IMPACT OF USING SOME BINDING MATERIALS TO REDUCE NEGATIVE EFFECTS OF MYCOTOXINS IN LACTATING ANIMAL DIETS

BY

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B.Sc. Agric. Sc. (Animal and Poultry production), Alex. Univ., 2003
M.Sc. Agric. Sc. (Animal nutrition), Ain Shams University, 2009

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ABSTRACT

Gouda Abd El-Haleam Gouda. Impact of Using Some Binding Materials to Reduce Negative Effects of Mycotoxins in Lactating Animal Diets. Unpublished Ph.D. Thesis Dissertation, Dept. of Animal Production, Fac. Of Agric., Ain Shams Univ., 2016.

In vitro, Four concentrations of each of four types of sorbent materials; (hydrated sodium calcium aluminosilicates (HSCAS), bentonite, montmorillonite and zeolite) (i.e., 5, I0, 20 and 40 mg/ml) were individually weighted into glass tubes (three replicates per sample) and the amount of aflatoxin B_1 (AFB_1) (0.5, 10 and 50 ppb) in aqueous solution were separately added. Also, two concentrations of each of two types of clay minerals (bentonite and montmorillonite) (i.e., I0 and 20 mg/ml) were individually weighted into glass tubes (three replicates per sample) and the amount of zearalenone (ZEN) (0.5 and 50 ppm) in aqueous solution were separately added. *In vivo,* fifteen lactating crossed (Nubian x Baladi) goats of about 2-2.5 years old with an average body weight of about 19-21 kg in the first week of lactation were used in the presented study. The animals were randomly assigned among three experimental treatments (five animals each) using repeated measurement design. The whole feeding period of this trial was 90 days divided into three experimental periods each of 30 days. Each period consisted of 27 days preliminary period followed by a period of 3 days for collection of the experimental samples. Animals were fed three experimental treatments: Control: 60% concentrate feed mixture (CFM) + 40% berseem hay. Bentonite: 60% concentrate feed mixture (CFM) + 40% berseem hay + 2% Bentonite clay as a percentage from concentrates. Montmorillonite: 60% concentrate feed mixture (CFM) + 40% berseem hay + 2% Montmorillonite clay as a percentage from concentrates.

In vitro results indicated that HSCAS showed the best results to adsorb AFB1 in aqueous solution typical to the results reported in the literature. Both Egyptian montmorillonite (EM) and Egyptian bentonite

(EB) had a high affinity to adsorb aflatoxin B_1 or zearalenone in vitro followed to HSCAS. These results revealed that these binding agents are promise candidates to be used in the chemoprevention against AFB_1 and ZEN in the contaminated animal feed when use at a dose as low as 2% (w/w). *In vivo* results showed that there were significant increase ($P \leq 0.05$) of DMI by using bentonite than the control group, while in montmorillonite group the dry matter intake (DMI) increased insignificantly ($P > 0.05$) than control and there were no significance ($P > 0.05$) between bentonite and montmorillonite groups in DMI. For the other nutrients; organic matter intake (OMI), crude protein intake (CPI), crude fiber intake (CFI), ether extract intake (EEI), nitrogen free extract intake (NFEI), neutral detergent fiber intake (NDFI) and acid detergent fiber intake (ADFI); individually, there were no significant differences ($P > 0.05$) among groups. There were significant increase ($P \leq 0.05$) of the digestibility of DM, OM, CP, CF, EE, NFE and NDF by using montmorillonite than bentonite than control group, respectively. However, the digestibility of ADF increased significantly ($P \leq 0.05$) using montmorillonite than control and insignificantly ($P > 0.05$) neither between bentonite and control nor between bentonite and montmorillonite groups. Bentonite treatment had no significant effects ($P > 0.05$) on total digestible nutrients (TDN), digestible energy (DE), metabolizable energy (ME) or the net energy required for lactation (NE_L) but montmorillonite treatment recorded the highest values ($P \leq 0.05$) compared with other groups. Treatments increased digestible crude protein (DCP) significantly ($P \leq 0.05$) than control by 1.48 and 4.74 % for bentonite and montmorillonite treatments, respectively. Feed efficiency (energy corrected milk (ECM)/DMI) was higher in montmorillonite supplemented group and lower in bentonite supplemented group compared to the control one.

Bentonite ration recorded the highest concentration of ruminal ammonia-N followed by control ration whereas montmorillonite ration recorded the lowest concentration ($P \leq 0.05$). The treatment rations

recorded higher ($P \leq 0.05$) content of total nitrogen (TN) than control ration. Control ration recorded the highest total volatile fatty acids (TVFA's) concentration ($P \leq 0.05$) followed by montmorillonite and then bentonite at zero time. While, at 3 hrs. post feeding and 6 hrs. post feeding montmorillonite ration recorded the highest concentration ($P \leq 0.05$) followed with bentonite then control. The respective values of rumen pH were 6.86, 7.13 and 6.48 for Control, bentonite and montmorillonite, respectively at zero time. The control groups recorded higher concentrations of AFB_1 than the treatment groups with significant differences ($P \leq 0.05$). Differences were not significant ($P > 0.05$) between the bentonite and montmorillonite groups. The bentonite group recorded lowest concentrations ($P \leq 0.05$) of ZEN compared to others. Differences between montmorillonite and control group were not significant ($P > 0.05$).

There were no negative effects of these additives on blood parameters (total protein, albumin, globulin, albumin: globulin ratio (A/G ratio), transaminases (AST, ALT), urea, creatinine, glucose, triglycerides, cholesterol and blood minerals (calcium, sodium, and potassium)). Montmorillonite or bentonite had beneficial effects on milk yield, energy corrected milk (ECM), total solids, fat, solids not fat, total protein, lactose and ash contents. The concentrations of aflatoxin M_1 (AFM_1) in milk at the beginning of the experiment (M0) were higher than the maximum permissible limits in milk of all animals. The concentration of AFM_1 in bentonite group has been decreased from 0.080 to 0.063 μg/L in M1 and 0.010 μg/L in M2 and not detected in M3. However, AFM_1 not detected in the group of montmorillonite after one month of treatment (M1). AFM_1 in control group has been increased gradually with time and recorded as 0.073, 0.116 and 0.157 μg/L at M1, M2 and M3 respectively. ZEN not detected in milk of all treatment groups even in control group.

In conclusion, the results of the *in vitro* study indicated that HSCAS showed the best results to adsorb AFB1 in aqueous solution typical to the results reported in the literature. Both EM and EB had a

high affinity to adsorb aflatoxin B_1 or zearalenone in vitro followed to HSCAS. These results revealed that these binding agents are promise candidates to be used in the chemoprevention against AFB_1 and ZEN in the contaminated animal feed when use at a dose as low as 2% (w/w). The *in vivo* evaluation of adding bentonite and montmorillonite at 2% of concentrates in lactating goats ration on the performance, rumen parameters, plasma blood chemistry or mycotoxin excretion in milk. It could be concluded that the addition of bentonite or montmorillonite to the ration had beneficial effects on the productive performance, no negative effects on rumen parameters or blood chemistry and significant reduction in AFB_1 excretion in milk of lactating goats under the field condition in Egypt.

Key words:

Aflatoxin B_1; zearalenone; montmorillonite; bentonite; HSCAS; zeolite; sorbent materials, lactating goats, nutrient digestibility, rumen parameters, blood plasma, milk production.

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LIST OF CONTENTS

LIST OF ABBREVIATIONS

Abbreviation	Mean
µg	Microgram
ACN	Acetonitrile
ADF	Acid detergent fiber
AFB_1	Aflatoxin B_1
AFB_2	Aflatoxin B_2
AFG_1	Aflatoxin G_1
AFG_2	Aflatoxin G_2
AFM_1	Aflatoxin M_1
AFM_2	Aflatoxin M_2
AFs	Aflatoxins
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATA	Alimentary Toxic Aleukia
CAST	Council for Agricultural Science and Technology
CDC	Centers for Disease Control and Prevention
CF	Crude fiber
CFM	Concentrate feed mixture
CH_2Cl_2	Di-Chloro methane
$CHCL_3$	Chloroform
CP	Crude protein

Da	Dalton (formerly atomic mass unit)
DCP	Digestible crude protein
DE	Digestible energy
DM	Dry matter
DMI	Dry matter intake
DNA	Deoxi Ribo Nucleic Acid
DON	Deoxynivelenol
EB	Egyptian bentonite
EC	European Commission
ECM	Energy corrected milk
EDTA	Ethylene Diamine Tetra-Acetic acid
EE	Ether extract
EFSA	European Food Safety Authority
ELISA	Enzme linked immunosorbent assays
EM	Egyptian montmorillonite
ERs	Estrogen receptors
FAO	Food and Agriculture Organization of the United Nations
FB_1	Fumonisin B_1
FCM	Fat corrected milk
FDA	Food and Drug Administration
FEEDAP	Panel on Additives and Products or Substances used in animal feed
FSA	Food Standards Agency

GOT	Glutamic oxalo acetic transaminase
GPT	Glutamic pyruvic transaminase
Hb	Hemoglobin
Hct	Hematocrit
HPLC	High Performance Liquid Chromatograghy
HSCAS	Hydrated Sodium Calcium Alumino Silicates
IARC	International Agency for Research on Cancer
kg	Kilogram
LDH	Lactic dehydrogenase
MCHC	Mean corpuscular hemoglobin concentration
ME	Metabolizable energy
MeOH	Methanole
mg	Milligram
MMN	Modified montmorillonite nano- composite
MOS	Mannan oligosaccharides
mRNA	Messenger Ribo Nucleic Acid
Na_2SO_4	Sodium Sulfate
NaCl	Sodium chloride
NaOH	Sodium Hydroxide
NDF	Neutral detergent fiber
NE_L	The net energy required for lactation
NFE	Nitrogen free extract