DETERMINATION THE EFFECTS OF VARYING LEVELS OF WHOLE COTTONSEED ON GOSSYPOL PLASMA AND PRODUCTION PARAMETERS IN EARLY LACTATING HOLSTEIN COWS

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Abstract

A study was carried out to determine the effects of varying levels of whole cottonseed on gossypol plasma and production parameters in early lactating Holstein cows. Sixteen early lactating Holstein cows (DIM=39±5) were used in Latin Square design with 4 block and 4 repeats. Animals in each group fed 1 of 4 experimental ration containing (0, 7, 14 and 21 % Whole cottonseed on dry matter base).The diets were similar as crude protein and NE_L on dry basis. Cows were fed with total mixed ration individually. Plasma Gossypol measurement was shown that diets were significant different ($P<0.05$), so that Plasma gossypol was higher for cows fed high whole cottonseed (14 and 20 % whole cottonseed) diets. Plasma gossypol of cows fed whole cottonseed were shown negative effects on milk fat and protein percentage and yields, but no effects on Dry matter intake and Milk production.

Key words: Early lactation, Holstein cows, Whole cottonseed, plasma gossypol, animal performance

Abbreviation Key:, WCS = whole cottonseed, BUN = blood urea nitrogen, TG = total gossypol, FG =free gossypol control, B, C, D diets respectively diets containing 0, 7, 14, 21 percentage WCS

INTRODUCTION

Whole cottonseed is a multinutrients feed that is extensively used as an energy and protein source in dairy cattle diets. It provides a unique blend of protein, energy and fiber compared with other feedstuffs (6, 8, 18, 23, 25). It is very importance in early lactation period that we have challenge with limit appetite and DMI and increasing energy demand for cows.

However whole cottonseed contains gossypol, a polyphenolic yellow pigment present in cotton plants, as a natural defense mechanism against in seed pets (2, 3, 6, 25). At least 15 gossypol pigments or derivatives have been isolated from extracts of WCS or cottonseed meal. The predominant, naturally occurring pigment ($C_{30}H_{30}O_8$) is a yellow highly reactive compound the exhibits acidic, phenolic and aldehydic properties (2, 7, 30).

In the intact whole cottonseed, gossypol is mostly found as free gossypol (30) .However, when cottonseed is processed, gossypol binds to proteins, possibly to lysine (2, 5). In addition to the free and bond forms, 2 distinct stero- isomers of gossypol occur in whole cotton seed, the plus (+) and minus (-) isomer (18, 22). These isomers appear to have different bioactivities and retained in body for longer period of time (16, 17). The minus (-) isomer appears to have higher biological activity (20). Generally in whole cottonseed , there is higher concentration of (-) isomer. Non- ruminants and young ruminant are risk to gossypol toxity. Mature ruminants, which process a functioning rumen, can detoxify gossypol and ability to metabolically tolerate gossypol coincides with the development of rumen function (20, 30).

It was long believed that the mechanism of gossypol detoxification by ruminant was by its binding to soluble protein and that the bond was resistant to enzymatic digestion (18, 20). However, reports of toxicity in mature cow, suggests that the capacity of rumen to detoxify gossypol can be exceeded

(22, 30). It is possible that feeding excessive amount of the toxin in the free form may overcome this protective mechanism and impair animal performance (17).

Feeding recommendations for cottonseed products have been based on nutrient content feeding levels of free and total gossypol in diets for lactating dairy cows. Also the effects of gossypol on males are well-known. However gossypol effects on female especially ruminant females (production performance), remain somewhat undefined (7)

The objective of this study was to determine the effect of feeding varying amount of whole cottonseed on plasma gossypol concentration and production performance in early lactating Holstein cows.

MATERIAL AND METHODS

Animals and Feeding: A sixteen multiparous Holstein cows in early lactation (DIM=39±5) were selected from Rezaei`s farm house in Karaj, Iran for 84-d experiment in which varying amounts of wcs were fed. Cows were assigned to 4 treatment diets (4 cows per diets). Periods were 21-d, the first 14-d were used an adaptation, and last 7-d were used for sampling. Composition of experimental diets is shown in table 1 and 2. Diets were fed as TMR and varied in amount of whole cottonseed (gossypol fed). Diets were similar in nutrients content and met the requirements for lactating Holstein cows (19).

Cows were fed TMR in two separate feeding at 0800 and 1600 prepared daily by hand mixing in manger and milked 3th daily (0400, 1200 and 1700) with milk yield measured and recorded at each milking. Milk samples were taken during 15-d and 17-d. Samples analyzed for milk fat, milk protein, milk lactose and milk SNF by Milko Scan (Milko Scan 134 A/B). Feed offered and refused were recorded daily to adjust feed offered for 10 % refusal.

Table 1. Ingredient composition of diets fed to cows during experimental periods diets

Ingredient	Diet[1]			
	control	B	C	D
Alfalfa hay	22.50	22.50	22	20.5
Corn silage	13.70	13.50	13.70	13.50
Corn gluten	4.50	4.50	4.10	3.70
Barley	27.50	27.50	22	20.50
Wheat bran	19.50	15.80	13.70	13.50
Whole cottonseed	-	7	14	21
Soybean meal	10.50	9	6.80	4.50
Calcium carbonate	0.9	0.9	1	1.5
Sodium bicarbonate	0.5	0.5	0.5	0.5
Vitamins A, D and E[2]	0.25	0.25	0.25	0.25
Nacl salt	0.25	0.25	0.25	0.25

[1] control = diet without WCS, B = diet containing 7 % WCS, C = diet containing 14 % WCS, D = diet containing 21 % WCS.

[2] contained 20,000 IU of vitamin A/kg, 6,235 IU of vitamin D/kg, and 98 IU of vitamin E/kg.

Body weights were recorded in beginning and final of each period after a.m. milking. Body condition score was recorded at beginning of experiment and then a beginning and final periods.

Laboratory Analysis: Weekly samples of feed offered were dried in a forced air oven at $100^{\circ C}$ to determine DMI. Samples were ground through a 2-mm screen indweller Mill (TOSHIBA, IRAN). Air dried samples were analyzed for DM, ash, OM, crude protein and ether extract according to methods of AOAC (1), Neutral Detergent Fiber, NDF, was determined according to the procedure Van soest (28). Blood samples were drawn from coccygeal vein of each animals into 10 ML vein inject tubes containing sodium Heparin (pars Darrow, Iran). Samples were obtained between 3h after morning feeding with vein inject tubes.

Table 2. Chemical composition of diets fed to cows during experimental periods

Item	Diet			
	control	B	C	D
Ash	6.1	6.4	5.9	5.5
NE_L, Mcal/kg of DM	1.65	1.64	1.66	1.66
CP	16	16	16	16
By pass protein(% of CP)	30.9	30.2	31.8	32.9
NDF	35	34	36.5	38.3
ADF	18.5	19.9	24.4	25
NFC[1]	38.9	40.8	36.5	33
FAT	3.7	2.6	4.9	6.2
Ca	0.91	0.87	0.85	0.98
P	0.45	0.47	0.49	0.51
Mg	0.28	0.30	0.31	0.33

[1] Nonfiber carbohydrate calculated by difference 100 – (%NDF + %CP + %Ash +%EE).

Blood samples were placed in ice immediately after collection, kept out of light, and transported to the laboratory within 5 min where samples were centrifuged at 3000 × g for 10 min. Plasma was recovered into a 5-ml screw cap mailer tube and stored at −20°C for subsequent gossypol analysis.

Samples were analyzed for plasma glucose, BUN, TCP, Ca, P, Mg and cholesterol. Samples of cottonseed were analyzed for TG and gossypol isomers by HPLC (15) and for free and TG by AOCS official methods (14, 15).

Whole cottonseeds were analyzed for FG and TG content. The Official Methods of the American Oil Chemists Society were used to determine FG (14) and TG (15) in WCS. Cottonseeds were decorticated before analysis, and the actual analyses were conducted on decorticated seed. High performance liquid chromatography was used to quantify the isomers of gossypol in WCS and plasma samples (4, 14). The HPLC procedure involved extraction of gossypol from WCS, or lyophilized plasma and formation of the 2-amino-1-propanol derivatives of (+) and (−) gossypol (4 ml). This was accomplished by heating the sample (98°C, 30min) in a complexing reagent consisting of 10% glacial acetic acid and 2% (R)-(−)-2-amino-1-propanol in N,Ndimethyl fornamide. The 2-amino-1-propanol derivatives were separated on a 4- × 100-mm Inertsil C18reverse-phase column operated isocratically with a mobile phase (87% methanol, 20% 10 mM KH2PO4 adjusted to pH 3.0 with H3PO4) with electronic detector (LKB, 2143SWEDEN) a flow rate of 1.0 ml/ min. Retention times were 2.3 and 3.8 min for the 2-amino-1-propanol derivatives of (+) and (−) gossypol isomers, respectively. Gossypol acetic acid containing 50%(+) and 50% (−) gossypol isomers was used as a standard. HPLC model is 1200Serirs,Ajilent Technology.

Statistical Analysis

Data were analyzed by covariance analysis. Data were analyzed by ANOVA including period, treatment, square and cow with in square using the general linear model procedures of SAS. Type III sums of squares were used and the residual served as error term for all tests.

The experimental design was a Latin Square design. Within treatment, cows were blocked according to DIM and milk production.

The following model was used:

$$Y_{ijkl} = \mu + T_i + P_j + B_k + B(k)_L + E_{ijkl}$$

Where: Yijkl = observation, μ = overall mean, Ti = treatment effect, Pj = period effect, Bk = square effect, B(k)l = cow with in square and Eijkl = residual effect

All means are least square means and differences were reported as significant when

$P < 0.05$. Differences among treatment mean for significant Effects were determined using the Duncan multiple range test .

RESULT AND DISCUSSION

The mean of different traits compartment are shown table 3.Diets were formulated to be isonitrogenous with 16%CP, isoenergetic with 1/65 mcal NE_L and to differ in their content of TG and FG from WCS. As expected, TG and FG intakes differed among dietary treatments, reflecting The different concentrations of FG and TG in the diets. The amount of TG consumed by cows increased as the Amount of TG from WCS in the diet increased. Intakes of DM did not differ among diets for cows (Table 3), and Yields of milk and 4% FCM did not differ among treatments (Table 3), and efficiency of utilization of NEL intake for milk production was similar across treatments. Feeding cottonseed in dairy rations do not alter DM intake of lactating cows. Coppock et al. (9) concluded that whole cottonseed can be added up to 25% of the Diet DM with no effects on intake. When the fiber content of the diet is maintained constant, adding whole cottonseed to the diet of lactating cows at 10 to 20% has usually no effect on DM intake. This is extremely important since nutrient intake is the driving force for yields of milk and milk components. In present study , similar Forage among diets was caused DMI hasn't difference. Main factor is determined rumen capacity is milk production (29). In results, diets have similar Milk production therefore another reason for similar DMI result among diets was it. Milk production was not different ($P > 0.10$) for cows fed treatment diets.

Similar milk production with increase WCS would be related similar DMI and NE_L density in diets. The results of study were different with previous studies (8, 10, 26, 27).

Table 3. Least square mean of performances of cows which were fed experimental diets

Item	Diet				SEM	effect	
	control	B	C	D		Diets	Period
DMI, kg/d	21.87	21.81	22.15	21.91	0.13	ns	0.4226
Milk, kg/d	33.47	33.40	33.84	33.08	0.32	ns	0.2374
Milk [1],kg/d	30.73	30.72	29.98	29.54	0.31	ns	0.44
Milk fat,%	3.44[a]	3.47[a]	3.21[b]	3.23[b]	0.026	0.0001	0.0001
Milk fat yield, kg/d	1.15	1.15	1.09	1.08	0.013	0.069	0.468
Milk protein,%	3.10[a]	3.07[a]	3.01[b]	2.93[b]	0.0194	0.021	0.492
Milk protein yield, kg/d	1.04	0.99	1.02	0.98	0.011	ns	0.55
Milk lactose,%	5.63	5.64	5.60	5.64	0.0199	ns	ns
Milk lactose yield,kg/d	1.88	1.88	1.89	1.86	0.02	ns	ns
BW change, kg/d	0.10[a]	0.12[a]	0.15[b]	0.19[b]	1.52	0.001	0.001
BCS[3] change	0.11[a]	0.20[b]	0.18[b]	0.21[b]	1.22	0.0001	0.012

a,b,c Means in row with different superscripts differ (p< 0.05).
n.s. = non significant
[1] Fat corrected milk for 4% fat(FCM)
[2] Milk productions per kg DMI
[3] body condition scores(five point scale where 1 = thin to 5 = fat)

In most studies (6, 9, 13), wcs was used as nonforage fiber source, while in current study forage percentage was constants in all diets. Therefore it can be concluded effect of WCS on production performance is function of forage percentage in rations.

Milk fat concentration was highest for second diet and was different significantly (P<0.05) among the diets and periods. Increasing TG and FG in diets 3 and 4 ,have negative effect on rumen fermentation and in result diets 3 and 4 indicated decreasing milk fat synthesis. A comprehensive review of the effects of whole cottonseed on lactation of dairy cows showed that including 10 to 30% of the diet as whole cottonseed increased milk fat content in 4 out of 13 trials (9). In general, adding whole cottonseed to the diet of lactating cows has minimal effect on milk fat content. Some have suggested that addition of whole cottonseed to diets high in corn silage can be 100 detrimental to milk fat content (25). This negative effect may be related to the fat content of the diet, as well as the availability of physically effective fiber in high corn silage diets.

Milk protein concentration was highest for Control diet and was different significantly (P<0.05) among the diets. When cottonseed is added to the diet of lactating cows and the fat content of the ration increases, usually a milk protein content depression is observed (9). However, yields of milk protein and fat change with addition of cottonseed to the ration. The effect of whole cottonseed on milk protein is usually negative when corn silage is the main forage. In early lactation cows, most factors that limited milk protein synthesis is lysine. Also in gossypol detoxification proccess, lysine is used (2, 30), therefore decreasing milk protein was predicted. Results of this experiment agreement with studies of coppeck (10) Clark and Armentano (6) and smith (26).

Table 4. Least square mean of Plasma metabolites of cows which were fed experimental diets

Item	Diet				SEM	effect	
	control	B	C	D		Diets	Period
Plasma Gossypol							
Total	0.08[a]	2.65[b]	3.01[c]	4.31[d]	0.23	0.0001	0.0018
Gossypol,mg/ml							
(+) Isomer, mg/ml	0.03[a]	1.39[b]	1.23[c]	2.52[d]	0.29	0.0094	0.0990
(-) Isomer, mg/ml	0.06[a]	1.71[b]	1.78[c]	2.81[d]	0.06	0.0001	0.0001
Plasma metabolites							
Ca, mg/d lit	12.37	12.57	12.66	14.37	0.53	0.3240	ns
P, mg/d lit	8.86[ab]	8.44[a]	9.81[ab]	10.74[b]	0.29	0.0094	0.0990
Mg, mg/d lit	2.25	2.17	2.05	2.21	0.06	ns	0.0001
Cholesterol, mg/dlit	228.56[a]	237.20[ab]	291.44[ab]	313[b]	26.5	0.0130	0.0010
BUN, mg/d lit	15.74[a]	17.15[ab]	17.33a[b]	18.35[b]	0.33	0.0612	0.0071

a,b,c Means in row with different superscripts differ (p< 0.05).
n.s. = non significant

Prieto et al. (20) observed no negative effect of increasing dietary gossypol by adding cracked Pima cottonseed to the diet on yields of milk and milk components.

Plasma metabolites concentrations hasn't significantly difference exception of BUN and cholesterol (table 4) increasing BUN with increasing WCS percentage of diets may have affected high rumen degradable CP of WCS, concentration ammonia in rumen or decrease microbial protein synthesis. Study (12) was shown diets containing WCS have higher BUN(20%) rather than control diet.

Plasma gossypol concentrations differed according to treatments, and gossypol intake increased mean PG concentrations during the 84-d study. Plasma gossypol concentrations directly reflect gossypol absorption in the gut and metabolism by the liver (23,25). When cottonseed is fed, gossypol in the free form that escapes detoxification within the fore stomachs is available for absorption in the small intestine. It is assumed that binding of gossypol to amino acids of dietary and bacterial 96 origins reduces gossypol availability. When cottonseed escapes rumen digestion, plasma gossypol concentrations increase dramatically. Mena et al. (17) fed lactating Holstein cows varying amounts of gossypol from whole linted Upland cottonseed or cottonseed meal. Gossypol in cottonseed is mostly in the free form and gossypol in cottonseed meal is mostly in the bound form. Plasma gossypol concentrations were directly related to intake of FG. Most plasma TG concentrations were well below the proposed safe upper limit of 5 μg/ml (4) no symptoms of gossypol toxicity such as decreased feed intake or milk production were observed.

The lack of symptoms of gossypol toxicity suggests that plasma TG concentration alone may be a nice indicator to determine safe levels of cotton seed in diets.

Another point to measure and compare plasma gossypol this study is that gossypol concentration of the fourth period noticeably decreased in some treatments. In other hand, the period effect is significant. The finding of these results concluded rumen micro flora gradually adapted and find more ability for detoxification (24,25).

CONCLUSION

Results of the present research indicate that using whole cottonseed in early lactating cow has benefit results in Body weight and Body condition score without affecting cow health, milk production and DMI. Most plasma TG concentrations were well below the proposed safe upper limit. no symptoms of gossypol toxicity such as decreased feed intake or milk production were observed. There is a linear relation between gossypol intake and plasma gossypol in short period. With previous researches, current Results indicate Amount of forage in diets affects on whole cottonseed efficiency. Intake total gossypol in diets decreasing milk protein percentage.

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REFERENCES

[1] A.O. A.C. 1990. Official methods of analysis. 15th ed. assoc. office. Anal. Chem., Arlington, v. a.

[2] Blauwiekel, R., S. Xu, J. H. Harrison, K. A. Loney, R. E. Riley, and M. C. Calhoun. 1997. Effect of whole cottonseed, gossypol, and ruminally protected lysine supplementation on milk yield and composition. J. Dairy Sci. 80:1358–1365.

[3] Cai, J., H. Zhang, Y. Zeng, et al., 2004. An optimized gossypol high-performance liquid chromatography assay and its application in evaluation of different gland genotypes of cotton. J. Biosci.29:1:67-71

[4] Calhoun, M. C., S. W. Kuhlmann, and B. C. Baldwin, 1995. Assessing the gossypol status of cattle fed cotton products. Proc. Pacific Northwest. Anim. Nutr. Conf. Portland, DR. Washington, state univ. pulman.

[5] Calhoun, M. S., and C. Holmberg, 2001. Availability of gossypol in soap stock fed to lambs. J. Am. oil. Chem. Soc. (abstr).

[6] Clark, P.W., E. Armentano, 1993. Effectivness of Neutral Detergent Fiber in whole cottonseed and dry distiller grain J. Dairy Sci. 76:2651.

[7] Colin-Negrete, J., H. E. Kiesling, T. T. Ross, et al, 1996. Effect of Whole Cottonseed on Serum Constituents, Fragility of Erythrocyte Cells, and Reproduction of Growing Holstein Heifers. J. Dairy Sci. 79:2016-2023

[8] Coppock, C. E., and D. L. Wilks, 1993. Feeding whole cottonseed and cottonseed meal to dairy and beef cattle, Texas. A&M univer. Public.

[9] Coppock, C.E., Lanham, J.K., Horner, J.I., 1987. A review of the nutritive value and utilization of whole cottonseed, cottonseed meal and associated by-products by dairy cattle. Anim. Feed Sci. Technol. 18, 89–129.

[10] Coppock, C.E., West, J., Moya, J.R., Nave, D.H., LaBore, J.M.,Thompson, K.G., Rowe Jr., L.D., Gates, C.E., 1985b. Effects of amount of whole cottonseed on intake, digestibility, and physiological responses of dairy cows. J. Dairy Sci. 68, 2248–2258.

[11] Depeters, E. J., S. J. Taylor, A. A. Frank and A. Agairre, 1985. Effect of feeding whole cottonseed on composition of milk. J. Dairy Sci. 68:897.

[12] Harrison, J. H., P. Kincail, et al, 1995. Effect of whole cottonseed and calcium of long chain fatty acids on performance of lactating dairy cows. J. Dairy Sci.78:181.

[13] Harvatine, D. I., J. L. Winkler, J. L. Firkins, 2001. Quantification of effectiveness of whole linted cottonseed as a forage substitution when fed with corn differing in ruminal starch availability, J. Dairy Sci. 82:213(abstr).

[14] Hron R J, Kuk M S and Abraham G 1990 Determination of gossypol by high performance liquid chromatography; J. Am. Oil Chem. Soc. 67 182–187

[15] Hron Sr R J, Kim H L, Calhoun M C et al 1999 Determination of (+)–, (–)–, and total gossypol in cottonseed by high performance liquid chromatography; J. Am. Oil Chem. Soc.76 1351–1355

[16] Kenar, J.A., 2006. Review Reaction Chemistry of Gossypol and Its Derivatives. JAOCS, Vol. 83, no. 4 (2006)

[17] Mena, H., J.E.P. Santos, J.T. Huber, J.M. Simas, M. Tarazon, and M.C. Calhoun. 2001. The effects of feeding varying amounts of gossypol

from whole cottonseed and cottonseed meal in lactating dairy cows. J. Dairy Sci. 84: 2231-2239.
[18] Mena, H., Santos, J.E.P., Huber, J.T., Tarazon, M., Calhoun, M.C., 2004. The effects of varying gossypol intake from whole cottonseed and cottonseed meal on lactation and blood parameters in lactating dairy cows. J. Dairy Sci. 87, 2506–2518.
[19] NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th Rev, Ed., Natl. Acad. Sci. Washington, DC. Pp.269-302.
[20] Prieto, J.G., E.J. DePeters, P.H. Robinson, J.E.P. Santos, et al. 2003. Increasing dietary levels of processed Pima cottonseed increase plasma gossypol but do not influence productive performance of lactating Holstein cows. J. Dairy Sci. 86: 254-267.
[21] Robinson, J.J., C.J. Ashworth, J.A. Rooke, et al.2006. Nutrition and fertility in ruminant livestock. Animal Feed Science and Technology 126 (2006) 259–276
[22] Rochfort, S., A.J. Parker, F.R.Dunshea.2008. Review Plant bioactive for ruminant health and productivity. Phytochemistry 69 (2008) 299–322
[23] Salehpour, M., M. Hajipour and K. Zand .2010. A book “Principles of animal Husbandry”, (Persian) Sepehr Public., Tehran, Iran
[24] Santos, J.E.P., M. Villasenor, E.J. DePeters, P.H. Robinson, and B.C. Baldwin. 2002. Type of cottonseed and gossypol in diets of lactating dairy cows: Lactation performance and plasma gossypol. J. Dairy Sci. 85:1491-1501.
[25] Santos, J.E.P., M. Villasenor, E.J. DePeters, P.H. Robinson, and C.H. Holmberg. 2003. Type of cottonseed and gossypol in diets of lactating dairy cows: Plasma gossypol, reproduction, and health. J. Dairy Sci. 86:892-905.
[26] Smith, W.A., Vosloo, L.P., 1994a. Effect of heat treatment of whole cottonseed on in vitro, in situ, and in vivo ruminant digestion characteristics. S. Afr. J. Anim. Sci. 24, 39–44.
[27] Smith, W.A., Vosloo, L.P., 1994b. Effect of whole cottonseed, heat treated whole cottonseed and whole cottonseed plus lanolin on milk production and composition. S. Afr. J. Anim. Sci. 24, 45–48.
[28] Vansoest, P.J. J.B.Robertson and B.A.Lewis. 1991. Method, for dietary fiber, neutral detergent fiber, and non Starch polysaccharides in relation to animal nutrition. J. Dairy sci.74:3583.
[29] Varga, G.A., DANN, and E.Ishler .1998.Use of fiber concentration formulation. J. Dairy Sci. 81:3063
[30] Zhang W., Z. Xu, X. Pan et al .2007.A review Article Advances in gossypol toxicity and processing effects of whole cottonseed in dairy cows feeding. Livestock Science 111 (2007) 1–9