of fat, protein, lactose, TS and SNF than the other tested rations (R2, R3 and R5), while insignificantly differed than control one. Milk ash content was significantly higher with both R3 and R5 than other experimental rations. Regarding milk yield and its constituents, the obtained result are in agreement with the findings reported by **Hassan *et al*.** (**2010**) who fed dairy cows a ration formulated with tomato haulms treated biologically by fungus (*Trichoderma reesie*) versus feeding rations of the untreated ones (fresh or dried as hay or ensiled). They concluded that the biological treatment with fungi or bacteria (silage) could be advisable in order to overcome the harmful effect of contaminantion with pestsides on tomato haulms, therefore, the productive performance of cows would improve significantly with well-being and harmlessly continuation of production. Additionally, **Abou Akkada *et al*. (1973)** demonstrated that rumen microbes could play a key role on detoxification mechanism for some pesticides to which ruminant animals might be exposed.

Table (4): Milk yield and milk composition for lactating goats fed the experimental rations.

Items	R1	R2	R3	R4	R5	SME	P Value
Live body weight	30.525	30.625	30.550	30.600	30.620	1.23	0.648
Milk yield (g/d)	914.75^{a}	739.42^{b}	539.74^{d}	934.62^{a}	619.62^{c}	41.62	<0.001
4%- FCM (g/d)	808.85^{a}	657.12^{b}	478.70^{d}	818.50^{a}	555.65^{c}	45.99	<0.001
Fat (g/d)	29.53^{a}	24.09^{b}	17.52^{c}	29.64^{a}	20.52^{bc}	1.68	0.001
Protein (g/d)	28.99^{a}	23.68^{bc}	15.84^{d}	29.15^{a}	19.55^{dc}	1.31	<0.001
Milk composition (%):							
Total solids	13.92^{a}	13.45^{b}	13.14^{c}	13.99^{a}	13.41^{b}	0.077	0.021
Solids not fat	10.46^{a}	10.14^{b}	9.95^{c}	10.55^{a}	10.15^{b}	0.061	0.016
Fat	3.46^{a}	3.31^{b}	3.19^{c}	3.44^{a}	3.26^{b}	0.084	0.019
Protein	3.44^{a}	3.23^{b}	2.87^{c}	3.41^{a}	3.10^{b}	0.078	0.01
Lactose	6.23^{a}	6.04^{c}	6.09^{bc}	6.26^{a}	6.12^{b}	0.066	0.027
Ash	0.79^{c}	0.87^{b}	0.99^{a}	0.88^{b}	0.93^{a}	0.023	0.012

$^{a,,b,c\ and\ d}$ *Means within rows with different superscripts are significantly different (P<0.05).*
R1: *50% CFM + 50% BH as control ration.*
R2: *50% CFM + 25% BH+25% WMVH (untreated WMV)*
R3: *50% CFM + 50% WMVH (untreated WMV)*
R4: *50% CFM + 25% BH+25% WMV treated with fungi*
R5: *50% CFM + 50% WMV treated with fungi*

Concentrations of pesticide residues (µg/kg on fat basis) in milk.

The concentrations of pesticide residues (µg/kg on fat basis) in milk of does fed the watermelon vines are presented in Table (5). The pesticide residues in milk of goats fed rations contain WMVH treated with fungi (*Trichoderma reesie*) (R4 and R5) showed zero values (not detectable) compared with those fed untreated ones, (R2 and R3) which showed significant values of different pesticide residues, while of course control group had nil concentration of pesticide residues in their milk. Biological treatment with fungi (*Trichoderma reesie*) is considered highly effective on reducing the level of pesticides. **Hassan *et al*. (2010)** showed that biological treatment with fungi or bacteria could be advisable to overcome the harmful effect of tomato haulms exposure to pesticides. **Deb Manadal *et al.* (2008)** reported that microbes

(fungi, bacteria and other microorganisms) could degrade or breakdown the pesticides whereas they used as effective detoxifying agents and consequently improve the quality of food. Moreover, **Quintero *et al*. (2008)** showed that white fungi species demonstrated a high capacity to degrade organic pollutants such as the insecticide lindance (y-HCH).

Table (5): Concentrations of pesticide residues (µg/kg on fat basis) of goat milk produced by the experimental rations.

Item	R1	R2	R3	R4	R5
Permethin	ND	0.09	0.15	ND	ND
Malathion	ND	0.11	0.14	ND	ND
Acetamiprid	ND	0.001	0.08	ND	ND
HCB*	ND	0.002	0.01	ND	ND
Lindine**	ND	0.001	0.02	ND	ND
p,p' DDE***	ND	0.001	0.005	ND	ND

** Hexachlorobenzene - ** gamma-hexachlorocyclohexan (γ-HCH)*

****Dichlorodiphenyldichloroethylene – ND (not detectable)*

Birth weight and daily gain

Data concerning birth weight and daily gain of kids from birth up to weaning are shown in Table (6). The values of both body weight and daily gain of kids at different phases of growth were significantly lower for 25% untreated WMVH- ration (R2), 50% untreated WMVH- ration (R3) and also, that contained 50% treated WMVF (R5), than those of control one (R1), while the 25% treated WMVF ration (R4) had similar values to those of control ration. Totally, among all tested rations, the best value was associated with dietary treatment (R4). Additionally, group R3 had the lowest value ($P<0.05$) of daily gain over the whole period (birth - 2nd month) yet, the biological treatment with fungi (*Trichoderma reesie*) could considered highly effective on reducing the level of pesticides, in vegetable by- products as well as the produced milk of lactating goats fed such agro by- products. **Hassan *et al*. (2010)** showed that biological treatment with fungi or bacteria could be advisable in order to overcome the harmful effect of tomato haulms exposure to the pesticides. Biological treatments using some fungi (**Khorshed, 2000)** were tested to improve the nutritive value and digestibility of poor quality roughages. **El-Ashry *et al.* (2003)** showed that enzymatic hydrolysis by fungi and biological conversions of cellulosic materials improve the nutritive value of residues especially crude protein and crude fiber.

Rumen Parameters

Results in Table (7) indicate that rumen liquor pH values did not significantly differ among treatments. The NH_3-N concentrations were significantly ($P < 0.05$) higher in R1 and R4 than the other experimental rations (R3 and R5), but insignificantly higher than R2. **Ørskov (1992)** and **Hassan *et al*. (2010)** reported that increased ruminal NH_3-N concentration could be a result of proteolytic activity in the rumen. **Yadov and Yaday (1988)** noticed that increased ruminal NH_3-N concentration may due to the higher intake of nitrogen and the better CP digestibility. Also, the highest values of TVFA's concentrations were observed with R1 and R4 followed by R2 and R5 while R3 recorded the lowest value among the experimental dietary treatments. Supporting to the obtained results**,** **Bassiouni (2001)** reported that watermelon vine hay had higher nutritive value, dry matter and protein degradability, as well as, it was better utilized than both wheat straw and rice straw by ruminants. Also, **Tripathi et al. (2008)** found that bio-processed mustard straw with C. versicolor (21 days) increased rumen TVFA after 6h of feeding to sheep

Table (6): Changes in body weight of goat kids fed the experimental rations.

	Body weight (kg)			Daily gain (g)		
Item	Birth weight	1st month weight	2nd month weight	Birth-1st month	1st month-2nd month	Birth-2nd month
R1	1.96 a	4.73a	8.75a	98.93 a	143.57 a	121.25 a
R2	1.74 b	3.78b	7.63b	72.86 b	137.50 b	105.18 b
R3	1.67 c	3.30 c	6.79 c	58.21c	124.64 c	91.43 c
R4	1.82 a	4.90 a	8.98a	110.00 a	145.71 a	127.86 a
R5	1.73 b	3.85 b	7.43 b	75.71 b	127.86 c	101.79 b
SME	0.45	0.52	0.33	0.18	0.71	0.37
P Value	0.033	0.023	0.012	0.025	0.015	0.020

a, b and c Means within column with different superscripts are significantly different (P<0.05)

Furthermore, cultured straw increased small holotricks but reduced large holotricks population in rumen liquor, while no effect on ruminal microbial enzyme activities was observed. These studies imply that most of the microbial converted feeds are safer and that the potential biohazards associated with them are very low for ruminants **(Villas-Bôas *et al*., 2002)**. Fermentation pattern, observed with fungal treated substrates upon microbial digestion, might favorably alter ruminal parameters because the bio- delignification of WMVH could enables faster accessibility by rumen microbes. Molar proportions of individual VFA and the acetate to propionate ratio were mostly affected significantly by some dietary treatments without clear trends among the experimental rations respecting the proportions of acetate, propionate and butyrate as well as the acetate to propionate ratio. Favorably, the values of such individual VFA of 25% treated WMVF ration (R4) were closely similar to those of control one (R1). Enteric methane (CH_4) production arises principally from microbial fermentation of hydrolyzed dietary carbohydrates such as cellulose, hemicellulose, pectin and starch. The amount of CH_4 produced during ruminal fermentation is dependent upon the nature of the substrate being fermented. Diet composition alters the digestion efficiency of animals thereby CH_4 production. In general, methanogens potential of ruminal micro-flora is greatest for the fermentation of structural carbohydrates compared to that of non-structural carbohydrates (**Boadi *et al*., 2004**). The goats fed R1, R4 and R5 had significantly (P<0.05) lower values of CO_2 and CH_4 compared with R2 and R3 groups. The gas production volume at 24h, organic matter digestibility, microbial protein, metabolizable energy, net energy and short chain fatty acids were significantly (P<0.05) higher in R4 than the other experimental rations. These results are in agreement with those detected by **Menke *et al*. (1979)** who suggested that gas volume at 24h after incubation is in indirect relationship with metabolisable energy of feedstuffs. Definitely, **Sommart *et al*. (2000)** suggested that gas volume is a good parameter to predict digestibility, fermentative end-product and microbial protein synthesis of the substrate by rumen microbes system. In addition report elsewhere (**Chumpawadee *et al*., 2007**) indicated that *in vitro* dry matter and organic matter digestibilities were shown to have high correlation with gas volume, as well as it has a close relationship with feed intake (**Blümmel *et al*., 1997**). Therefore, the higher gas volume that recorded with R4 group was likely to be caused by its reduced contents of cell wall, especially ADF and ADL. Obviously, the high lignin content in a ration of ruminant animals could be depressed or even impaired the digestibility due to its effect on lowering the rate of microbial colonization of such high fibre feed (**Van Soest,**

1994) and **(Okano *et al*., 2009)**. This implies good digestibility potential for the fungal treated rice straw when harnessed as feed resources for ruminant animals. Although gas production is metabolically consider wasteful product (**Mauricio *et al*., 1999**), it provides a useful basis from which ME, OMD and short chain fatty acids (SCFA) may be predicted. The higher OMD that observed in the 25% treated WMVF-ration (R4) could be meaning that the microbes in rumen and animal have high nutrient uptake. The higher fibre content in control group probably resulted in lower OMD since high NDF and ADL content in feedstuffs resulted in lower fibre degradation (**Van Soest, 1994**). Higher production of gas and the eventual preponderance of SCFA in the R4 probably showed an increased proportion of acetate and butyrate but may mean a decrease in proximate production. The estimated ME was found to be comparable to that reported for fungal treated millet stover (**Akinfemi *et al*., 2010)**. The *in vitro* gas production method has been widely used to evaluate the energy value of several classes of feed (**Getachew *et al*., 2002**). As cell wall components (high values of NDF and ADF) are known to have a negative correlation with gas production (**Sallam *et al*., 2007**) and thus readily available soluble carbohydrate fractions found in fungal treated substrates are expected to produce more gas (**Chumpawadee *et al*., 2007**) and short chain fatty acids (SCFA), with an increased ME contents (**Mahesh, 2012**). **Mahesh (2012)** observed a reduction in CH_4 (%) from fungal treated wheat straws which contained lesser fibre fractions (NDF and ADF) than untreated straw. This could probably due to indirect effect via fibre digestion leading to lesser residency of feed particles in the rumen (**Sallam *et al*., 2007**).

Table (7): Rumen liquor parameters of lactating goats fed the experimental rations.

Item	R1	R2	R3	R4	R5	SME	*P* Value
pH	6.32	6.29	6.17	6.23	6.2	0.11	0.847
NH_3- N (mg/100 ml)	13.64^{a}	13.47^{ab}	12.08^{c}	13.70^{a}	12.99^{b}	0.18	0.031
TVFA's (meq/100 ml)	10.99^{a}	9.09^{b}	8.33^{c}	10.61^{a}	9.12^{b}	0.36	0.026
Acetate (meq/100 ml)	55.31^{c}	57.35^{ab}	57.97^{a}	55.79^{c}	56.11^{bc}	0.47	0.033
Propionate (meq/100 ml)	26.34^{a}	24.37^{b}	22.85^{c}	26.86^{a}	24.00^{b}	0.31	0.016
Butyrate(meq/100 ml)	7.70^{b}	8.88^{a}	9.07^{a}	7.83^{b}	7.93^{b}	0.28	0.041
Acetate: propionate ratio	2.10^{c}	2.53^{a}	2.54^{a}	2.08^{c}	2.34^{b}	0.11	0.013
Gas production volume at 24 h	23.6^{b}	20.4^{c}	18.1^{d}	28.2^{a}	21.3^{c}	1.34	0.001
CO_2	45.78^{b}	48.08^{a}	48.31^{a}	45.86^{b}	45.95^{b}	0.49	0.027
CH_4	24.92^{d}	27.02^{a}	27.81^{a}	25.97^{c}	26.02^{b}	0.21	0.011
OMD, %	39.09^{b}	35.97^{c}	33.30^{d}	43.47^{a}	36.66^{c}	2.27	0.001
Microbial protein (g/h/d)	75.45^{b}	69.41^{c}	64.28^{d}	83.90^{a}	70.75^{c}	3.11	0.001
ME (MJ /Kg DM)	5.03^{b}	4.46^{c}	3.99^{d}	5.64^{a}	4.41^{c}	0.38	0.014
NE (Mcal/lb)	2.34^{a}	2.24^{b}	2.13^{c}	2.41^{a}	2.21^{b}	0.07	0.036
Short chain fatty acids	51.97^{b}	44.86^{c}	39.76^{d}	62.18^{a}	46.86^{c}	2.65	0.001

$^{a,,b,c\ and\ d}$ *Means within rows with different superscripts are significantly different (P<0.05).*

Blood parameters

Data of serum glucose, cholesterol and triglyceride are presented in Table (8). Results showed that ration (R3) which formulated of 50% untreated WMVH caused significant increases in glucose, cholesterol and triglyceride levels in comparison with the other experimental rations. The lowest levels respecting these metabolites were occurred with the control ration (R1). The differences among dietary treatments R4, R5 and R1 respecting the previous items were not significant. **Hassan *et al*. (2010)** reported that 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). **Ferrando and Andreu-Moliner (1991)** showed that exposure to pesticides 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 carbohydrates metabolism may be responsible of the toxic action of pesticides. Additionally, exposure of animals to pesticides may interfere with transport of glucose that crosses the gastrointestinal canal into blood stream and make its certain actions. (**Carlson and Kolmodin- Hedman, 1972**). **Hassan *et al*., 2010)** found that the increase in level of serum cholesterol may be responsible of 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. Earlier, **Wasserman and Wasserman (1970)** reported that high levels of liver cholesterol due to proliferation of smooth surface endoplasmic reticular induced by chlorinated pesticides. The data concerning serum total protein, albumin and globulin concentrations are presented in Table (8). The values of total protein and its fractions were comparable for R1, R4 and R5 and those being significantly higher than those of R2 and R3 rations. So, the biological treatments of WMVH had significant favor effects on these blood constituents. The reduction of serum protein, particularly albumin, in animals fed R2 and R3 treated by pesticides could be attributed to changes in protein and free amino acid metabolism and their synthesis in the liver (**Rivarola and blegeno, 1991**). Pesticides are capable of inhibiting RNA synthesis, breaking strand 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 purpose (**Murthy and Priyamvada Devi, 1982**). **Kabeer Ahamed *et al.* (1978)** reported that the free amino acids (FAA) are found to play a vital role in synthesis of enzymes and hormone and that increasing their levels in liver and kidney indicate stepped up proteolysis, fixation of ammonia and keto acids resulting in amino acid formation. Similar effect was also found by **Hassan *et al*. (2010)** who fed the dairy cows on ration involved tomato haulms treated with fungi. Regarding the effect of experimental rations on kidney functions, feeding of R3 ration caused a significant ($P<0.05$) increase in urea and creatinine levels followed by R2 and R5 then R1 and R4 rations. Supporting to these results, **Rodwell (1979)** and **Hassan *et al*. (2010)** reported that the increases in blood urea and creatinine concentrations should be due to the pesticide treatments. Elevated blood urea is known to be resulted of more efficient conversion of ammonia to urea as a result of increased synthesis of enzyme involved in urea production. Also, **Janardhan *et al. (*1988)** showed that the increase in blood urea was closely correlated to histopathological changes in the kidney which considered as degenerative bioprocess and these changes caused disturbance in the transport system of biochemical constituents in the kidney. **Bhatia *et al.* (1972)** reported that urea content increased in the tissues of mice treated with lindance could cause an excess of liver ammonia that 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 of AST and ALT of lactating goats fed the experimental rations are presented in Table (8). Results showed that the values of AST and ALT clearly behaved the same trends of urea

and creatinine concentrations among the dietary treatments. The R4 values were closely similar to those of control one, while the values of the other tested rations (R2, R3 and R5) were significantly higher than those of control ration (R1). These results are in agreement with those obtained by **Rajined *et al.* (1990)** and **Hassan *et al.* (2010)** who showed that an elevation of aminotransferase enzyme in blood could be used as an indicator of altered permeability of plasma membrane and /or cellular damage. In addition, **Shakoori *et al.* (1994)** found that the increased activities of serum AST and ALT could considerably be used as an indicator for liver damage and disruption of normal liver function. **Navarro *et al.* (1993)** indicated that the increments of the activities of AST and ALT in plasma and the reduction of such activities in liver are mainly due to the leakage of these enzymes from the hepatic cytosol into the blood stream, which gives an indication on the hepatotoxic effect of lindance which leads to the liver damage.

Table (8): Blood serum parameters for lactating goats fed the experimental rations.

Item	R1	R2	R3	R4	R5	SME	P value
Glucose (mg/dl)	50.89 ^{c}	53.86^{b}	57.46^{a}	51.15 ^{c}	52.07^{c}	0.53	0.034
Cholesterol (mg/dl)	40.77 ^{c}	44.79 ^{b}	50.18 ^{a}	41.53 ^{c}	41.99 ^{c}	0.45	0.026
Triglyceride(mg/dl)	46.90 ^{c}	51.12 ^{b}	54.67 ^{a}	47.00 ^{c}	47.20 ^{c}	0.38	0.035
Total Protein (g /dl)	7.81 ^{a}	6.12 ^{b}	6.03 ^{b}	7.78 ^{a}	7.67 ^{a}	0.21	0.041
Albumin (g /dl)	3.49 ^{a}	3.23 ^{b}	3.16 ^{b}	3.38 ^{a}	3.38 ^{a}	0.18	0.019
Globulin (g/dl)	4.32 ^{a}	2.89 ^{b}	2.87 ^{b}	4.40 ^{a}	4.29 ^{a}	0.25	0.027
Urea (mg/dl)	38.95 ^{c}	43.79 ^{b}	45.09 ^{a}	39.76 ^{c}	42.08^{b}	0.17	0.039
Creatinine (mg/dl)	0.82 ^{c}	0.92 ^{b}	1.06 ^{a}	0.83 ^{c}	0.92 ^{b}	0.10	0.045
AST (U/L)	30.57 ^{c}	32.30 ^{b}	35.25 ^{a}	30.77 ^{c}	31.93 ^{b}	0.32	0.013
ALT (U/L)	18.92 ^{c}	20.97 ^{b}	24.19 ^{a}	18.57 ^{c}	21.08 ^{b}	0.42	0.022

$^{a,,b, and c}$ *Means within rows with different superscripts are significantly different (P<0.05).*

Feed intake, feed conversion and economic evaluation:

Data of feed intake, feed conversion and economic evaluation of the experimental rations are presented in Table (9). The quantity of TDMI was markedly higher in both R1 and R4 than that R2, R3 and R5 groups, where R3 ration that had 50% untreated WMNH was the poorest one. Concerning feed conversion, ration R4 recorded the best efficacy (P<0.05) of feed conversion compared with those of control and the other experimental groups. Results revealed that daily feed cost was lower for untreated and treated WMVH with different ratios (25 and 50 % contained in rations) than that of control one. The substitution of berseem hay by WMVH treated with fungi at 50% level (R4) resulted in better economic return in comparison with that of the other experimental treatments. Likewise**, Saleh *et al.* (2003)** reported that better economic efficiency with rations contained WMV might be due to the decrease in feed cost of these rations compared to control ration that free from such ingredient. The best relative economic efficiency value was detected with (R4), being 106.66% when compared with the control group (100%), due to the great differences in the productive performance among them. The same trend was observed by **Bendary *et al.* (1996) and Saleh *et al.* (2000)**. **Hassan *et al.* (2010)** showed that biological treatment with fungi or bacteria could be advisable to overcome the harmful effect of tomato haulms exposure to pesticides.

CONCLUSION

It could be concluded that biological treatment with fungi could be advisable to overcome the harmful effect of WMV exposure

to pesticides. This study suggested the possibility of replacing berseem hay by treated WMVH with fungus in rations of lactating goats up to 50% as a cheap ingredient and it could be used safely and economically in formulating ruminant's rations. These results strongly exhibit the high potential of biologically treated WMVH as by-product feedstuff efficiently replaces high quality forage like berseem hay.

Table (9): Feed intake, feed conversion and economic evaluation for lactating goats fed the Experimental rations

Item	R1	R2	R3	R4	R5	SEM	*P Value*
CFM	500	500	500	500	500	-	-
BH	543.75	330	-	303.75	-	-	-
WMV	-	124.58	284.58	211.25	389.17	-	-
TDMI (g/h/d)	1043.75	954.54	784.58	1015.00	889.17	-	-
Milk yields g/d	914.75^{a}	739.42^{b}	539.74^{d}	934.62^{a}	619.62^{c}	41.62	0.001
4%- FCM (g)	808.85^{a}	657.12^{b}	478.70^{d}	818.50^{a}	555.65^{c}	45.99	0.001
Feed conversion (g/g):							
TDMI / Milk yields (g /g)	1.14 ^{c}	1.29 ^{b}	1.45 ^{a}	1.09 ^{c}	1.44 ^{a}	0.39	
TDMI/FCM (g/g)	1.29 ^{c}	1.45 ^{b}	1.64 ^{a}	1.24 ^{c}	1.60 ^{a}	0.99	
Economic evaluation:							
Daily feed cost, L.E	2.39	2.15	1.44	2.14	1.50	-	-
Price of daily milk yield, L.	9.15	7.39	5.40	9.35	6.20	-	-
Economic return, L.E	6.76	5.24	3.96	7.21	4.70	-	-
Economic return,(h/d) %	100	77.51	58.58	106.66	69.53	-	-

$^{a,b,c \text{ and } d}$ *Means within rows with different superscripts are significantly different (P<0.05)*

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MICROBIAL DEGRADATION OF PESTICIDE RESIDUES IN WATERMELON VINES AND ITS EFFECT ON PRODUCTIVE PERFORMANCE OF LACTATING GOATS

التحلل الميكروبى لبقايا المبيدات فى عروش البطيخ و تأثيره على الأداء الانتاجى للماعز الحلاب .

أمل محمد عبد المجيد فايد - أيمن عبد المحسن حسن - محمد سمير محمود خليل - عفاف حسن زيدان

معهد بحوث الانتاج الحيوانى - مركز البحوث الزراعية – الدقى – جيزة - مصر

تهدف هذه الدراســة الى بحث مدى تاثير ســمية بقايا المبيدات فى عروش البطيخ من خلال الإحلال الجزئى او الكلى لدريس البرســيم بعروش البطيخ على صــورة دريس او دريس معامل بفطر التريكوديرما فى علائق االماعز الحلاب على ادائها الانتاجى وكذلك مكونات الدم ووظائف الكرش. لذلك تم استخدام خمسة وعشرين عنزة قسمت الى خمسة مجاميع متساوية عمر ٢- ٤سنوات وبمتوســط وزن ٣٠,٥٨ كيلو جرام (٥ حيوانات فى كل مجموعة). حيث غذيت حيوانات المجموعة الاولى على عليقة تحتوى على ٥٠٪ دريس البرسيم+ ٥٠٪ علف المركز (كنترول)، والمجموعات من ٢ الى ٥ غذيت على علائق تحتوى نسبة ٢٥ او ٥٠ % على التوالي من عروش البطيخ المجففة شــمســيا و الغير معاملة بالفطر وعروش البطيخ المعاملة بفطر التريكودرما على التوالى, بدءت التجربة قبل الولادة بشهرين واستمرت حتى الفطام . و اوضحت النتائج ارتفاع نسبة البروتين الخام من ٦٣و٨٪ فى العروش الغير معاملة بالفطر الى نسبة ٥٧و١٤٪ فى العروش المعاملة بالفطر مقارنا بنسبة ٧٦و١٢٪ فى دريس البرسيم .و كانت نسبة الالياف الذائبة فى المحلول المتعادل و كذلك الالياف الذائبة فى المحلول الحامضى منخفضة فى العروش المعاملة بالفطرعن نسبة كلا منهما فى العروش الغير معاملة وانخفضت ايضا تركيز بقايا المبيدات فى العروش المعاملة بالفطر الى نسبة ضيئلة جدا مقارنا بالعروش الغير معاملة وايضا لم يكتشف اى اثر لبقايا المبيدات فى لبن الماعز فى المجموعات التى غذيت على العلائق التى تحتوى نســبة ٢٥ او ٥٠ % على التوالي من عروش البطيخ المعاملة بفطرالتريكودرما بالمقارنة بالمجموعات التجريبية الاخرى التى تتغذى على العلائق المحتوية على عروش البطيخ الغير معاملة. حققت المجموعة التجريبية الرابعة التى على ٢٥٪ دريس البرسيم+ ٢٥٪ عروش البطيخ المعامة بالفطر + ٥٠٪ علف المركز افضل النتائج بالنسبة لانتاج اللبن و مكوناته و كان لها أعلى محصــولا لكلا من البروتين و الدهن فى اللبن من المجموعات التجريبية الاخرى بدون فروق معنوية مع مجموعة الكنترول و ســجلت أيضــا هذه المجموعة (الرابعة) ارتفاع فى معدل الزيادة اليومية فى الوزن بالنســبة للجداء خلال الفترة من الميلاد حتى الفطام .

ولوحظ زيادة فى تركيز الامونيا فى المجموعتين الاولى و الرابعة اكثر من المجموعتين الثالثة و الخامســة و لكن بدون فروق معنوية مع المجموعة الثانية و زيادة ايضا فى تركيز الاحماض الدهنية الطيارة الكلية فى الكرش بالنسبة للمجموعتين الاولى و الرابعة , ثم المجموعتين الثانية و الخامسة و اقل تركيز قد سجل بواسطة المجموعة الثالثة. ولا توجد اى فروق معنوية بالنسبة لدرجة الحموضة فى الكرش.

بالنســبة لمكونات الدم ســجلت المجموعة الرابعة التى تتغذى على عليقة تحتوى على ٢٥٪ عروش البطيخ المعاملة بالفطر نقص معنوى فى تركيز كلا من الجلوكوز و الكوليسترول وترى الجلسريد واليوريا و الكرياتينين و بعض انزيمات الكبد AST, ALT. سجلت المجموعات التجريبية الرابعة و الخامسة اعلى قيم معنوية بالنسبة لتركيز كلا من البروتين الكلى والالبيومين من المجموعات التجرية الثانية و الثالثة بدون اى فروق معنوية مع مجموعة الكنترول.

يستخلص من هدا البحث انه يمكن الاستفادة من عروش البطيخ المعاملة بالفطر باستخدامها بنجاح فى علائق االماعز الحلاب و ذلك باستبدال ٥٠٪ من الدريس بهذا المخلف بعد المعاملة بالفطر للتخلص من الاثر الضار للمبيدات دون اى تاثيرات سلبية على الإنتاجية و الحالة الصحية للحيوان كما يحسن من الكفاءة الغذائية وانتاج البن. مما يساهم فى التغلب على مشكلة تغذية الحيوانات والاستفادة من المخلفات الزراعية مع الحفاظ على نظافة البيئة .