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THE EFFECT OF TOTAL AND PARTIAL SUBSTITUTION OF FERMENTED AND UNFERMENTED *ECHINOCHLOA CRUS-GALLI* SEEDS BY *PLEUROTUS OSTREATUS* INSTEAD OF YELLOW CORN CONTAMINATED WITH AFLATOXINS ON SOME PRODUCTIVE TRAITS OF BROILER MEAT

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Abstract

This research was conducted to determine the effect of total and partial substitution of fermented and unfermented *Echinochloa crus-galli* seeds by *Pleurotus ostreatus* instead of yellow corn contaminated with aflatoxins on some productive and physiological traits of broiler meat. Experiments were undertaken to feed broiler meat with fermented and unfermented *E. crus-galli* seeds for five weeks. Results showed that T3 (10% unfermented seeds) was significantly higher in the average of body weight (gm) of broiler meat in all breeding weeks and amounted 1893.37gm compare to 1745.80gm in the control treatment (10% corn grain). The average of body weight in T3 was significantly higher than T4 (5% contaminated corn + 5% fermented barnyard grass seeds) and T5 (10% fermented seeds by *P. ostreatus*) and mathematically than negative treatment T2 (10% contaminated corn with aflatoxin). Results also showed that the highest feed conversion ratio (FCR) was recorded in T5 in all breeding weeks including cumulative nutritional conversion. T3 was recorded the lowest FCR in weeks 1, 2 and 4 in comparison with T5, while there was no difference in FCR in all treatments in week 3. T3 was the highest in cumulative feed consumption rate followed by T4, T2, T5 and T1 respectively and weeks 2 and 3 did not show any significant differences in all treatments. *P. ostreatus* was inhibited *A. flavus* that producing aflatoxins greatly by Bell scale.

***Keywords*:** *Echinochloa crus-galli*, *Pleurotus ostreatus*, barnyard grass, broiler meat, aflatoxin.

Introduction

Bush contaminated with mycotoxins is among the most important challenges that facing poultry production in developing countries due to the lack of modern scientific methods in agriculture and storage of yield (Yiannikouris and Jouany, 2002; Manafi *et al*., 2012). Hence, poultry diets have received wide attention because they are the main variable that determines the success or failure of poultry projects as they constitute large proportions of the production cost 65-70% (Devegowda and Murthy, 2005).

Barnyard grass (*Echinochloa crus-galli*) is a harmful weed native to Europe and from there it spread globally with diverse living environments including Asia, Australia and America. This weed is considered one of the most malignant weeds associated with rice cultivation and depending on the intensity of this weed, rice production is greatly reduced (Dang *et al*., 2018). Prietto *et al*. (2017) mentioned that barnyard grass seeds contain a high content of fat, minerals and dietary fiber and it is considered a source of nutrients in human and animal food. *P. ostreatus* has the ability to grow and composite mycelium very quickly on wide range of media under different conditions and contains salts, vitamins and nutrients (Zinabu *et al*., 2015). *A. flavus* has a high ability to produce six types of aflatoxins including B1, B2, G1, G2, M1, and M2 (Mounir, 2019). These aflatoxins are secondary metabolites can cause cancer mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus* in agricultural food such as corn and animal feed (Jalili, 2016). Mwakinyali *et al*. (2019) reported that B1AFB1 aflatoxin is carcinogen of the first type and causes serious effects on food industry.

Yellow corn is considered third grain crop worldwide in term of productivity and in Iraq corn grains used widely in animal feed and bush poultry which make it the most polluted grain by mycotoxins (Salomy, 2007; Abbas and Shier, 2009) therefore, it consider the main source of fungal mycotoxins as it presents 50-60% of bush poultry contents and works as a perfect media for fungi to produce mycotoxins. The productivity of yellow corn in Iraq reached 63307 thousand ton in 2018 (CSO, 2019). The cost of imported corn has risen recently particularly in Europe as it used to produce biofuel (ethanol) (Abdulrahman *et al*., 2017; Alshukri *et al*., 2019). Due to the importance of mycotoxins and its impact on human food and health, this study was conducted to determine the effect of total and partial substitution of fermented and unfermented *E. crus-galli* seeds by *P. ostreatus* instead of yellow corn contaminated with aflatoxins on some productive and physiological traits of broiler meat.

Materials and Methods

Culture media used in this study

Potato Sucrose Agar (P. S.A.)

This medium was prepared following the procedure of (Koneman *et al*., 1978). The medium was poured into sterile plates and kept at 4°C until used for growing *P. ostreatus*, *A. flavus* and in inhibition experiment.

Pleurotus ostreatus fungus

Pure cultures of *P.ostreatus* were obtained from postgraduate laboratory, Department of Plant Protection, Faculty of Agriculture University of kufa, Iraq. The fungus was re-cultivated on Potato Dextrose Agar (P.D.A.) and local millet seeds.

Fermented *Echinochloa crus-galli* seeds by *Pleurotus ostreatus*

Fermentation solid state was used according to Semeniuk *et al*., (1970) procedure after that seeds were autoclaved then left to cool and inoculated with 5% of *P.ostreatus* (Balakrishnan and Nair, 1995).

The effect of the ability of *P. ostreatus* to inhibit *A. flavus* isolated from yellow corn grain.

The ability of *P. ostreatus* to inhibit *A. flavus* was tested by double implant method in petri plates on P.S.A. medium using 1-5 scale (Bell *et al*., 1982).

Field experiment

A field experiment was conducted in the poultry field in Faculty of Agriculture, University of Kufa from 27/5/2019 to 1/7/2019. 225 1 day old chicks of broiler meat (Ross-308) were used and randomly distributed into five treatments in cages with 45 chicks for each treatment and three replicates with 15 chicks for each replicate as follows:

T1: (Positive control) 10% healthy yellow corn.

T2: 10% local yellow corn contaminated with 35.9 ppb (negative control).

T3: 10% local barnyard grass (*E. crus-galli*) seeds.

T4: 5% local barnyard grass (*E. crus-galli*) seeds fermented by *P. ostreatus* + 5% local yellow corn contaminated with 35.9 ppb.

T5: 10% local barnyard grass (*E. crus-galli*) seeds fermented by *P. ostreatus* only.

Data were analyzed using Duncan test (Torrie and Steel, 1980). Comparisons between means were made using L.S.D. test at 5% level of significance (P>0.05) (AlRawi and Khalafala, 2000).

Feed composition

Feed was prepared in feed factor of Department of Animal production, Faculty of Agriculture, University of Kufa when chicks were feed with primary bush (starter) for 21days (Table 1) and with final bush (finisher) from day 22 to day 35 (Table 2) then chemical contents of bush were estimated using NRC (1994) procedure.

Table 1 : Primary bush (starter) contents in the first 21 days for all treatments.

Feed name	Treatments				
	T1	T2	T3	T4	T5
Crushed wheat%	54.89	54.89	54.49	55.19	54.19
Yellow corn uncontaminated with aflatoxins %	10	-	-	-	-
Yellow corn contaminated with aflatoxins %	-	10.0	-	5.0	-
Barnyard grass (*E. crus-galli*) seeds	-	-	10.0	-	-
Barnyard grass (*E. crus-galli*) seeds fermented by *P. ostreatus*	-	-	-	5.0	10.0
Soybean meal (47% protein)	31.0	31.0	30.0	30.0	30.0
Premix (Turkish)	2.5	2.5	2.5	2.5	2.5
Sunflower oil (Iraqi)	-	-	1.4	0.7	1.7
Mono calcium phosphate	0.5	0.5	0.5	0.5	0.5
Limestone	1.0	1.0	1.0	1.0	1.0
Synthetic lysine	0.1	0.1	0.1	0.1	0.1
Methionine	0.01	0.01	0.01	0.01	0.01
Total	100	100	100	100	100
Metabolic energy kc/kg	2907.0	2907.0	2910.0	2901.7	2902.3
Raw protein%	23.50	23.50	23.50	23.40	23.50

Table 2 : Final bush (finisher) contents for 22-35 days of all treatments.

Feed name	Treatments				
	T1	T2	T3	T4	T5
Crushed wheat%	60	60	59.7	58.2	58.4
Yellow corn uncontaminated with aflatoxins %	10	-	-	-	-
Yellow corn contaminated with aflatoxins %	-	10	-	5	-
Barnyard grass (*E. crus-galli*) seeds	-	-	10	-	-
Barnyard grass (*E. crus-galli*) seeds fermented by *P. ostreatus*	-	-	-	5	10
Soybean meal (47% protein)	23	23	22	22.5	22
Premix (Turkish)	2.5	2.5	2.5	2.5	2.5
Sunflower oil (Iraqi)	2.5	2.5	3.8	4.8	5.1
Mono calcium phosphate	0.69	0.69	0.69	0.69	0.69
Limestone	1.0	1.0	1.0	1.0	1.0
Synthetic lysine	0.1	0.1	0.1	0.1	0.1
Methionine	0.01	0.01	0.01	0.01	0.01
Iodine-fortified salt	0.2	0.2	0.2	0.2	0.2
Total	100	100	100	100	100
Metabolic energy kc/kg	3101.0	3101.0	3102.0	3105.3	3102.7
Raw protein%	20.1	20.1	20.0	20.02	20.0

Results and Discussion

Testing the antagonistic ability of *P. ostreatus* against *A. flavus* producing B1, B2 and G2 aflatoxins.

Results of double implant method of *P. ostreatus* and *A. flavus* on P.D.A. showed that highest antagonistic ability of *P. ostreatus* against *A. flavus* measured by 1-5 Bell scale (Fig 1). This finding is in agreement with Kermasha, (2017) who mentioned that the highest antagonistic ability of *P. ostreatus* against fungi that contaminated bush poultry including *A. flavus*, *A. niger*, *Penicillium* sp., *A. parasiticus*, *A. terreus*, *Alternaria* sp. and *Rhizopus* sp. and *Fusaruim* sp. came second. Chowdhwy *et al.* (2015) and Vamanu, (2012) also reported that *Pleurotus* species distinguished by its containment of many anti-microbial, antioxidants and effective biomaterials, in addition to high potential for growth and colonization on different media.

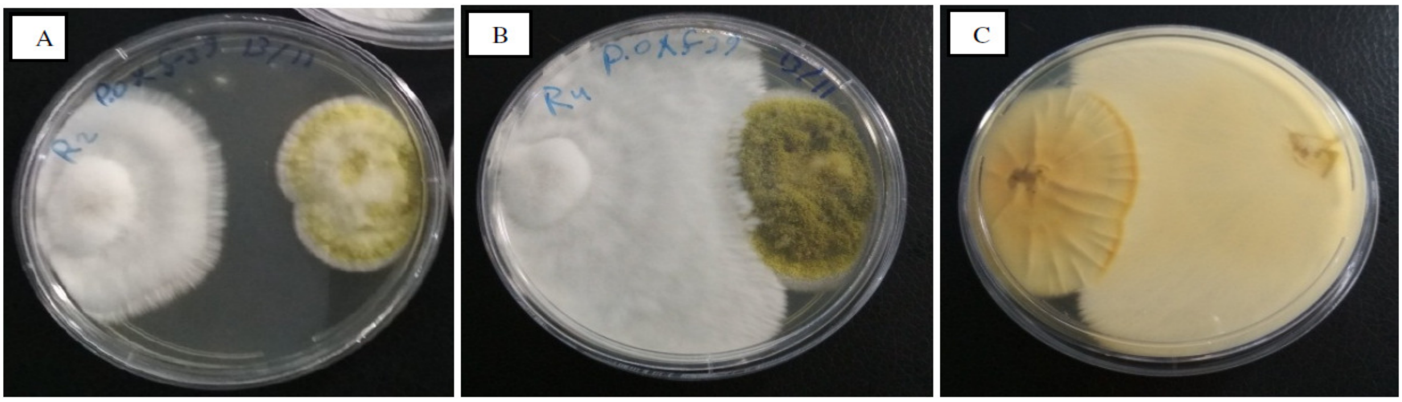

Fig. 1 : The antagonistic ability of *P. ostreatus* against *A. flavus* on P.D.A. A= antagonists after 3 days, B= antagonists after 5 days, C= antagonists after 6 days.

Field experiment results

The effect of total and partial substitution of fermented and unfermented *E. crus-galli* seeds by *P. ostreatus* instead of yellow corn contaminated with aflatoxins on weekly average of body weight (gm)

Table 3 results showed that T3 treatment (10% unfermented seeds) was significantly higher in the average of body weight of broiler meat in all breeding weeks and amounted 1893.37gm compare to 1745.80gm in the control treatment. The average of body weight in T3 was significantly higher than T4 and T5, and mathematically than the negative treatment T2 which amounted 1781.62gm. Also T3 was significantly higher compare to positive control in all breeding weeks except first week.

Table 3 also showed T4 (5% contaminated corn + 5% fermented barnyard grass seeds) and T5 (10% fermented seeds by *P. ostreatus*) were not significantly differ from positive and negative treatments especially T4 which amounted 135.68, 315.02, 717.93, 1088.42 and 1765.01gm from 1-5 weeks respectively compare to 135.11, 320.53, 716.93, 1119.37 and 1745gm in T2(negative control). T5 (10% fermented seeds by *P. ostreatus*) was not significantly differ from positive and negative treatments in week five. This result was reduced the proportion of yellow corn contaminated with aflatoxins in poultry feed. T5 was significantly differed in reducing the average of body weight compare to positive and negative control in week 2, 3 and 4 and differed from positive control in week 1. The reason for body weight reducing may occurred due to the lack of ether extract as a result of *P. ostreatus* consuming which lead to reduce metabolic energy (ME) in fermented seeds or consumption of the nutritional content of barnyard grass seeds by *P. ostreatus* during it growth, which results in seeds being discharged from material leaving seeds cover as a result of the food preference of the fungus. The increasing of body weight may attributed to the fact that barnyard grass seeds contain organic acids that have improved the small intestine environment and increased the beneficial bacteria that living in the intestine which prefer acid medium and that lead to improve absorption of material feed. This has already been confirmed by Prietto *et al.* (2017) who mentioned that barnyard grass seeds contain multiple fatty acids particularly Linoleic acid estimated 61.03% which consider one of essential fatty acids in poultry feeding. Another study also reported that Linoleic acid lead to increasing of body weight and improving feed conversion ratio (Ibraheem, 2013).

Table 3 : The effect of total and partial substitution of fermented and unfermented *E. crus-galli* seeds by *P. ostreatus* instead of yellow corn contaminated with aflatoxins (35.9ppb) on weekly average of body weight (gm) of broiler meat.

Treatments	Age in weeks				
	1	2	3	4	5
T1 (positive control) 10% healthy yellow corn	141.28 ab*	327.46 b	722.93 b	1118.00 b	1745.80 b
T2 (negative control) 10% contaminated yellow corn	135.11 cb	320.53 b	716.93 b	1119.37 b	1781.62 ab
T3 10% unfermented barnyard grass seeds	148.35 a	367.64 a	794.48 a	1206.15 a	1893.37 a
T4 5% fermented barnyard grass seeds + 5% contaminated yellow corn	135.68 bc	315.02 b	717.93 b	1088.42 b	1765.01 b
T5 10% fermented barnyard grass seeds	130.62 c	279.06 c	656.66 c	983.60 c	1666.26 b

*= different letters in one column indicate significant differences at P>0.05.

These results are consistent with the finding of Subeh, (2018) who mentioned that improving the body weight when Japanese quail were feed with fermented millet by *P. ostreatus* at (25, 50, 75 and 100%). Moreover, Kermasha, (2017) reported significant increasing in body weight when broiler meat were feed with 50% fermented barley by *P. ostreatus*, also Hassan, (2015) mentioned that there were significant differences in weight increasing rate when 10% of *P. ostreatus* extract was added to drinking water.

Table 4 showed that there were no significant differences in weekly weight increasing rate of broiler meat in week 5. As for the cumulative weight increase, T3 exceeded by obtaining the highest cumulative weight increase during the trial period and amounted 1848.87gm compare to T1, T4 and T5 that recorded 1701.31, 1720.51 and 1621.76gm respectively. While T3 was mathematically excel T2 which reached 1737.12gm in cumulative weight increase and the treatments T1, T2, T4, T5 were not differ from each other. Results of Table 4 also showed that T3 was significantly higher in cumulative weight increase of chicks than all treatments in week 2 and three of the experiment. T5 was recorded the lowest weekly weight increase particularly in week 2, 3 and 4 of breeding. The reason for this decreasing may be due to the decreasing of nutritional value of barnyard grass seeds as a result of the growth of *P. ostreatus* on this seeds for 21 days before the substitution with yellow corn in poultry feed. That led to defect in filling the need of chicks from proteins, amino acids and vitamins in addition to the high proportion of fibers. This finding is consistent with Malik *et al*. (2016) who reported that the increasing of fibres proportion decreased the advantage from nutrition in bush poultry.

The decreasing of proteins and amino acids occurred as a result of total substitution of 10% fermented barnyard grass seeds. While the increasing of cumulative weight in T3 was gave a high advantage of proteins (Alkassar, 2018). Moreover, barnyard grass seeds contain multiple fatty acids particularly Linoleic acid estimated 61.03% which consider one of essential fatty acids in poultry feeding in addition to another 14 fatty acids such as Oleic, Palmatic and Stearic (Prietto *et al*., 2017). Studies mentioned that there were a proportion of fatty acids in the food which improve the structure and the test of broiler meat (Alkassar, 2012; Ibraheem, 2013).

Table 4 : The effect of total and partial substitution of fermented and unfermented *E. crus-galli* seeds by *P. ostreatus* instead of yellow corn contaminated with aflatoxins (35.9ppb) on weekly average of cumulative weight increase (gm) of broiler meat.

Treatments	**Age in weeks**					**Cumulative weight increase (gm)**
	1	**2**	**3**	**4**	**5**	
T1 (positive control) 10% healthy yellow corn	96.78 ab*	186.18 b	395.47 bc	395.07 ab	627.80 n.s**	1701.31 b
T2 (negative control) 10% contaminated yellow corn	90.61 bc	185.42 b	396.40 bc	402.44 ab	662.25 n.s	1737.12 ab
T3 10% unfermented barnyard grass seeds	103.85 a	219.29 a	426.84 a	411.67 a	687.22 n.s	1848.87 a
T4 5% fermented barnyard grass seeds + 5% contaminated yellow corn	91.18 bc	179.34 b	402.91 b	370.49 b	676.59 n.s	1720.51 b
T5 10% fermented barnyard grass seeds	86.12 c	148.44 c	377.60 c	326.94 c	682.66 n.s	1621.76 b

*= different letters in one column indicate significant differences at P>0.05. **= there were no significant differences.

The effect of total and partial substitution of fermented and unfermented *E. crus-galli* seeds by *P. ostreatus* instead of yellow corn contaminated with aflatoxins on weekly average of feed consumption rate.

Table 5 showed that there were no significant differences in week 2 and 3 for all treatments. Cumulative feed consumption results showed that T3 was significantly higher than all other treatments and consumed the highest amount of feed 3059gm and differed from T1 and T5 which consumed 2735, 2852gm respectively. T3 was not differ in cumulative feed consumption from T2 and T4 that amounted 2892, 2939gm respectively. T4 and T5 were varied in week 5 compare to other treatments and with T1, T2 in cumulative feed consumption. This in turn consistent with Subeh, (2018) who mentioned that fermented millet by *P. ostreatus* was affected the weekly cumulative feed consumption in Japanese quail.

Table 5 also showed that in week 5 T3 was the highest in the average of feed consumption when amounted 1293.70gm and significantly differ from T1, T2, T5 which recorded 1092.87, 1173.65, 1148.93gm respectively. This has been explained as a result of taste improving or the nutritional need to feed on this bush poultry due to the adding of unfermented barnyard grass seeds. Prietto *et al*., (2017) findings supported this results when reported that barnyard grass seeds contain multiple fatty acids particularly Linoleic acid estimated 61.03% which consider one of essential fatty acids in poultry feeding in addition to another 14 fatty acids such as Oleic, Palmitic and Stearic. Studies mentioned that there were a proportion of fatty acids in the food which improve the structure and the test of broiler meat (Alkassar, 2012; Ibraheem, 2013). T3 was also mathematically excelled all other treatments and reached 705.24gm compare to 617.90gm in positive control. While in week 1, T3was the lowest in the average of feed consumption than all other treatments when consumed only 120gm of feed. This may be due to the weak enzymatic ability of chicks to break down and take advantage of fermented barnyard grass seeds. Bolu *et al*., (2010) mentioned significant decrease in the amount of feed consumed when using *A. flavus* with drinking water for broilers. While Ebenebe *et al*., (2011) noted a decrease in the amount of feed consumed when 2% of *Termitomyces microcarpus* was added.

Table 5 : The effect of total and partial substitution of fermented and unfermented *E. crus-galli* seeds by *P. ostreatus* instead of yellow corn contaminated with aflatoxins on weekly average of feed consumption rate (gm) of broiler meat.

Treatments	Age in weeks					Cumulative feed consumption (gm)
	1	2	3	4	5	
T1 (positive control) 10% healthy yellow corn	128.00 b*	262.22 n.s**	636.97 n.s	617.90 b	1092.87 c	2735 b
T2 (negative control) 10% contaminated yellow corn	130.35 a	270.48 n.s	659.46 n.s	660.70 ab	1173.65 bc	2892 ab
T3 10% unfermented barnyard grass seeds	120.97 c	295.72 n.s	646.53 n.s	705.24 a	1293.70 a	3959 a
T4 5% fermented barnyard grass seeds + 5% contaminated yellow corn	125.75 b	270.13 n.s	658.44 n.s	695.55 a	1191.7 abc	2939 ab
T5 10% fermented barnyard grass seeds	126.04 b	273.64 n.s	642.73 n.s	663.97 ab	1148.93 bc	2852 b

*= different letters in one column indicate significant differences at P>0.05. **= there were no significant differences.

The effect of total and partial substitution of fermented and unfermented *E. crus-galli* seeds by *P. ostreatus* instead of yellow corn contaminated with aflatoxins on weekly average of feed conversion ratio of broiler meat.

Results showed that there were no significant differences in the average of feed conversion ratio between all treatments in 1-5 weeks (Table 6). T5 was gave the highest feed conversion ratio for all experiment weeks including total FCR except week 5 when T3 was significantly differ 1.87g from T2 and T5 which recorded 1.77, 1.65gm respectively. In week 4 of broiler age, T4 and T5 were not significantly differ in feed conversion ratio and T5 was significantly different from T1, T2, T3 while T4 was significantly different from T1 and T2 only. In week 1, T5 was significantly higher 1.46gm and in week 2 was different from T1, T2, T3, and T4. These findings are in agreement with Subeh, (2018) who mentioned that there were no significant differences between treatments in week 2, 3 and 5 but week 4 showed significant differences in feed conversion ratio when Japanese quail were feed with fermented millet by *P. ostreatus*. Table 6 showed that T3 was mathematically gave the best feed conversion ratio in the first three weeks and recorded 1.16, 1.35, 1.51gm respectively. This result supported a previous study by Prietto *et al*., (2017) who mentioned that barnyard grass seeds contain multiple fatty acids particularly Linoleic acid estimated 61.03% which consider one of essential fatty acids in poultry feeding. Another study also reported that Linoleic acid lead to increasing of body weight and improving feed conversion ratio (Ibraheem, 2013). Alsudany, (2005) reported that improving the feed conversion ratio may occurred due to the increasing of beneficial bacteria which works as a probiotic and enhances bird health. Adding 0.3% of the probiotic *A. oryzae* to bush poultry was improved the feed conversion ratio significantly (Shim *et al*., 2010). This result is consistent with Ali –Mursyid *et al*., (2009) who used fermented cassava-waste by *Trichoderma*.

Table 6 : The effect of total and partial substitution of fermented and unfermented *E. crus-galli* seeds by *P. ostreatus* instead of yellow corn contaminated with aflatoxins on weekly average of feed conversion ratio of broiler meat.

Treatments	Age in weeks					The average of feed conversion ratio (1-5 weeks)
	1	2	3	4	5	
T1 (positive control) 10% healthy yellow corn	1.32 ab*	1.40 b	1.61 n.s*	1.56 b	1.74 ab	1.60 n.s
T2 (negative control) 10% contaminated yellow corn	1.44 a	1.45 b	1.66 n.s	1.64 b	1.77 b	1.66 n.s
T3 10% unfermented barnyard grass seeds	1.16 b	1.35 b	1.51 n.s	1.71 bc	1.87 a	1.65 n.s
T4 5% fermented barnyard grass seeds + 5% contaminated yellow corn	1.38 a	1.50 b	1.63 n.s	1.88 ac	1.76 ab	1.70 n.s
T5 10% fermented barnyard grass seeds	1.46 a	1.84 a	1.70 n.s	2.03 a	1.65 b	1.75 n.s

*= different letters in one column indicate significant differences at P>0.05. **= there were no significant differences.

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