EFFECT OF PROBIOTIC TREATMENT ON CHEMICAL COMPOSITION, GAS PRODUCTION AND DETOXIFICATION OF CORN SILAGE

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SUMMARY

This study aimed to examine the impact of probiotic treatment (ZAD) on in vitro dry matter digestibility (IVDMD), gas generation, and detoxification of corn silage (CS) contaminated with aflatoxins (5ppb). The samples were divided into 7 different groups whereas control (C) contaminated sample, T1: C+ 1ml ZAD/ Kg DM, T2: C+ 1ml ZAD/ Kg DM, T3: C+ 2ml ZAD/ Kg DM, T4: C+ 2ml ZAD/ Kg DM, T5: 3ml ZAD/ Kg DM for and T6: C+ 3ml ZAD/ Kg DM. T1, T3 and T5 were incubated for one week after adding ZAD, while T2, T4 and T6 incubated for two weeks. Measurements were taken for aflatoxin, in vitro dry matter digestibility (IVDMD), gas generation, Ruminococcus bacterial count, and chemical analysis. The crude protein (CP) values for all treatments (7.3, 7.6, 9.9, 10.1, 10.3 and 10.9% respectively) were considerably greater than the control (3.2%). The crude fiber (CF) levels in all treatments (24.4, 24, 23, 22.5, 21.5 and 18% respectively) were significantly lower than the control (28.5%). Evaluation of (IVDMD) occurred at 6, 24 and 48 hours. The control group had percentages of 31%, 35.5%, and 42% at each period. T1 had percentages of 33%, 36%, and 44.7%. T2 had percentages of 33.4%, 45%, and 42.7%. T3 had percentages of 36.5%, 46.6%, and 54%. In vitro dry matter disappearance was increased gradually by treatment at all time points. This indicates that the application of ZAD treatment significantly improves the in vitro dry matter digestibility (IVDMD). Notable variations in gas generation were seen among the control group and all treated groups at different time points of IVDMD. Probiotic ZAD effectively eradicates aflatoxin at a concentration of 3 ppb in T6.

Keywords: Probiotic, ZAD, Gas production, Chemical analysis, Aflatoxins.

INTRODUCTION

The silage of corn whole-plant has grown in importance and popularity as a feed ingredient for dairy production during the past 25 years. Around the world, whole-plant corn silage (WPCS), has supplanted other forages as the most common feed for dairy cattle (Ferraretto *et al.*, 2018). Dairy animals require a consistent supply of high-quality nutrients throughout the year to ensure their health and optimal performance. Feed preservation for use in times of underproduction is a global issue. Providing their livestock with feed throughout the year, both in terms of quantity and quality, is a difficulty that faces all farmers worldwide (Aragón, 2012). The previous investigations demonstrated that maintaining feedstock quality is a critical issue for every farm that wants to run profitably (Murad *et al.*, 2009) Also, feed preservation on farms is crucial to preserving feed's nutritional content and preventing losses from microorganisms and unwanted toxin contamination, such as mycotoxin. Poor harvests in major rice-producing nations, supply shortages in rice-growing economies, and the rapidly increasing demand for biofuel have all contributed to a steady increase in grain prices. It is not anticipated that prices will drop in the upcoming years. So, farmers should be use abundantly available locally produced feedstuffs, like pastures, silages,

and industrial byproducts, to optimize animal performance. A popular method of preserving of feeds is to control the amount of oxygen present or absent. Hay and grains are often maintained aerobically by adding various preservatives. One well-known instance of an anaerobic preservation method is ensiling. Ensiling was first employed as a management technique, mostly in ruminant production, to meet the need for feed by keeping any surplus feed resources from times of overproduction for use at a later time during times of shortage. But lately, its significance has grown, particularly in high input "zero-grazing" systems that raise animal productivity per unit of land (Ellis *et al.*, 2016). Lately Producers have focused more on silage additives.

Aragón (2012) revealed that improving of silage fermentation helps in increasing silage's nutritional value through reduce nutrients losses and increase the aerobic stability of silage after opening silos. According to Ellis *et al.*, 2016 in study on effect of 4 strains of lactic acid bacteria (LAB) as probiotic and silage additive for different silage substrates on gas production and in-vitro OM disappearance, they found that the gas production and OM disappearance vary with type of LAB strain and substrate incubated.

According to a study conducted by Nataša Hojnik *et al.* (2017), contamination by aflatoxins can arise as a result of malpractices occurring before, during, and after harvest of plant, as well as through the application of specific biological treatments. Moreover, the management, preservation, and conveyance of agricultural commodities have the potential to raise the levels of aflatoxin contamination. Aflatoxins (AFs) are secondary metabolites synthesized by specific Aspergillus species that infect crops. Aflatoxins are chemicals that exhibit toxicity, carcinogenicity, and immunosuppressive properties. Rohit *et al.* (2018) have shown that these secondary metabolites are not altered to be lower toxicity by the processes of pasteurization and heating. Multiple studies have shown that probiotics possess the capacity to produce enzymes that can efficiently break down toxins, leading to the formation of less harmful or non-toxic substances (Zhang *et al.*, 2018). Probiotics have significant potential as an effective tool to reduce the risk of toxin exposure in ruminant animals (Huang *et al.*, 2018). Kholif *et al.* (2019) found that AFM1 and total aflatoxins are both significantly reduce due to the favorable impact of ZAD probiotic treatment. The fermentation of dietary carbohydrates in the rumen leads to the production of gases which is a major environmental concern due to its emissions. Yanti and Yayota (2017) found that burning by-products in agricultural areas releases carbon monoxide and methane, causing serious environmental problems.

Agricultural byproducts, namely straw and hay, contain a substantial amount of fiber. This finding suggests that feed has a higher propensity to create methane than other feed types like grass and legumes. Ruminants are recognized as a significant contributor to methane emissions, particularly when they have a large population. The main objective of improving feed efficiency has long been centered around minimizing the total gas production during rumen fermentation (Abrar *et al.*, 2016). According to Yanti and Yayota (2017), gases production reduction can be accomplished by adding a mixture of agricultural by-products and other nonstructural carbohydrates to the total mixed ration (TMR).

The aim of this study was to investigate the influence of cellulolytic bacteria (ZAD) on the processing of whole corn silage, with the goal of maximizing the utilization of by-products in animal feed. Furthermore, this study seeks to find possible approaches for manufacturing animal products that are safe and either free of Aflatoxins or have very low amounts of its residues.

MATERIAL AND METHODS

The current study was carried out in the laboratories of the Faculty of Agriculture - Ain Shams University in Egypt, and the Animal Production Research Institute and the Animal Health Research Institute of the Agricultural Research Center in Giza, Egypt. Two experiments have been done in the current study:

The first experiment involved evaluating the effects of using ZAD as a probiotic on the chemical composition and toxin levels of contaminated whole corn silage.

The second experiment include conducting in-vitro studies to investigate the impact of ZAD addition on, rates of dry matter degradation, and gas production in both treated and untreated corn silage (CS) samples.

The probiotic levels and incubation time used to produce seven different corn silage as follow:

Control group (C): corn silage contaminated with aflatoxin (5ppb) without treatment, (T1): control + 1ml ZAD/ Kg for 1 week, (T2): control + 1ml ZAD/ Kg for 2 weeks, (T3): control + 2ml ZAD/ Kg for 1 week, (T4): control + 2ml ZAD/ Kg for 2 weeks, (T5): control + 3ml ZAD/ Kg for 1 week, and (T6): control + 1ml ZAD/ Kg for 1 week.

Chemical analysis:

Dry matter was determined by drying both treated and untreated samples in a forced-air oven for 72 hours at 65°C. To measure ash content, samples were burned at a temperature of 550°C for 4 hours in a muffle furnace. Crude protein (CP) and ether extract (EE) fractions measured by using the Kjeldahl and Soxhlet techniques, respectively, following the methodology described by (AOAC 2023). Also, crude fiber (CF) was determined according to (AOAC 2023) standard. The evaluation of NFE was determined by using the formula: NFE = 100 - (CP + EE + CF + Ash).

Mycotoxin analysis:

The mycotoxins in animal feeds were quantified using high-performance liquid chromatography (HPLC) following the guidelines outlined by the Association of Official Analytical Chemists (AOAC 2023)

Bacterial count:

Quantification of Ruminococcus bacteria was conducted using following methodology:

One gram of each experimental medication was mixed with 9 ml of saline solution (SS) in a sterile test tube. A total of twelve dilutions were reproduced by using the same original process. Three sets of each dilution (ranging from 2 to 12) were distributed into a Petri plate containing 10–15 ml of potato dextrose medium. The dishes were Incubated at a temperature of 37° C for 48 hours. The colonies were visually evaluated without using magnification (Abd El Maksoud, 2019).

First experiment:

Contaminated corn silage (CS) samples were combined with exogenous enzymes and an anaerobic bacterium mixture (Ruminococcus flavefaciens, 1x109 CFU/L) ZAD. The humidity of the samples was increased to 65% by adding a urea solution at a rate of 10 g per kilogram, along with the addition of sugarcane molasses at a rate of 50 g per kilogram of dry matter and distilled water. The experiment entailed putting all items in anaerobic incubation at three concentrations (1, 2, and 3 ml ZAD/kg of sample) for durations of one or two weeks. Once the samples have been compressed and isolated by rubbing them up against plastic sheets, samples were stored under normal environmental conditions. Chemical analyses were performed using a representative sample acquired from each iteration.

Second experiment:

In-vitro duration of 72 hours was utilized to calculate the rate of dry matter breakdown per hour. Mertens (1977) described the transformation of the data into a linear form using the natural logarithm approach. Each individual sample was incubated in three different tubes for the following durations of time: 0, 6, 12, 24, 30, and 48 hours. In-vitro dry matter disappearance was measured according to Tilley and Terry (1963). After the incubation period, samples were filtered by filter paper. Conduct a comprehensive rinse of the tubes using heated distilled water. The filter paper and residue must undergo a drying procedure at a temperature of 60°C for a period of 24 hours, or alternatively, at a temperature of 100°C for a minimum of 6 hours. Samples were transferred to a desiccator and let it cool for a period of 2 to 4 minutes before measuring its weight.

Calculation:

% IVDMD = 1 – [(Residue + filter paper) – filter paper] -blank (sample weight) (DM) Blank = (Blank residue + filter paper) – filter paper.

In vitro gas generation:

Vials were kept under controlled conditions for a period of 120 hours to examine the rate at which gas is produced. Gas output was measured using plastic syringes at certain time intervals of 6, 12, 24, 30 and 48 hours. Two empty vials were incubated for each inoculum to adjust the gas production quantities and account for endogenous gas generation.

Statistical analysis:

The statistical analysis of the original research was conducted using version (V0.2) of the SPSS statistics program (Verma JP 2013). The statistical analysis system employed the general linear models (GLM) methodology and conducted an analysis of variance (ANOVA). Duncan, 1955 multiple range test was employed to detect statistically significant disparities between means at a significance level of P <0.05. Pearson correlation coefficient was computed to assess the relationship between several measurements, to chemical analysis, IVDMD and gas production, using the following model:

$$Y_{ij} = \mu + T_i + e_{ijk}$$

Where: Y_{ij} : is the observation of the model, μ : overall mean, Ti: the effect of probiotic dilution, e_{ijk} : is an abbreviation for experimental error.

RESULTS AND DISCUSSION

Data in table (1) shows significant differences in dry matter (DM) content between control group and T1, T3 T4 and T5. Control group and T6 recorded least (29.5%) while the highest for T3 (36.5%). On the other hand, significant differences were found among treatment levels except T1 and T4 and T2 and T6. The dry matter content of T1 higher than T2, T3 higher than T4 and T5 higher than T6. On the other hand, there is no clear trend for Ash content despite significant differences were found between control and different levels of ZAD. T1 recorded the highest value (17%) while the least was recorded for C group (8%). Significant differences were shown between T1 and another treatment levels. While no significancy between T2, T4, T5 and T6. As well as T3 and T4 had no significant differences. On the other hand, the Ash content of T2 and T5 were higher than T6 but lower than T3 and T4.

The amount of crude fiber (CF) significantly decreased as the incubation period and probiotic doses (ZAD) increased in the proximate analysis of corn silage. The observed impact exhibits statistical significance when comparing the control group to all treatment groups. While no significant differences were observed between groups T1, T2, T3, T4 and T5. The current study corroborates the findings of prior research undertaken by Gado *et al.* (2017), Asmare (2020), and Lydia *et al.* (2023). Previous investigations demonstrated that incorporating ZAD during the ensiling of sugar cane bagasse, wheat straw, and maize stalks improved ruminal digestion and increased rates of fiber degradation, reaching a value of 3 L/ton. Consequently, the use of agricultural waste resulted in the creation of a more purified animal feed product. The main factors contributing to the effects were the nitrogen levels in the bacteria present in the silage samples and the nitrogen levels in the added urea, which were approximately 1 g/kg. One potential explanation for this phenomenon may be that the increase in single-cell protein, resulting from bacterial growth, occurred at the cost of CF, which had a significant reduction in the opposite direction of the CP trend. In El-Mahy's (2001) study, maize stalks were treated with ZAD at doses of 1 and 3 Kg/ton for 1, 2, and 4 weeks. The results showed a substantial rise in crude protein (CP) levels in the treated samples compared to the control. This increase in CP may be ascribed to the presence of a specific cell protein.

However, the quantities of crude protein were increased due to the combined effects of probiotic administration and a longer incubation period. The statistical analysis revealed significant differences between the control group and all treatment groups. The findings of the present study align with the research carried out by Gado *et al.* (2017), Asmare (2020), and Lydia *et al.* (2023) demonstrated that a decrease in fiber levels can be linked to a decrease in structural polysaccharide components. Enzymatic treatments enhance the process of dry matter biodegradation, increasing the percentage of degradation and expediting the rate of fiber breakdown in fibrous feeds. The mechanism by which enzymes facilitate biodegradation is a subject of current debate (Togtokhbayar *et al.*, 2015). Studies conducted by Asmare (2020) and Lydia *et al.* (2023) have emphasized the benefits of utilizing biological therapy for the breakdown of fiber in crop residue. More precisely, they have focused on applying white rot fungi, which can decompose complex fibers into easily digestible soluble sugars. This facilitates the improvement of DM digestion.

Either extract increased significantly by treatment. It observed significant differences between control group and ZAD levels. The highest value was recorded for T6 (3.2%) while the least recorded for C (2.4%). No significancy between T1, T2 and T3 as well as T5 and T6. T4 was higher than T1, T2 and T3. While it was lower than T5 and T6.

Treatment	DM	Ash	CF	СР	EE	NFE
Control	29.5 ^d	8 ^e	28.5°	3.2 ^d	4 ^d	4.8
T1	32.5°	17 ^a	24.4 ^b	7.3°	10.5 ^{bc}	11.3
Т2	29.8 ^d	11.6 ^{cd}	24 ^b	7.6 ^c	9.7°	14.9
Т3	36.1ª	14.3 ^b	23 ^b	9.9 ^b	9.6 ^c	13.4
T4	33.3°	13 ^{bc}	22.5 ^b	10.1 ^b	12.5 ^b	5.9
Т5	34.9 ^b	11.3 ^{cd}	21.5 ^b	10.3 ^b	11.8 ^{ab}	11.8
T6	29.5 ^d	9.3 ^{de}	18.6 ^a	10.9 ^a	12.7ª	13.5
SE	±0.34	±0.76	±1.15	±0.43	±0.52	

Table (1): Effect of ZAD level on chemical composition for corn silage (%).

a, b, c and d in the same column mean significant deference, Sig=.001, SE= standard error, DM= dry matter, CF= crude fiber CP= crude protein, EE= Either extract, NFE= non fiber extract, C= control group, T1= control group+1mlZAD/1week, T2= control group+1mlZAD/2weeks, T3= control group+2mlZAD/1week, T4= control group+2mlZAD/2weeks, T5= control group+3mlZAD/1week and T6= control group+3mlZAD/2weeks.

Table (2) displays the results of the in vitro dry matter disappearance (IVDMD), emphasizing a significant correlation between the treatment and the in vitro time period. This discovery is consistent with the research carried out by Asmare (2020), Yanti and Yayota (2017), and Abrar et al. (2016). Arriola and Adesogan (2013) found that using an external fibrolytic enzyme for anaerobic pre-treatment can lead to an interaction between enzymes and feed before ingestion. This interaction has the ability to partially break down through hydrolysis and improve the use of fiber fraction in biological processes. Togtokhbayar et al. (2015) conducted a study in which they proposed a prospective method to improve the initial rate of anaerobic digestion of dry matter (DM) by using exogenous fibrolytic enzymes. An alternative strategy entails promoting the increased colonization of feed particles by anaerobic bacteria. Valdes et al. (2015) found that the addition of external enzymes has the capacity to promote an increase in the overall population of viable anaerobic bacteria. Consequently, this can result in an increase in the decomposition of fiber and an enhancement in the capacity of rumen anaerobic bacteria to uptake and process feed. Arriola and Adesogan (2013) suggest that there may be a connection between the better breakdown of feeds and the better functioning of anaerobic enzymes in the rumen fluids. This could be due to the release of a greater amount of easily soluble carbohydrates from undigested feed particles. Studies have shown that an increase in the amount of soluble carbohydrates released from cell walls can provide extra energy for microbial growth and reduce the time needed for microbial colonization in the rumen. Moreover, prior research has shown that it can increase the attraction of bacteria to the gastrointestinal tract and improve their capacity to stick to undigested meal particles (Gado et al., 2017).

Table (2) demonstrates a significant improvement in the in vitro dry matter disappearance when comparing the control group to the treatment group at all treatment doses. This agreement aligns with the conclusions stated by Asmare (2020), Yanti and Yayota (2017), and Abrar *et al.* (2016).

Treat	6h	12h	24h	30h	48h	DR
С	31 ^b	32.6 ^e	35.5 ^d	42 ^e	42 ^d	.005
T1	33 ^b	34.8 ^d	36 ^d	42.6 ^d	44.7°	.007
Т2	33.4 ^b	36 ^d	45°	49 ^c	49.7 ^b	.008
Т3	36.5 ^a	44.4 ^c	46.6 ^{bc}	49 ^b	54 ^a	.008
T4	38.4 ^a	46.7 ^b	$47^{\rm abc}$	50.7 ^b	54 ^a	.009
Т5	39.5 ^a	48.7 ^{ab}	49^{ab}	51.4 ^{ab}	56.1 ^a	.009
T6	39.6 ^a	49.3 ^a	49.6 ^a	55.8ª	57.3 ^a	.009
SE	<u>+</u>	<u>+</u>	<u>+</u>	<u>+</u>	±	
SE	1.28	0.73	0.84	0.45	0.89	

Table (2): Effect of ZAD level and incubation in vitro time on IVDMD (%) for corn silage.

a, b, c, d, e and f in the same row / column mean significant deference, Sig= .001, DR= Digestion rate, C= control group, T1= control group+1mlZAD/1week, T2= control group+1mlZAD/2weeks, T3= control group+2mlZAD/1week, T4= control group+2mlZAD/2weeks, T5= control group+3mlZAD/1week and T6= control group+3mlZAD/2weeks

Data in table (3) demonstrates a significant decrease in total gas production at all treatment doses when comparing the control group to the treatment group. Yanti and Yayota (2017) detected a direct relationship between the fiber content of feed and the release of methane gas. Microbial fermentation in the rumen produces gases. Dietary carbohydrates, including cellulose, hemicellulose, pectin, and starch, play a crucial role in facilitating the total gas production in the rumen.

Treatment	6h	12h	24h	30h	48h
Control	0	0	0	5.3ª	14 ^a
T1	0	0	0	2.7 ^{bc}	14 ^a
T2	0	0	0	2.7 ^{bc}	13.7 ^a
Т3	0	0	0	0.7^{d}	12.7 ^{ab}
T4	0	0	0	3.3 ^b	12.3 ^{ab}
Т5	0	0	0	2.7 ^{bc}	12^{ab}
T6	0	0	0	1.7°	11.3 ^b
SE	± 0.0	±0.0	± 0.0	± 2.49	± 2.49

Table (3): Effect of ZAD level and incubation in- vitro time on total gas production (ml) for corn silage.

a, b, c, d, e, f and g in the same row/ column mean significant deference, Sig=.002, C= control group, T1= control group+1mIZAD/1week, T2= control group+1mIZAD/2weeks, T3= control group+2mIZAD/1week, T4= control group+2mIZAD/2weeks, T5= control group+3mIZAD/2weeks

Table (4) displays the data about the number of bacteria found on corn silage, together with the increase in bacterial count seen due to the manipulation of probiotic doses and the incubation period. The results clarify that the improvement of dry matter digestibility and gas production in a laboratory setting can be achieved by increasing the quantities of zero-acid detergent and prolonging the incubation time.

Table (4): Effect of ZAD level of	n bacterial count	(CFU)) for corn silage.
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Treatment	Control	T1	T2	T3	T4	Т5	T6
Bacterial count (CFU)	8*10^10	8*10^12	10*10^12	10*10^12	42*10^12	100*10^12	<100*10^12
C = control group, group+2mlZAD/lweek.		0 1			0 1		

group+2mIZAD/1week, T4= control group+2mIZAD/2weeks, T5= control group+3mIZAD/1week and T6= control group+3mIZAD/2weeks

Data in table (5) showed that the corn silage control group got rid of all the aflatoxin by using 3ml/L of probiotic ZAD for two weeks incubation time. The concentration of aflatoxin dropped to 3 parts per billion (ppb) in T6. The decrease in toxin levels that was detected is statistically significant, consistent with the findings published by Liu *et al.* (2017), Zhang *et al.* (2018), Zhou *et al.* (2019) and Kholif *et al.* (2019). A study conducted by Kholif *et al.* (2019) demonstrated a significant reduction in total aflatoxin levels, specifically aflatoxin M1 in milk, when the ZAD probiotic, which includes a combination of bacteria and exogenous enzymes, was used. Huang *et al.* (2018) reported the discovery that the probiotic strain Bacillus subtilis and the mycotoxin degradation enzyme had the ability to breakdown Aflatoxin B1 and zearalenone.

Multiple studies offer evidence that demonstrates the effectiveness of probiotics in treating toxin-induced diseases in ruminant animals. Huang *et al.* (2018) found that the probiotic strain Lactobacillus plantarum can effectively reduce the toxicity caused by aflatoxin B1, a very powerful mycotoxin. Huang *et al.* (2018) showed that the probiotic strain Bacillus subtilis can absorb ochratoxin A, another mycotoxin, in the gastrointestinal system of pigs.

The most efficient medium was found to be a maize steep media, originally designed for penicillin synthesis, but modified to have a pH of 7.6 and incubated at 25 degrees Celsius. Glucose was used as a substitute for lactose. In the process of synthesizing endotoxins, the inclusion of inorganic salts is also necessary, as described by Hesseltine *et al.* in 1966. The information provided indicates that several factors, including probiotics, cellulose bond breaking, the presence of nutrients necessary for fungal cell growth, and the inability of probiotics to inhibit the growth of these harmful organisms, may all contribute to the rise in mycotoxin levels at 1 and 2 ml ZAD/week.

The probiotic exhibited inhibitory effects on the proliferation of these hazardous microorganisms at a dosage of 3 ml ZAD each week, and shown a mitigating effect on their growth at a dosage of 3 ml ZAD every two weeks

Moreover, numerous in vivo studies have been carried out to assess the efficacy of probiotics in treating toxininduced diseases in ruminants, which complement the previously described in vitro experiments. On another hand, ZAD doses under 3ml/L had no positive effect on aflatoxin levels for any incubation time.

Treatment	С	T1	Τ2	Т3	T4	Т5	T6
Toxin levels (ppb)	5 ^{bc}	10 ^a	7 ^b	10 ^a	7 ^b	5 ^{bc}	3°
SE	± 0.69	± 0.69	± 0.69	± 0.69	± 0.69	± 0.69	± 0.69

C= control group, T1= control group+1mlZAD/1week, T2= control group+1mlZAD/2weeks, T3= control group+2mlZAD/1week, T4= control group+2mlZAD/2weeks, T5= control group+3mlZAD/1week and T6= control group+3mlZAD/2weeks

CONCLUSION

In conclusion, the aforementioned points collectively substantiate the view that. Probiotics have demonstrated efficacy in augmenting the nutritional quality of corn silage for ruminant feeding. Moreover, it is considered a potentially efficacious tool for treating toxin-induced illnesses in ruminant animals. These organisms utilize diverse mechanisms to break down poisons present in bovine diets and within the rumen. Extensive studies have shown that they are effective in reducing the harmful effects of toxins, as proven by studies conducted both in laboratory settings and in living organisms.

Additional research is necessary to fully understand the mechanisms by which probiotics aid in toxin detoxification and to develop effective ways to use probiotics for detoxifying poisons in ruminant animals. However, based on the available facts, it can be inferred that probiotics serve as a valuable tool for enhancing the health and productivity of ruminant animals in the presence of toxin contamination.

REFERENCES

- Abd El Maksoud, A.M. (2019). Effect of some ruminal bacteria on digestion characteristics in ruminant. M.Sc. Thesis, Ain- Shams Univ. Egypt. pp 72-90.
- Aragón Y. A. (2012) The Use of Probiotic Strains as Silage Inoculants chapter 1.
- AOAC (2023) Official Methods of Analysis, 22nd Edition. George W Latimer (Ed) Oxford University press
- Abrar, A.; M. Kondo; T. Kitamura; T. Ban-Tokuda and H. Matsui (2016). Effect of Supplementation of Rice Bran and Fumarate Alone or on Combination on In Vitro Rumen Fermentation, Methanogenesis and Methanogens. Animal Science Journal 87, 398-404
- Arriola K, and A. T. Adesogan (2013) Effect of Fibrolytic Enzyme Application on the Digestibility of Corn Silage, Alfalfa Hay, Concentrates, and Complete Diets Under Simulated Ruminal and Preruminal Conditions. Animal Nutrition and Feed Technology 13, 537-550
- Asmare B. (2020) Biological Treatment of Crop Residues as An Option for Feed Improvement in the Tropics: A Review. Animal Husbandry, Dairy and Veterinary Science, 4, 1-6
- Duncan, D.B. (1955). Multiple range and multiple F test. Biometrics, 1955 (11): 1 41.
- Ellis J. I.; A. Bannink, I. K. Hindrichsen, R. D. Kinley, W. F. Pellikaan, N. Milora and J. Dijkstra (2016) The Effect Of Lactic Acid Bacteria Included As A Probiotic Or Silage Inoculant On In Vitro Rumen Digestibility, Total Gas And Methane Production. The journal of animal feed science and technology 211, 61-74.
- El-Mahy, M. A. M. (2001) Effect of Some Biological Treatments On The Nutritive Value Of Agricultural By-Products. M. Sc. Thesis, Menofiya Univ. Egypt. pp 95-104

- Erasmus, L. J. (2006). Methane production from various concentrates in simulated rumen incubations. Livestock Science, 99(1-3), 189-197.
- Ferraretto L. F.; R. D. Shaver, and B. D. Luck (2018) Silage review: Recent advances and future technologies for whole-plant and fractionated corn silage harvesting. Journal of dairy science 101 (5), 3937-3951.
- Huang W.; J. Chang, P. Wang, C. Liu, Q. Yin, Q. Zhu, F. Lu and T Gao, (2018) Effect of the Combined Compound Probiotics with Mycotoxin–Degradation Enzyme on Detoxifying Aflatoxin B1 and Zearalenone. The Journal of Toxicological Sciences 43, 377-385
- Hesseltine, C. W.; O. L. Shotwell; J. J. Ellis and R. D. Stubblefield (1966). Aflatoxin Formation by Aspergillus flavus. (bacteriological reviews). American Society for Microbiology, 30, 795-804.
- Gado. H M.; M. M. Y. Elghandour, M. Cipriano, N. Odongo and A. Z. M. Salem, (2017) Rumen Degradation and Nutritive Utilization of Wheat Straw, Corn Stalks and Sugarcane Bagasse Ensiled with Multienzymes. Journal of Applied Animal Research, 45, 485- 489
- Kholif, M. A.; H. M. Gado, H. M. Metwally, R. M. Abdelgawad and A. M. Kholif, (2019), Effect of Anaerobic Probiotic And/or Biological Antitoxin Supplementations on Productive and Reproductive Performance of Lactating Cows. Arab Universities Journal of Agricultural Sciences, Special Issue 27, 357-365
- Lydia K. O.; S. I. Omoanghe, P. A. Dele. F. N. Anike, K. A. Ike, Y. Shaw, R. M. Brice, O. E. Orimaye, M. Wuaku, B.G. Essick, N. Holt, N. S. Udombang, J. O. Enemudo, K. Subedi and U. Y. Anele, (2023) Effects of the Incubation Period of Pleurotus Ostreatus on the Chemical Composition and Nutrient Availability of Solid-State-Fermented Corn Stover. Animals, 13, 2587- 2600 https://doi.org/10.3390/ani13162587
- Liu, S.; H. Zhang, J. Wang, Y. Zhao and Q. Meng, (2017). Saccharomyces cerevisiae ZDY005 suppresses the growth of Escherichia coli and alleviates lipopolysaccharide-induced inflammation in the rumen of cattle. Journal of Animal Science and Biotechnology, 8(1), 6.
- Mertens, D.R. (1977). Dietary Fiber Components: Relationship to Rate and Extent of Ruminal Digestion. Fed. Proc. 36,187-192
- Moss, A.; R., J. P. Jouany and J. C. Newbold, (2000). Methane production from ruminants: its contribution to global warming and mitigation strategies. Journal of the Science of Food and Agriculture, 80(2), 1001-1020.
- Murad H H.; M. A. Hanfy, A. M. Kholif, M. H. Abdel Gawad and H. A. Murad (2009). Effect of cellulases supplementation to some low quality roughages on digestion and milk production by lactation by lactating goats. J Biol. Environ. Sci. 4: 791-809.
- Nataša H.; U. Cvelbar, G. Tav^{*}car-Kalcher, L. W. James and K. Igor, (2017) Decontamination of Food: Cold Atmospheric Pressure Plasma versus "Classic" Decontamination. Toxins 9, 5-15
- Rohit P.; N. Kumar, V. Kashyap, R. C. R. Chand and K. R. K. Rajeev, (2018) Aflatoxin M1 Detoxification Ability of Probiotic Lactobacilli of Indian Origin in In vitro Digestion Model. Probiotics and Antimicrobial Proteins, 11, 460-469
- Tilley J. M. A. and R. A. Terry, (1963) A Two-Stage Technique for the in Vitro Digestion of Forage Crops. Journal of British Grassland Society, 18, 104-111. https://doi.org/10.1111/j.1365-2494.1963.tb00335.x
- Togtokhbayar, N; A. María, C. Errillo, M. M. M. Y. Elghandour, A. Z. M. Salem, C. Urankhaich C, S. Jigjidpurev, N. E. Odongo and A. E. Kholif, (2015) Effect of Exogenous Xylanase on Rumen In Vitro Gas Production and Degradability of Wheat Straw. Animal Science Journal. 86, 765–771
- Valdes K. I.; A. Z. M. Salem, S. Lopez, M. U. Alonso, N. Rivero, M. M. Y. Elghandour, I. A. Domínguez, M. G. Ronquillo and A. E. Kholif, (2015) Influence of Exogenous Enzymes in Presence of Salix Babylonica Extract on Digestibility, Microbial Protein Synthesis and Performance of Lambs Fed Maize Silage. Journal of Agriculture Science (Cambridge), 153, 732-742
- Verma, J. P. (2013) One-Way ANOVA: Comparing means of more than two samples. Data analysis in management with SPSS software, 221-254.
- Yanti Y and M. Yayota, (2017) Agricultural By-Products as Feed for Ruminants in Tropical Area: Nutritive Value and Mitigating Methane Emission. Reviews in Agricultural Science, 5, 65-76
- Zhang, X.; X. Zhou, Y. Li, J. Wang and Q. Meng, (2018). Bacillus subtilis ZDY007 adsorbs ochratoxin A in vitro and in vivo. Journal of Agricultural and Food Chemistry, 66(11), 2804-2810
- Zhou, X.; L. Zhang, T. Wu, H. Zhang, J. Wang and Q. Meng, (2019). Lactobacillus plantarum ZDY006 degrades aflatoxin B1 and reduces its cytotoxicity in vitro and in vivo. Frontiers in Microbiology, 10, 904.

تقييم سيلاج الذرة المعامل بالبروبيوتك على التركيب الكيميائي و انتاج الغاز و ازالة السموم

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تهدف هذه الدر اسه إلى در اسة تأثير المعاملة بالبر وبيوتيك (ZAD) على هضم المادة الجافة في المختبر (IVDMD)، وتوليد الغاز، و إز الة السموم من سيلاج الذرة (CS) المصاب بالأفلاتوكسين (ppb5). تم تقسيم العينات إلى 7 مجمو عات مختلفة، حيث: (C) المجموعة غير المعاملة، 11: 1 مل من سيلاج الذرة (CS) المصاب بالأفلاتوكسين (ppb5). تم تقسيم العينات إلى 7 مجموعات مختلفة، حيث: (C) المجموعة غير المعاملة، 11: 1 مل ZAD/ كجم مادة جافة، 27: 2 مل ZAD/ كجم مادة جافة، 27: 2 مل ZAD/ كجم مادة جافة، 27: 2 مل ZAD/ كجم مادة جافة، 27: 1 مل ZAD/ كجم مادة جافة، 27: 2 مل ZAD/ كجم مادة جافة، 27: 2 مل ZAD/ كجم مادة جافة، 27: 3 مل ZAD/ كجم مادة جافة، 27: 2 مل ZAD/ كجم مادة حافقتر تم تحضين 11 و 3 مليوع واحد بعد إضافة ZAD/، وعدد بكتيريا وعدد بكتيريا وعدد بكتيريا وعدد بكتيريا والت والت والت الأفلاتوكسين، و هضم المادة الجافة في المختبر (IVDMD)، وتوليد الغاز، و عدد بكتيريا وعدد بكتيريا و 2 من يعنيا معاملات (IVDMD)، وتوليد الغاز، و عدد بكتيريا وعدد بكتيريا وعدد بكتيريا وعدد بكتيريا وعدد بكتيريا والت والت والت والت الأوليان الخام (CP) لجميع المعاملات (7.5، 6.5، 9.0)، و 10.1، 3.01 و 10.1، كانت مستويات الألياف الخام (CP) في ع و 20 مليان الحبوم عد غير المعاملة على نسب كثير من العينة غير المعاملة (2.2%). كانت مستويات الألياف الخام (CD) في 6 و 2 و 2 و 40 ساعة. حصلت المجموعة غير المعاملة (2.2%). كانت مستويات الألياف الخام (CD) في 6 و 2 و 2 و 2 و 20، 20، 20.5، 20.5 و2.5، 20.5 و2.5 و 2.5%، و 2.5%،