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EVALUATION OF SODIUM BENTONITE EFFECTS ON AFLATOXIN B1 TOXICITY: IN VITRO STUDY

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ABSTRACT

The study aimed to investigate the impact of different concentrations of aflatoxin B1 in concentrated animal feeds on the digestion of dry matter and organic matter, as well as the production of greenhouse gases (total gas and methane) after 24 hours of digestion in a laboratory setting. To mitigate the negative effects of aflatoxin, various proportions of sodium bentonite clay (SB) were added. Four treatments were prepared and labeled as T1, T2, T3, and T4, with varying concentrations of aflatoxin (0, 20, 40, and 60 parts per billion, respectively). Different percentages of sodium bentonite clay (0%, 3%, 5%, and 7%) were added to each treatment. Statistical analysis revealed significant increases (P<0.05) in the values of dry matter digestion among the different treatments. T1 recorded the highest value (84.09), while T4 had the lowest (65.08) at a concentration of 0%. Similar trends were observed in organic matter digestion, with T1 showing the highest value (92.38), and T4 the lowest (78.54) at 0% concentration. The addition of 7% sodium bentonite clay resulted in the highest digestion rate (89.26), followed by 5% (88.45), 3% (87.21), and 0% (84.29). Regarding total gas production after 24 hours, significant increases were observed (P<0.05) among the different treatments. T1 had the highest value (45.66), while T4 had the lowest (15.66) at a concentration of 0%.In summary The study showed that adding sodium bentonite clay to concentrated animal feed resulted in a partially reduction of the negative effects of aflatoxin B1, leads to improved bacterial digestion through laboratory digestion.

Keywords: Aflatoxin B1, Sodium Bentonite Clay, Greenhouse Gases, Concentrated Feeds, Rumen Fluid.

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تقييم تأثيرات بنتونيت الصوديوم على سمية الأفلاتوكسين B1: دراسة مخبرية

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الخلاصة

هدفت الدراسة إلى معرفة تأثير تراكيز مختلفة من الأفلاتوكسين B1 في الأعلاف الحيوانية المركزة على هضم المادة الجافة والمادة العضوية وكذلك إنتاج الغازات الدفيئة (الغاز الكلي والميثان) بعد 24 ساعة من الهضم في بيئة مختبرية للتخفيف من الآثار السلبية للأفلاتوكسين، تم إضافة نسب مختلفة من طين بنتونيت الصوديوم. تم إعداد أربع معاملات ووصفت بأنها T1 وT2 وT3 وT4، بتراكيز متفاوتة من الأفلاتوكسين (0، 20، 40، و60 جزءًا في البليون، على التوالي). تم إضافة نسب مختلفة من طين بنتونيت الصوديوم (0%، 3%، 5%، 7%) لكل معاملة. أظهر التحليل الإحصائي وجود زيادات معنوية (P<0.05) في قيم هضم المادة الجافة بين المعاملات المختلفة. سجلت T1 أعلى قيمة (84.09)، في حين سجلت T4 أقل قيمة (65.08) بتركيز 0%. ولوحظت اتجاهات مماثلة في هضم المواد العضوية، حيث أظهرت T1 أعلى قيمة (92.38)، وأدنى قيمة 78.54) T4) عند تركيز 0٪. أدت إضافة طين بنتونيت الصوديوم 7% إلى أعلى معدل هضم (89.26)، تليها 5% (88.45)، 3% (87.21)، و0% (84.29). أما فيما يتعلق بإجمالي إنتاج الغاز بعد 24 ساعة، فقد حدثت زيادات كبيرة. لوحظ (P<0.05) بين المعاملات المختلفة. وكان T1 أعلى قيمة (45.66)، في حين كان T4 أقل قيمة (15.66) بتركيز 0٪. وخلاصة القول، أظهرت الدراسة أن إضافة طين بنتونيت الصوديوم إلى الأعلاف الحيوانية المركزة ادى الى تخفيف الآثار السلبية للأفلاتوكسين B1 بصورة جزئية، ويؤدي إلى تحسين الهضم البكتيري من خلال الهضم المختبري.

الكلمات المفتاحية: أفلاتوكسين B1، طين بنتونيت الصوديوم، غازات دفيئة، أعلاف مركزة، سائل الكرش.

INTRODUCTION

Animal feed is considered a crucial component of farm animal nutrition systems. Therefore, preserving its quality and safety has become essential in today's times, especially given the increased importance of this issue due to the activity of microorganisms such as fungi during storage**(Al-Samaraae, Hassan, & Hashim, 2008; Al-Samaraee, 2019)**. These microorganisms can cause significant damage to both the nutritional value and safety of animal feed**(Hussein & Slomy, 2012;Jabr&Al-Salahi,2005)**. The danger lies not only in the microorganisms themselves but also in the toxins they produce during their growth and development on these materials **(Al-Samaraee, *et al*., 2019; Hussain & Hussein, 2020)**. Research has shown that the consumption of animal feed contaminated with these toxins leads to reduced growth rates, milk productivity, fertility **(Al-Ani *et al*., 2007)**. One of the challenges facing livestock farmers is the limited availability and high cost of animal feed, particularly during drought periods **(Hassan& Al-samaraee, 2020)**. Studies have indicated that the fungus A. Flavus is the most common in feed grains, especially in peanuts **(Hussein & Al-wahab, 2020)**. Researchers have referred to non-nutritive feed additives, where bentonite clay stands out due to its ability to absorb and enhance rumen fermentations **(Azadbakht *et al*., 2017)**. Bentonite plays a role in reducing the effect of toxins in feed through ion exchange **(Kirovski *et al*., 2015)**. Many researchers have indicated that the use of bentonite in feeding farm animals can enhance their productive performance **(Al-Neuamy *et al*., 2020).**

MATERLALS AND METHODS

Study Site and Sample Preparation

This experiment was conducted in two distinct phases. The initial stage occurred at the Fungal Toxins Laboratory within the Department of Plant Protection, College of Agricultural Engineering Sciences University of Baghdad. During this phase, an isolate of Aspergillus flavus, known for its aflatoxin B1 production, was obtained and registered in the global gene bank. This fungal isolate was utilized to contaminate concentrated feed, which was subsequently cultivated under standard humidity and temperature conditions to mimic natural environmental settings. The concentration of toxins present in the contaminated feed was quantified using High-Performance Liquid Chromatography (HPLC) at the Environmental and Water Laboratories under the Ministry of Science and Technology.The second stage of the experiment was conducted at the Nutrition Laboratory situated within the Department of Animal Production, College of Agricultural Engineering Sciences, University of Baghdad in Al-Jadriya. The primary objective of this stage was to investigate the impact of incorporating varying levels (0% 3%, 5%, 7%) of sodium bentonite (SB) into the concentrated feed contaminated with aflatoxin. The feed had been previously contaminated with Aspergillus flavus fungus, which produces aflatoxin B1, at concentrations ranging from (0, 20, 40, 60) ppb.

Preparation of Raw Materials and Experimental Feeds

The initial materials were prepared and blended based on the specified ratios, which included barley (40%), wheat bran (10%), soybean meal (15%), yellow corn (33%), and vitamins and minerals (2%). These composite materials underwent analysis using the method referenced as (**AOAC *et al.*, 2012),** which was carried out in the laboratories associated with the Quality Control Department within the Animal Wealth Division of the Ministry of Agriculture.Sodium bentonite clay was procured from local markets situated in the Najaf province. Following the preparation of the concentrated feed and its division into two equal portions, one of these portions was intentionally contaminated with aflatoxin B1. Meanwhile, the other portion was subjected to dilution to achieve various concentrations of the toxin.

Measurement of Toxin Concentration in Aflatoxin B1 Contaminated Feed

The measurement of toxin concentration was carried out at the laboratories of the Ministry of Science and Technology - Environment and Water Department, following the methodology detailed in reference **(Liu *et al.*, 2012).**To accomplish this, high-performance liquid chromatography (HPLC) equipment of the SYKAMN model was employed. The HPLC system utilized in this analysis originated from Germany. The mobile phase used in the chromatography process consisted of a blend of acetonitrile and distilled water, with a ratio of 70:30. A C18 - ODS separation column measuring 25cm in length and 4.6mm in diameter was employed for phenolics separation. Fluorescence detection was utilized, with excitation set at 365 nm and emission at 445 nm. The flow rate of the mobile phase was maintained at 0.7 ml/min.

Rumen Fermentations

Characteristics Studied for Rumen Fluid

Estimation of In Vitro Dry Matter Digestibility (IVDMD)

The in vitro digestion process was carried out in two sequential stages to account for both microbial and enzymatic digestion, given the presence of concentrated feed in the diet. In the

first stage, 0.5 grams of experimental feed were weighed and mixed with 20 ml of artificial saliva and 10 ml of filtered rumen fluid. The mixture was placed in laboratory digestion tubes, and carbon dioxide gas was introduced twice daily. Subsequently, the samples were incubated in a water bath at a consistent temperature of 39.5°C for 48 hours, with the tubes being agitated twice daily. In the second stage, pepsin (2 ml) was added to the digestion tubes, followed by an additional 48-hour incubation for enzymatic digestion. This process adheres to the methodology detailed by **(Tilley&Terry, 1963)**. Afterward, the samples underwent filtration, and the undigested feed residues were collected in porcelain crucibles. These residues were then dried in an electric oven at 105°C for a full day.

The formula for calculating IVDMD (%) is as follows.

IVDMD (%) = (Dry Matter in Feed - Undigested Dry Matter - B) / Dry Matter in Feed × 100

Estimation of In Vitro Organic Matter Digestibility (IVOMD)

In this stage, in vitro organic matter digestibility was determined by incinerating the previously dried samples at 600°C for 3 hours to measure ash content. The organic matter in the feed was estimated by subtracting the ash content from the initial dry matter The formula for calculating IVOMD (%) is as follows.

IVOMD (%) = (Organic Matter in Feed - Undigested Organic Matter - B) / Organic Matter in Feed × 100

These procedures are employed to estimate the digestion of both dry matter and organic matter in the feed samples.

The estimation of total gas production in the laboratory

Three replicates were performed for each sample according to the method of Menke and Steingass (1988). Two hundred milligrams of the experimental feed material were measured and supplemented with 20 ml of artificial saliva and 10 ml of filtered rumen fluid obtained from a recently slaughtered sheep's rumen. These samples were placed in 100 ml glass syringes, and carbon dioxide gas was introduced into each syringe only once before sealing with a plunger to eliminate all air. To prevent liquid from escaping the syringe, the needle was sealed with a plastic cap. The syringes were then incubated in a water bath at a temperature of 39°C for 24 hours, with blanks included for each incubation stage. The syringes were agitated twice daily to simulate rumen activity. Subsequently, the syringes were withdrawn to calculate the total gas production. Additionally, 4 ml of 4% sodium hydroxide were added to the samples to estimate methane gas production using the method outlined by **(Fievez *et al*., 2005).**

Statistical Analysis

The text appears to describe a statistical analysis using the Statistical Analysis System **(SAS, 2018).** The analysis aimed to study the effect of different factors on the studied characteristics in a 4x4 factorial experiment conducted with a Completely Randomized Design (CRD). The analysis involved using the following mathematical model, and significant differences between means were compared using the Duncan multiple range test **(Duncan, 1955).** $Yijk = \mu + Ti + Cj + TC(ij) + eijk$

RESULT AND DISCUSSION

Rumen Fermentations

Degradable Organic Matter (DOM)

The discusses the outcomes of a research study that investigated the impact of different concentrations of sodium bentonite in aflatoxin B1-contaminated concentrated feed on the organic matter digestion coefficient in animals' rumen. The main findings of the study are:

Table (1) showed the interaction between different levels of sodium bentonite and aflatoxin B1 contamination significantly affected the organic matter digestion coefficient in the rumen. Treatment T2 displayed the highest value (92.38), while treatment T4 recorded the lowest value (78.54) with no sodium bentonite.

Across all treatments, significant differences were observed in the organic matter digestion coefficient. Treatment T1 had the highest value (91.44), followed by T2 (90.29), T3 (85.25), and T4 (82.23), which had the lowest digestion coefficient.The addition of sodium bentonite had varying effects on the organic matter digestion coefficient. The inclusion of 7% sodium bentonite led to the highest digestion coefficient (89.26), followed by 5% (88.45), 3% (87.21), and 0% (84.29). The positive effect of 7% sodium bentonite could be attributed to its role in mitigating the negative impacts of aflatoxin B1 and enhancing the microbial population in the rumen, thus improving organic matter digestion.

The study also highlighted the temporary storage properties of minerals like bentonite and their role in cation exchange. Previous research supports the positive impact of sodium bentonite on organic matter and dry matter digestion.In conclusion, the research demonstrates that sodium bentonite, particularly at a 7% concentration, can effectively enhance the organic matter digestion coefficient in the presence of aflatoxin B1 in concentrated feed **(Khalifeh *et al*., 2012)**. This improvement is likely due to its influence on the ruminal microbial population. **(Mohsen & Tawfik, 2002).**

Table (1): The effect of the interaction between the addition of different levels of sodium bentonite to the concentrated diet contaminated with different levels of aflatoxin B1 on the value of the organic matter digestibility.

average aflatoxin	**Levels of sodium bentonite**				**Treatments**
	(7 %)	(5 %)	(3 %)	(0 %)	
91.44a ±0.12	91.71A ±0.01	91.58A ±0.15	91.37A ±0.22	91.11A ±0.39	**T1**
90.29b ±0.80	92.38A ±0.17	91.75A ±0.13	91.09A ±0.28	85.95C ±1.02	**T2**
85.25c ±0.85	88.93B ±0.69	86.24C ±0.13	84.27D ±1.01	81.56E ±0.30	**T3**
82.23d ±0.73	84.03D ±0.73	84.24D ±0.27	82.10E ±0.67	78.54F ±0.68	**T4**
	89.26a ±1.01	88.45b ±0.99	87.21c ±1.26	84.29d ±1.45	**average sodium bentonite**

The means bearing different letters differ significantly ($P < 0.05$) between them T1 (control treatment without contamination), T2 (feed contaminated with aflatoxin B1 at a concentration of 20 ppb), T3 (feed contaminated with aflatoxin B1 at a concentration of 40 ppb), T4 (feed contaminated with aflatoxin B1 at a concentration of 60 ppb).

Degradable Dry Matter (DDM)

Table (2) showed the effect of the interaction between the addition of different levels of sodium bentonite to the concentrated diet contaminated with different levels of aflatoxin B1 on the dry matter digestibility value ± the standard error of the rumen fluid, as it is noted that there is a significant increase (P<0.05) in the dry matter digestibility coefficient value. Among the different treatments, the first treatment, T1, recorded the highest value (84.09) among the treatments compared to the fourth treatment, which recorded the lowest value in the dry matter digestibility coefficient (65.08) at 0% concentration. It is also noted from Table (2) that there are significant differences in the rate of the dry matter digestion coefficient among all the experimental treatments, where the first treatment T1 (81.87) was superior, followed by the effect of the treatment T2 (79.27), followed by the effect of the treatment T3 (72.23) and then the treatment T4 (68.19), which The lowest rate was recorded among the treatments in the value of the coefficient of dry matter digestion. As for the average levels of sodium bentonite addition to the diet on the value of the dry matter

digestion coefficient, the rate of 7% was higher (79.04), followed by the rate of 5%, which recorded (75.92), followed by the rate of 3%, which recorded (74.48), which did not differ significantly with the rate of 0%, which recorded The lowest average value of the coefficient of dry matter digestion (72.12). The reason for the high digestibility coefficient of the dry matter may be due to the provision of a suitable environment for microorganisms to digest the diet in the laboratory by reducing the harmful effects of aflatoxin B1 when adding bentonite by 7%, as indicated by **(Singh & Kumar Saini, 2022)**, to the increase in the digestibility coefficient of dry matter when adding bentonite Sodium was added to the diet contaminated with aflatoxin, as it led to the mitigation of the harmful effects resulting from the poison.

Table (2): The effect of the interaction between the addition of different levels of sodium bentonite to the concentrated diet contaminated with different levels of aflatoxin B1 on the value of the dry matter digestibility.

Treatments	Levels of sodium bentonite				average aflatoxin
	(0 %)	(3 %)	(5 %)	(7 %)	
T1	80.87B ±0.21	81.40B ±0.23	81.14B ±0.30	84.09A ±0.62	81.87a ±0.42
T2	74.54E ±0.62	78.62CD ±0.35	80.40BC ±0.36	83.52A ±0.28	79.27b ±0.99
T3	68.00IJ ±1.07	70.72GH ±0.40	72.96EF ±0.54	77.23D ±0.58	72.23c ±1.06
T4	65.08K ±0.87	67.17J ±1.45	69.19HI ±0.67	71.33FG ±0.23	68.19d ±0.80
average sodium bentonite	72.12d ±1.86	74.48c ±1.76	75.92b ±1.53	79.04a ±1.58	

The means bearing different letters differ significantly (P < 0.05) between them T1 (control treatment without contamination), T2 (feed contaminated with aflatoxin B1 at a concentration of 20 ppb), T3 (feed contaminated with aflatoxin B1 at a concentration of 40 ppb), T4 (feed contaminated with aflatoxin B1 at a concentration of 60 ppb).

Gas Production and Microbial Biomass Production

Total gas after 24 hours

Table (3) showed the effect of the interaction between adding different levels of sodium bentonite to the concentrated diet contaminated with different levels of aflatoxin B1 on the total gas after 24 hours ± the standard error of the rumen liquid, as it is noted that there is a significant increase (P<0.05) in the value of the total gas after 24 hours between the different treatments, as the first treatment T1 recorded the highest value (45.66) among the treatments compared to the fourth treatment, which recorded the lowest value in total gas after 24 hours (15.66) at 0% concentration. It is also noted from Table (3) that there are significant differences in the total gas rate after 24 hours between all the treatments of the experiment, where the first treatment T1 excelled (41.91), followed by the effect of the treatment T2 (22.25), followed by the effect of the treatment T3 (18.08) and then the treatment T4 (16.50). Which recorded the lowest rate among transactions in the value of total gas after 24 hours. As for the average levels of sodium bentonite addition to the diet on the total gas value after 24 hours, the rate of 7% (25.33) was higher, followed by the rate of 5%, which recorded (24.58), which did not differ significantly with the rate of 3% (24.50), which did not differ significantly either. With the rate of 0%, which recorded the lowest rate in the total gas value after 24 hours (24.33). The reason for the difference in the total gas value between the treatments in terms of height, as we mentioned earlier, may be attributed to the fact that the addition of sodium bentonite at a rate of 7% has led to an improvement in the biological function of the bacterial content of the rumen fluid, which led to an increase in the total gas value after 24 hours, as previous studies indicated that the metabolic functions Fermented by microbial organisms of rumen liquid leads to the mineralization of large polysaccharide chains in the feed into small dispersible components, where volatile fatty acids, carbon dioxide and hydrogen accumulate in the rumen through feed fermentation **(Morgavi *et al*., 2010)**. Previous studies also indicated the effectiveness of mineral binders. Clays with aflatoxin binding capacity **(Boudergue *et al*., 2009), (Kozieł *et al*., 2021).**

Table (3): The effect of the interaction between the addition of different levels of sodium bentonite to the concentrated diet contaminated with different levels of aflatoxin B1 on the total gas after 24 hours.

Treatments	Levels of sodium bentonite				average aflatoxin
	(0 %)	(3 %)	(5 %)	(7 %)	
T1	45.66A ±0.33	43.33B ±0.66	41.33C ±0.33	37.33D ±0.66	41.91a ±0.94
T2	18.66HI ±0.66	20.66G ±0.33	22.33F ±0.33	27.33E ±0.66	22.25b ±0.99
T3	17.33IJK ±0.66	17.66IJK ±0.33	18.00HIJ ±0.0	19.33GH ±0.33	18.08c ±0.28
T4	15.66L ±0.33	16.33KL ±0.33	16.66JKL ±0.66	17.33IJK ±0.66	16.50d ±0.28
average sodium bentonite	24.33b ±3.73	24.50b ±3.31	24.58b ±2.98	25.33a ±2.38	

The means bearing different letters differ significantly ($P < 0.05$) between them T1 (control treatment without contamination), T2 (feed contaminated with aflatoxin B1 at a concentration of 20 ppb), T3 (feed contaminated with aflatoxin B1 at a concentration of 40 ppb), T4 (feed contaminated with aflatoxin B1 at a concentration of 60 ppb).

Methane after 24 hours

Table (4) shows the effect of the interaction between adding different levels of sodium bentonite to the concentrated diet contaminated with different levels of aflatoxin B1 on methane gas after 24 hours ± the standard error of the rumen fluid, as it is noted that there is a significant increase ($P<0.05$) in the value of methane gas after 24 hours between the different treatments, as the first treatment T1 recorded the highest value (17.66) among the treatments compared to the fourth treatment, which recorded the lowest value in methane gas after 24 hours (3.00) at a concentration of 0%. It is also noted from Table (4) that there are significant differences in the rate of methane gas after 24 hours between all the treatments of the experiment, where the first treatment T1 (15.75) was superior, followed by the effect of the treatment T2 (7.83), followed by the effect of the treatment T3 (4.50) and then the treatment T4 (3.66). Which recorded the lowest rate among the treatments in the value of methane gas after 24 hours. As for the rates of levels of sodium bentonite addition to the ration on the value of methane gas after 24 hours, the rate of 7% was higher (9.16), followed by the rate of 5%, which recorded (8.25), followed by the rate of 3% (7.41), which did not differ significantly with the rate of 0%, which recorded (7.41). The lowest average value of methane gas was recorded after 24 hours (6.91). It may be that the rise in methane gas after 24 hours has been attributed to the reduction of the harmful effects of mycotoxins present in contaminated diets on the bacterial biomass of rumen fluid and the improvement of bacterial digestion when sodium bentonite was added by 7% as it binds aflatoxins, as previous studies have mentioned

due to the ability of Clays that are high on cation exchange, can bind or adsorb aflatoxins in the interlayer voids, outer surfaces, and edges through a different mechanism of action, including chemisorption and ion exchange, i.e., between clay cations and carbonyl groups of aflatoxins. **(Elliott, *et al*., 2020), (Kozieł *et al*., 2021)**.

Table (4): The effect of the interaction between the addition of different levels of sodium bentonite to the concentrated diet contaminated with different levels of aflatoxin B1 on methane gas after 24 hours .

Treatments	Levels of sodium bentonite				average aflatoxin
	(0 %)	(3 %)	(5 %)	(7 %)	
T1	17.66A ±0.33	16.33B ±0.33	15.33B ±0.33	13.66C ±0.33	15.75a ±0.46
T2	5.33GH ±0.66	6.33FG ±0.33	8.00E ±0.0	11.66D ±0.33	7.83b ±0.74
T3	1.66K ±0.33	3.66IJ ±0.33	5.66G ±0.33	7.00EF ±0.57	4.50c ±0.63
T4	3.00J ±0.57	3.33IJ ±0.33	4.00IJ ±0.0	4.33HI ±0.33	3.66d ±0.22
average sodium bentonite	6.91c ±1.92	7.41c ±1.59	8.25b ±1.30	9.16a ±1.12	

The means bearing different letters differ significantly ($P < 0.05$) between them T1 (control treatment without contamination), T2 (feed contaminated with aflatoxin B1 at a concentration of 20 ppb), T3 (feed contaminated with aflatoxin B1 at a concentration of 40 ppb), T4 (feed contaminated with aflatoxin B1 at a concentration of 60 ppb).

CONCLUTIONS

It was concluded that aflatoxin contamination of feed (Concentrated diet) at 60 ppb level significantly affected the in vitro rumen fermentation in terms of reduced truly degradable dry matter, truly degradable organic matter, gas production, Inclusion of sodium bentonite by (7%) to the aflatoxin contaminated feed partially ameliorated the adverse effects of aflatoxin on in vitro rumen fermentation parameters.

REFERENCES

1. Al- Ani, R. A., Hamad, R. A., & Al-Hadithi, A. N. (2007). Evaluation of bentonite and activated charcoal in adsorbing T2 toxin from feeds. *Iraqi Journal of Agricultural Sciences*, 38(1), 23-28.
2. Al-Neuamy, A. M., Abed, M. A., & Hassan, A. A. A. (2020). Effect of Addition of Different Concentrations of Bentonite to the Ration on Concentration of Blood Minerals and Ruminal Fluid Traits of Awassi Lambs. *Al-Anbar Journal of Veterinary Sciences*, 13(1),20-25.

3. Al-Samaraee, W. H. (2019). Effect of Adding Different Level of Nigella Sativa And Rosemary. *Biochemical and Cellular Archives.*19(1),1123-1126.
4. Al-Samaraee, W. H., Ahmed, A. A., Hussein, H. Z., Alwaeli, S. N., & AL-SAMARAEE, W.(2019). Effect of Trichoderma Harzianum, on Chemical Composition and in Vitro Digestibility of Crop Residues. *Plant Archives*, 19(2), 3623-3628.
5. AOAC. (2012). *Association of Official Analytical Chemists, Official Methods of Analysis* (14th ed.). Washington, D.C., U.S.A.
6. Azadbakht, S., Norouzian, M. A., & Khadem, A. A. (2017). Assessing the Protective Effect of Bentonite Against Lead Toxicity in Growing Lambs. *Environmental Science and Pollution Research*, 24(35), 27484-27489.
7. Boudergue, C., Burel, C., Dragacci, S., Favrot, M. C., Freme, J. M., Massimi, C., ... & Avantaggiato, G. (2009). Review of Mycotoxin-Detoxifying Agents Used as Feed Additives: Mode of Action, Efficacy and Feed/Food Safety. *EFSA Supporting Publications*, 6(9), 22-76
8. Duncan, D. B. (1955). Multiple Range and Multiple F Tests. *Biometrics*, 11(3), 1-42.
9. Elliott, C. T., Connolly, L., & Kolawole, O. (2019). Potential Adverse Effects on Animal Health and Performance Caused by The Addition of Mineral Adsorbents to Feeds to Reduce Mycotoxin Exposure. *Mycotoxin Res*., 36(2), 115–126.
10. Fievez, V., Babayemi, O. J., & Demeyer, D. (2005). Estimation of Direct and Indirect Gas Production in Syringes: a Tool to Estimate Short Chain Fatty Acid. *Animal Feed Science and Technology*, 123(1), 197-210.
11. Hashim, A. J., Hassan, S. A., & AL-Samaraae, W. H. (2008). Using of Microbial treatment To Improve The Nutritive Value Of Ground *and* Chopped Frond. *Iraqi Journal of Agricultural Sciences*, 39(2), 94-111.
12. Hassan, A. A., & Al-samaraee, W. H. (2020). Improvement Nutritive Value of Oyster Mushrooms (Pleurotus Pulmonarius) Residues Silage by Urea or Poultry Litter Supplementation. *Biochemical & Cellular Archives*, 20(2),4957-4961.
13. Hussain, A. H., & Hussein, H. Z. (2020). Evaluation of Agaricus sp. and Pleurotus sp. Extracts Efficiency in Aspergillus Flavus Growth Inhibition and Aflatoxin B1 Reduction. *Systematic Reviews in Pharmacy*, 11(10),564-569.
14. Hussein, H. Z., & Al-wahab, A. A. (2020). Assessing the Efficacy of Certain Nano, Natural and Chemical Materials in Fungal Inhibition and Af b1 Toxin Reduction of Aspergillus Flavus Isolated From Peanut on PDA media. *Plant Archives*, *20*(1), 1051-l057.
15. Hussein, H. Z., & Slomy, A. K. (2012). The Detection of Zeralenone Toxin in Maize and Its DetoxifIcation. *Iraqi Journal of Agricultural Sciences*, 43(2), 18-26.
16. Jaber, K. S., & Al-Salahi, M. A. (2005). Estimation of Fungal Biomass and Aflatoxin B1 in Imported Wheat Seeds and The Potential Control Using Organic Acids. *Iraqi Journal of Agricultural Sciences*, *36*(2),127-134.
17. Khalifeh, M. J., Mohammadabadi, T., Chaji, M., Salari, S., & Khalil, M. (2012). The Effect of Different Levels of Sodium Bentonite on in Vitro Fermentation and Digestibility of Soybean Meal. *Pakistan Veterinary Journal*,32(2), 225-228.
18. Kirovski, D., Adamovic, M., Radivojevic, M., Samanc, H., Vujanac, I., Prodanovic, R., & Sladojevic, Z. (2015). Effects of Bentonite on Weight Gain, Feed Consumption, Blood Metabolites and Ruminal Protozoa in Dairy Calves. *Animal nutrition and feed technology*, 15(1), 11-20.

19. Kozieł, M. J., Kowalska, K., & Piastowska-Ciesielska, A. W. (2021). Nrf2: A main Responsive Element in Cells to Mycotoxin-Induced Toxicity. *Archives of Toxicology*, 95(5), 1521-1533.
20. Liu, L., Jin, H., Sun, L., Ma, S., & Lin, R. (2012). Determination of Aflatoxins in Medicinal Herbs by High-Performance Liquid Chromatography–Tandem Mass *Spectrometry*. *Phytochemical Analysis*, 23(5), 469-476.
21. Mohsen, M. K., & Tawfik, E. S. (2002). Growth performance, rumen fermentation and blood constituents of goats fed diets supplemented with bentonite. Fac. Agric. Tanta Univ, 33(2), 16-26. .
22. Morgavi, D. P., Forano, E., Martin, C., & Newbold, C. J. (2010). Microbial Ecosystem and Methanogenesis in Ruminants. *Animal*, 4(7), 1024-1036.
23. SAS Institute Inc. (2018). Statistical Analysis System, User's Guide (Statistical Version 9.6th ed.). Cary, NC, USA: SAS Institute Inc.
24. SINGH, R., & KUMAR SAINI, A. (2022). Efficacy of Sodium Bentonite (SB) to Ameliorate Adverse Effects of Aflatoxin on In Vitro Rumen Fermentation of Wheat Straw. *The Indian Journal of Animal Sciences*, *92*(7), 876–880.
25. Tilley, J. M. A., & Terry, R. A. (1963). A Two-Stage Technique for in-vitro Digestion of Forage Crops. *Journal of the British Grassland Society*, 18, 104-111.