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EFFECT OF ANAEROBIC PROBIOTIC AND/OR BIOLOGICAL ANTITOXIN SUPPLEMENTATIONS ON PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF LACTATING COWS

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ABSTRACT

Forty-eight lactating Holstein Friesian cows were utilized to assess impact of supplementing probiotic ZAD (mixture of live bacterial cells and enzymes) compared with T5X (antitoxins product) on some productive, reproductive and antitoxins activity parameters. we have divided the animals to three experimental categories (16 each). The first category, control treatment, was fed basal diet without any supplements. The second category, ZAD treatment, was fed basal diet supplemented with ZAD probiotic (1.1 L/ton). The third category, T5X treatment, was fed basal diet supplemented with T5X antitoxins product (1.1 Kg/ton). Milk yield was recorded. we have collected and analyzed milk samples, blood samples and feed samples in order to constitutes. Time-interval between calving and fertilizing artificial insemination and pregnancy rate were recorded. The gained outcomes demonstrated that milk yield was significantly raised by ZAD treatment than other treatment. Milk components yield were significantly raised by ZAD probiotic and T5X treatments than control. Blood serum total protein, albumin, globulin, glucose, urea and total lipids were not significantly influenced by treatments. Total aflatoxins in concentrate mixture and aflatoxin M1 in milk were significantly diminished by ZAD and T5X than control. Number of days between calving and fertilized artificial insemination were diminished by treatments than control. Pregnancy rate was significantly raised by ZAD probiotic than T5X and control treatments, respectively. The overall conclusion of this study illustrated that Probiotic ZAD, potentially, has antitoxins activity leading to reinforcing the productive and reproductive performance of lactating cows.

Keywords: Probiotic, Antitoxins Activity, Milk Yield, Reproductive Performance, Blood, Concentrate Mixture

INTRODUCTION

Mycotoxins are secondary metabolites created by several of fungi species. These substances are produced fundamentally through five species of fungi (Fusarium sp, Penicillium sp. Aspergillus sp. Claviceps sp. Alternaria sp.) **(Xiong et al 2015 and Zouagui et al 2017).** Aflatoxins is One of the most common kinds of mycotoxins exist in livestock feeds. Aflatoxins are produced fundamentally in members of *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* through the polyketide pathway **(Battacone et al 2012 and Xiong et al 2018).** Natural forms of aflatoxin, involving forms B1, B2, G1, and G2, are mostly found in feeds.

Aflatoxin M1 (AFM1), the monohydroxylated derivate of aflatoxin B1 (AFB1), occurs in milk from dairy cows fed an AFB1 contaminated diet and perhaps subsequently transferred into other dairy products **(Battacone et al 2005, Firmin et al 2011 and Xiong et al 2015).** Since, AFB1 and AFM1 are categorized as a hepatotoxic and carcinogenic substances, and are considered as Group 1 human carcinogens by the International Agency for Research on Cancer (IARC) of the World Health Organization **(IARC, 2002),** a lot of trials utilizing various manners

to minimize the transfer of aflatoxins from feed to milk involving physical, chemical, and biological manners. Organic and inorganic adsorbents have been utilized to reduce absorbance of AFB1 and transforming as AFM1 to milk (**Khattab et al 2009, Xiong et al 2015 and Zouagui et al 2017).** furthermore, the antitoxic activity for microorganisms have been documented. some studies indicated that certain strains of bacteria and yeasts have parietal structures capable of binding to mycotoxins **(Zouagui et al 2017).** According to **Chiquette (2009) and Xu et al (2017),** Probiotics are "live microorganisms that have a positive impact by improving the balance and activity of the intestinal microflora and thereby host health and productivity". For ruminants, probiotics have been shown to be utilized as pH stabilizing agent, thereby, preventing from acute and sub-acut ruminal acidosis **(Nocek and Kautz 2006 and Chiquette et al 2008).** Also, probiotics had been demonstrated to raise dry matter intake (2.6 kg/day) and (2.3 kg/day) milk yield (**Nocek et al 2003, Nocek & Kautz 2006)**. Similarly, **Lehloenya et al (2007)** pointed that a 9% raise in milk yield when a mixture of yeast and Propionibacterium was fed to dairy cows.

on the other hand, **Fukuda et al (2006)** indicated that a several bacteria with very specified missions in the rumen like a *Butyrivibrio fibrisolvens*, which produce conjugated linoleic acids (CLA) from linoleic acid, have been suggested as probiotics for ruminants. The probiotic product, ZAD, is defined as a biotechnical product made from anaerobic bacteria which convert the polysaccharide into monosaccharide by specific enzymes. ZAD improved nutrients digestibility, live body weight gain and feed conversion of wheat straw in sheep and increased milk production **(Gado et al 2009, Salem et al 2011 and Khattab et al 2011)**

As mentioned earlier, utilizing bacterial probiotics generates an acidic environment which it injurious to pathogens. Also, the production of bacteriocins by several probiotic strains protects intestinal health and working as adsorbents for mycotoxins **(Firmin et al 2011 and Zouagui et al 2017)**. In this context, the primary goals of this study were evaluating the antitoxic activity for ZAD® probiotic product comparing to the commercial one (T5X). Productive and reproductive performance of lactating cows were evaluated.

MATERIALS AND METHODS

This study was implemented on El-Gabry farm for animal production, Al-Fayoum Governorate, Egypt. The field experiment was extended for three months from Feb., 2016 to May, 2016. Feed chemical composition, milk chemical composition, blood serum analysis, and feed and milk mycotoxins content analysis were conducted on dairy production department, National research center, Egypt.

Materials

The purpose of this study is estimating the efficiency of ZAD probiotic product as antitoxic agent. Probiotic ZAD is a mixture of bacteria and exogenous enzymes in liquid formula prepared to supply to ruminants diets. It comprises anaerobic bacteria and mixture of enzymes as cellulase, xylanase, alpha amylase and protease in balanced effective formula. Probiotic ZAD product was compared to a commercial antitoxic product, T5X (mixture from different types of clays as well as vitamins produced by Neovia, vanes cedex, France and imported by Multivita animal nutrition company, Egypt).

Animals, Feeding and management

In this experiment, we utilized a set of 48 Holstein-Frisian cows (120±3 DIM) in the first season of lactation and averaged 500 Kg body weight. The animals were divided to three equal categories; the first, Control treatment and it fed on the farm basal diet. The second, ZAD treatment and was fed on basal diet supplemented with 1.1 liter of ZAD mixture/ton. The third group, T5X treatment and it was fed on basal diet supplemented with 1.1 Kg/Ton T5X product. Cows were fed as a group open feed with free access to water. The nutrient requirements were calculated according to NRC, 2001 and recalculated biweekly according to the changing in body weight and milk production. The chemical composition of basal experimental rations is presented in **Table 1.**

Animals, Feeding and management

Total of 48 Holstein-Frisian cows (120±3 DIM) in the first season of lactation and averaged 500 kg body weight were used in this experiment.

Table 1. Total mixed ration ingredients (kg/ton) and its chemical composition

Ingredient[1]	Treatments		
	Control	ZAD	T5X
Corn silage	400	400	400
Alfalfa hay	110	110	110
Water	140	140	140
Glutofeed 16%	75	75	75
Soybean meal 44%	100	100	100
Yellow corn	160	160	160
Limestone	7	7	7
Vitamin mixture[2]	0.75	0.75	0.75
Minerals mixture[2]	1.5	1.5	1.5
Sodium bicarbonate	7	7	7
T5X (kg/Ton)	0	0	1.1
ZAD (L/Ton)	0	1.1	0
Total DM offered (kg/d)	20	20	20
TMR chemical composition %			
DM	84.76		
CP	17		
CF	19		
EE	3.1		
NDF	30		
Forage NDF	23.1		
ADF	18.7		
NFC	43.9		
Ca	0.38		
P	0.24		
ME	2.42 Mcal /kg		
NE l	1.52 Mcal /kg		
NE g	1.14 Mcal /kg		
DCAD	236 mQE/kg		

The animals were divided to three equal groups; the first, control treatment and it fed on the farm basal diet. The second, ZAD treatment was fed on basal diet supplemented with 1.1 liter of ZAD mixture/ton. The third group, T5X treatment was fed on basal diet supplemented with 1.1 kg/Ton T5X product. Cows were fed as a group open feed with free access to water. The nutrient requirements were calculated according to **NRC, (2001)** and recalculated biweekly according to the changing in body weight and milk production. The chemical composition of basal experimental rations are presented in **Table (1).**

Feed, milk and blood Sampling and analysis

During the experiment, represented samples of TMR were collected monthly and analyzed for DM, OM, CP, EE, CF and Ash according to **A.O.A.C. (2000)** methods. Neutral detergent fiber (NDF) Acid detergent fiber (ADF) were determined according to the procedure of **Van Soest et al (1991)**. Cows were milked 2 times daily at 8 a.m. and 5 p.m. Milk yield for all cows were recorded daily using Delaval milk manager model sortie. Represented milk samples were collected biweekly. Milk constituents of total solids, fat, protein, lactose, solids-non-fat, and ash were determined by Bentley 150 infrared milk analyzer (Bentley Instruments, Chaska, MN, USA). The energy-corrected milk (ECM) equation is commonly used (Tyrrell and Reid, 1965), where: ECM (kg)= (0.3246 * milk kg)+ (12.86 * fat kg) + (7.04 * protein kg)

Mycotoxins analysis in feed and milk

Determinations of total mycotoxins in feeds were implemented according to **AOAC (1995)** utilizing HPLC. For AFB1, a C18 Luna II, stainless steel, 5 µm column (150 x 4.6 mm; Phnomenex, The Netherlands) connected to two high precision pumps (Gynkotek model 300) set at a flow rate of 2 ml/min and controlled by a Chromeleon-Gynkotec HPLC software (Softron). The mobile phase consisted of acetonitrile: methanol: water (1: 3: 6, v: v: v). Fluorescence detection was performed with a FP 920 fluorescence detector (Jasco, Japan) set at 360 nm excitation wavelength and 450 nm emission wavelength. Milk was analyzed for mycotoxins (AFM1) according to **Navas et al (2005)** using HPLC (a C18 Luna II, stainless steel, 5 µm column (150 x 4.6 mm; Phnomenex, The Netherlands) connected to two high precision pumps (Gynkotek model 300) and set at a flow rate of 1 ml/min and controlled by a Chromeleon-Gynkotec HPLC software (Softron). Mobile phase was consisted of 2% acetic acid: acetonitrile: methanol (40:35:25, v/v/v). Fluorescence detection was performed with a FP 920 fluorescence detector (Jasco, Japan) set at 360 nm excitation wavelength and 430 nm emission wavelength.

Statistical analysis

Data collected were subjected to statistical analysis as one-way analysis of variance using **SAS (2004)** according the following model:

Yij = μ + Ti + eij.

Where:

Yij =the observation

μ= overall mean

Ti= effect of treatment

eij=experimental error

The significant differences between means of studied groups were tested according to Duncan's New Multiple Ranges Test **(Duncan, 1955).**

RESULTS AND DISCUSSION

Blood serum parameters

Data in **Table (2)** presented the findings of several blood parameters influenced by ZAD probiotic and T5X antitoxin supplementation to lactating cows' diets.

Also, the findings demonstrated that utilizing ZAD probiotic and T5X antitoxins didn't have any significant impacts on blood serum parameters. It is noted that Globulin value was raised by treatments than control, leading to the same trend with total protein, however, the differences were not significant. It seems that is happened as a reflection for utilizing antitoxins agents, nevertheless it still within the normal range. These findings are in agreement with those reported by **Khattab et al (2009).** They utilized bentonite as antitoxins agent and they found that plasma total protein was insignificantly (P>0.05) raised by treatment than control. Plasma urea-N concentration insignificantly raised with utilizing ZAD probiotic than control and T5X treatment. In contrast to these results, blood urea-N was reduced utilizing tafla or bentonite as antitoxins agents with sheep, goats and buffaloes **(Hassona et al 1995, Abou'l Ella, 2007 and Khattab et al 2009).** Blood urea N may serve as an indicator of ruminal protein degradability and post ruminal protein supply **(Roseler et al 1993).** This could explain the increasing in blood urea-N with using ZAD product which consist of various kinds of enzymes and live bacteria. These compounds lead to raise rumen fermentation activity and ammonia production, thereby blood urea-N. in general, the Urea-N values for all treatments are still in the normal range (10-50 mg/dl) as reported by **Kaneko (1989).** Also, the same trend was noted with total lipid values which increased by treatments than control but this raise was not significant. However, Glucose ratio was slightly reduced by treatments but also it was not significant. Also, liver function enzymes, Alanin aminotransferase (ALT) and aspertat aminotransferase (AST) were not significantly influenced by treatments, meaning that dairy animals were not negatively influenced by utilizing ZAD probiotic and T5X antitoxins agents. These outcomes are in consistent with those reported by **Abd El-Baki et al (1988) and Khattab et al (2009).** However, **Zouagui et al (2017)** indicated that utilizing the anti-mycotoxin product (yeast enzymes and clays minerals mixture) had a positive and significant impact on the transaminase activities, liver function of treated animals and total protein, whereas, a significant reduce on reduce of blood urea and total bilirubin were recorded. They attributed these outcomes to the binding properties to mycotoxins of the clay and of the inactivated bacteria and yeast present in the utilized anti-mycotoxin product.

Table 2. Effect of supplemented ZAD probiotic and T5X antitoxin on blood parameters of lactating cows

Parameter	Diets			SEM	*P* value
	Control	ZAD	T5X		
Total proteins (g/dL)	4.13	4.61	4.99	0.289	0.165
Albumin (g/dL)	2.73	2.68	2.68	0.127	0.959
Globulin (g/dL)	1.40	1.93	2.31	0.298	0.191
Albumin/globulin	1.95	1.39	1.16	0.214	0.148
Urea-N (mg/dL)	14.3	16.5	14.4	1.34	0.464
Glucose (mg/dL)	70.8	67.2	67.5	6.54	0.911
Total lipids (mg/dL)	215	315	291	31.0	0.114
Alanin aminotransferase (ALT) (Units/L)	39.5	42.1	40.9	2.22	0.714
Aspartate aminotransferase (AST) (Units/L)	41.8	41.9	39.1	1.23	0.254

Milk yield and composition

Table (3) presented the impact of ZAD probiotic and T5X antitoxins agents on milk yield and milk composition of lactating cows. The outcomes obviously administrated that milk yield was significantly raised by ZAD treatment by 11.2%, 12.9% than control and T5X treatment, respectively, however, no significant difference was shown between T5X treatment and control. Also, milk components yield (Kg/d) were calculated. Milk total solids yield, solids not fat yield, fat yield, protein yield and lactose yield were significantly raised by ZAD probiotic treatment, however, it reduced significantly by T5X treatment. Milk energy output (MJ/d) was, also, raised by ZAD probiotic treatment but it negatively influenced by T5X treatment comparing to control and the differences were significant. Mycotoxins are known to influence dairy cow performances, involving milk production **(Zouagui et al 2017)**. Utilizing antitoxins agents can be help to overcome these negative impacts via inhibiting mycotoxins actions. The outcomes of recent study reported that ZAD probiotic has a potential to inhibit mycotoxins activity as well as raising nutrient intake and digestibility, extent of ruminal fermentation and microbial protein synthesis**,** Consistent findings were gained by **Gado et al (2011)** who pointed that ZAD probiotic improve the nutrient digestibility and feed conversion which will led to rais milk production. In addition to, **Zouagui et al (2017)** utilized clay and the inactivated bacteria and yeasts utilized as ingredients of the anti-mycotoxin mixture and they found that within the peak of lactation, the treated cows produced an average of 32.9 liters per day of milk against 23 liters for the control cows. In contrary, utilizing antitoxin T5X in this study didn't impact milk yield (table 3), which consistent with the findings gained by various studies showed no impact on milk yield and milk components when they utilized different materials and clays as antitoxins agents **(Kutz *et al* 2009, Queiroz et al 2012 and Xiong et al 2015).**

Table 3. Effect of ZAD probiotic and T5X antitoxins agents on milk yield and milk composition of lactating cows

Item	Diets			SEM	*P* value
	Control	ZAD	T5X		
Milk production (kg/d)					
Yield	20.4^{b}	22.7^{a}	20.1^{b}	0.30	<0.001
Energy corrected milk	21.5^{a}	20.3^{b}	17.8^{c}	0.28	<0.001
Milk components (g/kg)					
Total solids	120.1^{a}	114.4^{ab}	108.0^{b}	1.86	0.044
Solids not fat	80.5	75.8	73.0	1.36	0.065
Fat	39.6^{a}	38.6^{ab}	35.0^{b}	0.69	0.035
Protein	36.0	34.1	32.0	0.82	0.090
Lactose	43.3	39.6	39.0	0.77	0.053
Milk energy content (MJ/kg)	2.92^{b}	3.08^{a}	2.72^{c}	0.033	<0.001
Milk components yield (kg/d)					
Total solids	2.45^{b}	2.59^{a}	2.17^{c}	0.034	<0.001
Solids not fat	1.64^{b}	1.72^{a}	1.46^{c}	0.023	<0.001
Fat	0.81^{b}	0.87^{a}	0.71^{c}	0.011	<0.001
Protein	0.73^{b}	0.77^{a}	0.64^{c}	0.010	<0.001
Lactose	0.88^{a}	0.90^{a}	0.78^{b}	0.012	<0.001
Milk energy output (MJ/d)	0.063^{b}	0.066^{a}	0.055^{c}	0.001	<0.001

a,b,c: means with different letters differ significantly. (p<0.01)

Antitoxins activity

Data in **Table (4)** presented Probiotic ZAD antitoxic activity comparing to T5X antitoxins product results

The findings obviously administrate that ZAD probiotic has a positive and significant impact on both total aflatoxins and AFM1. Their concentration was reduced by 50% and 26.6% for ZAD and T5X treatments than control, respectively. Aflatoxins are secondary metabolites, produced by some species of the genus Aspergillus fungus that contaminate plants and plant products utilized in animals feeding **(Cheraghali et al 2007, Iqbal et al 2010, Xiong et al 2015).** Aflatoxin B1 is one of the most dangerous aflatoxins. Itis metabolized in animals' rumen to Aflatoxin M1. Different, physical, biological and chemical treatments are utilized to reduce transferring aflatoxins from feed to milk.

According to the outcomes of recent study, ZAD probiotic as one of biological treatments has a potential effect to lessen the aflatoxins from aflatoxins contaminated feed to milk. These outcomes in consistent with those reported by **Zouagui et al (2017)** who reported that utilizing antitoxin containing inactivated bacteria and yeast reduced the transferring aflatoxin B1 to milk as AFM1.

Table 4. Effect of ZAD probiotic and T5X antitoxin supplementation on TMR total aflatoxins content and milk AFM1 content.

Mycotoxin	Treatment			SEM	*P* value
	Control	ZAD	T5X		
Total aflatoxins (ppm)	22.02	14.84	16.70		0.017
AFM1 (ppm)	17.52	8.76	12.85	0.77	

Reproduction performance

The findings in **Table (5)** presented the effect of probiotic ZAD and T5X antitoxins supplemented to lactating cow's diets on some reproductive parameters.

The findings in table 6 displayed the average required time from calving up to fertilized insemination was reduced by ZAD probiotic treatment, then T5X treatment than control but the differences were not significant. The overall pregnancy rate was significantly increased by antitoxins treatments than control. The highest value was attained by ZAD probiotic treatment and it was 69.2%. These findings are in agreement with those reported by **Zouagui et al (2017)** who reported that the time-intervals between calving and first artificial insemination (AI) and between calving and fertilizing AI revealed that these times were shorter for cows got diets supplemented with antitoxins containing inactivated bacteria and yeasts with clays. Also, in this study treated cows also had the highest overall pregnancy rate (50% against 27.2% for the control group). They attributed the poor reproductive performances in non-treated cows to zearalenone aflatoxin present in the mixed feeding ration. Zearalenone is a major toxin produced by the Fusarium molds and its chemical structure is similar to that of the estrogen hormones and this chemical structure is well known by its estrogenic activities**.** So, it causes several reproductive disorders and different modifications at the genital organs, mainly when its concentration in feed is near to 400 ppb **(Whitlow and Hagler, 2000).** Depending on this interpretation, the findings of raising pregnancy rate with ZAD probiotic treatment in the recent study may give an initial indication about the role of ZAD probiotic on inhibiting zearalenone toxin. This relation should be investigated within further studies.

Table 5. Effect of ZAD probiotic and T5X supplementation on some reproduction parameters of lactating cows

Item	Treatment			SEM	*P* value
	Control	ZAD	T5X		
Calving.-FAI (days)*	77.7	66.3	73.8	7.93	0.592
Pregnancy rate (%)	38.5^c	69.2^a	53.8^b	1.39	0.037

Calving.-FAI (days)* Time-interval between calving and fertilizing artificial insemination

a,b,c: means with different letters differ significantly. ($p<0.01$).

CONCLUSION

The outcomes of recent study could assure the outcomes gained by various studies reporting that different species of probiotic bacteria has a potential to have antitoxins activity as well as its a key role in improving the productive and reproductive performance of dairy cows.

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تأثير إضافات البروبايوتيك اللاهوائي مع / أو مضاد السموم البيولوجي علي الآداء الإنتاجي والتناسلي للأبقار الحلابة

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الموجـــــــز

تم استخدام ثماني وأربعين من الابقار الهولشتين فريزيان لتقييم تأثير اضافة البروبايوتيك ZAD (خليط من الخلايا البكتيرية الحيه والانزيمات) مقارنه مع T5X (منتج حيوى مضاد للسموم) علي بعض مقاييس النشاط الانتاجي والتناسلي والنشاط المضاد للسموم. تم تقسيم الحيوانات الي ثلاث مجموعات تجريبية (16 رأس لكل مجموعه). المجموعة الأولى:- للمقارنة (الكنترول)، تم تغذية عليقة المحطة بدون اي اضافات. المجموعة الثانية:-تم تغذية عليقة الكنترول مع اضافة ZAD بروبايوتيك (1,1 لتر/ طن). المجموعة الثالثة تم تغذية الكنترول مع اضافة T5X كواقع (1,1 كجم/طن). تم تسجيل انتاج الحليب وتجميع عينات لبن ودم وعلف وتحليل مكوناتها. تم تسجيل الفترة بين ولادتين والتلقيح الاصطناعي ومعدل الحمل. اظهرت النتائج التي تم الحصول عليها أن إنتاج اللبن زاد معنوياً بالمعاملة بـ ZAD عن المعاملات الأخرى. تم زيادة مكونات اللبن معنويا في معاملة ZAD وT5X عن المجموعة الأولى. لم يتأثر البروتين الكلي لمصل اللبن والالبيومين والجلوبيولين والجلوكوز واليوريا والدهون الكلية للمعاملة. انخفاض إجمالي الافلاتوكسين في العليقة وافلاتوكسين M1 في اللبن بشكل كبير في ZAD وT5X عن المجموعة الأولى غير المعاملة. انخفاض عدد الايام بين الولادة والتلقيح الاصطناعي في المعاملات عن المجموعة الأولى. زيادة معنويه في معدل الحمل في معاملة ZAD وT5X والمجموعة الأولى علي التوالي. يمكن الاستنتاج أن ZAD يمكن ان يكون له نشاط مضاد للسموم مما يؤدي الي تحسين الاداء الانتاجي والتناسلي للأبقار الحلابة.

الكلمات الدالة: بروبايوتيك، مضادات السموم، محصول اللبن، الأداء التناسلي، الدم

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