Egyptian J. Anim. Prod.(1994) 31(1):149-160.

EFFECT OF FAT SOURCE, LEVEL AND CALCIUM SUPPLEMENTS ON IN VITRO FIBER DIGESTION

Sabbah M. Allam[1], H.M. El-Hussiny[2], T.M. El-Bedawy[1] and F.H. Shahin[2]

1) Department of Animal Production, Faculty of Agriculture, University of Cairo, Giza, Egypt 2) Animal Production Reserach Institute, Ministry of Agriculture, Cairo, Egypt

SUMMARY

Series of in vitro incubations were carried out to study the effect of three sources of fat (sunflower oil, hydrogenated vegetable oil (HVO) and tallow), five levels of supplementations (0, 5, 10, 15 and 20%) with or without 1% calcium addition on in vitro digestibility of DM, OM, NDF, ADF, hemicellulose and cellulose. Data of Seventeen replicates for each cell with a total number of 510 samples were used to calculate IVDMD. Residues from each cell were pooled in two samples for OM, NDF, ADF, cellulose and hemicellulose determination.

Increasing fat level decreased the digestibilities of DM and OM and all cell wall constituents. The decrease in the measured parameters were greater in case of vegtable oil than the other fat sources. Calcium supplement did not improve the in vitro digestibilities. Cellulose digestibility were the most affected parameter by fat supplement. The results might indicate the specific inhibatory effect of fat on cellulytic microorganisms.

Keywords: Fat supplement, calcium addition, in vitro digestibility

INTRODUCTION

High fat supplements were reported to depress in vitro

digestibility (Doreau et al., 1991). However, Bock et al. (1991) observed no effect or an increase (Firkins et al., 1990). The decrease in dry matter digestibility was related to the adverse effect of supplemental fat on fiber digestibility (Ikwuegbu and Sutton 1982), but fiber is not unformed matter includes different components e.g. cellulose, hemicellulose, lignin each has different digestion process in the rumen (Van Soest, 1982). This base could explain the different response of digestion to forage type, fat source and level of supplementation. Use of in vitro technique allows to study the effect of high levels of fat are not applicable in in vivo studies then accurate quantification for the relalionships between fat source; levels and nutrient digestibilities will be possible.

The objectives of this study are to 1) determine what feed component (s) is the most affected by fat supplement; 2) to evaluate the role of calcium addition to allivate the decrease in nutrient digestibilities; 3) to quantify the effect of fat supplements on each nutrient digestibility; 4) to verify the different hypothesis based to explain these effects and 5) to recognize the recommended level of fat from viewpoint of in vitro digestibilitity.

MATERIALS AND METHODS

Series of one-stage in vitro incubation (Tilly and Terry, 1963) in 3-factor factorial design were carried out to study the effect of three sources of fat (sunflower oil, HVO and tallow), four levels of supplementations (5, 10, 15 and 20%) with or without 1% calcium addition on in vitro digestibility of DM, OM, NDF, ADF, hemicellulose and cellulose of berseem hay . Data of Seventeen replicates for each cell with a total number of 510 samples were used to calculate IVDMD. Residues from each cell were pooled in two samples for OM, NDF, ADF, cellulose and hemicellulose determinations.

Substrates were prepared by substituation of hay by fat sources on dry matter basis. Calcium was added in form of calcium carbonate as 2.5% calcium carbonate providing 1% calcium. Rumen fluids were collected from three whethers fed berseem hay to be used in the in

vitro incubations at 39°C for 48 hours.

Chimical composition of substrates and residues were determined according to A.O.A.C (1975) and Goering and Van Soest (1970).

Data collected were statistically analysed using General Linear model (GLM) according to SAS (1986). Results were presented as the change in the in vitro digestibility of each nutrient from its control to avoid the initial differences in the control values.

RESULTS AND DISCUSSION

Chemical composition and in vitro nutrient digestibilities of fat unsupplemented berseem hay with or without 1% calcium are shown in Table 1. Calcium addition decreased the in vitro digestibility of cell wall constituents (IVDCWC) but without remarkable effect on dry matter or organic matter digestibilities.

Table 1. Dry matter composition and average in vitro nutrient digestibilities of berseem hay supplemented with or without 1% calcium

Nutrient	Chemical composition,%		In vitro digestibility,%	
	0% calcium	1% calcium	0% calcium	1% calcium
Dry matter	90.88	89.91	61.20	61.24
Organic matter	87.65	86.20	61.47	60.43
NDF	68.74	63.41	50.87	46.27
ADF	42.52	37.84	44.15	36.02
Cellulose	33.59	30.25	52.94	50.59
Hemicellulose	26.22	25.57	64.48	61.41

Data in Table 2a showed the change in vitro digestibilities of cell wall constituents (NDF, ADF, cellulose and hemicellulose) as a difference from each control value. The analyses of variance of these data are presented in Table 2b.

Effect of fat source on IVDCWC

Data in Table 2 (a & b) showed significant (P=0.0001) difference among the three fat sources in their effect on the in vitro digestibilities of cell wall constituents. Oil and HVO supplements decreased the in vitro digestibilities of NDF, ADF, cellulose and

hemicellulose. However, tallow supplement had no such

Table 2a. Effect of fat source, level and calcium supplements on in vitro change in digestibility of cell wall constituents

Level,%	source	Oil		HVO		Tallow		Level mean
	Ca,%	0	1	0	1	0	1	effect
Neutral detergent fiber, (NDF)								
5		- 6.98	- 2.21	-14.95	- 6.01	0.45	7.34	- 3.37
10		- 2.51	- 8.33	- 9.38	-16.70	- 9.46	9.69	- 6.11
15		-11.18	-23.50	-16.03	-12.73	5.82	13.50	- 7.35
20		-17.20	-14.45	-25.50	-13.45	- 2.70	- 7.21	-11.01
Acid detergent fiber, (ADF)								
5		-12.41	- 2.84	-17.07	- 8.15	- 1.97	8.92	- 2.87
10		-25.15	-11.74	- 3.85	-13.42	- 5.81	16.51	- 7.24
15		-22.20	-22.63	-11.86	- 4.57	18.63	24.56	- 3.01
20		-18.77	-29.83	-22.60	-10.25	- 5.56	- 2.86	-13.13
Cellulose								
5		.24	- 2.62	- 6.62	1.60	- 6.53	3.90	- 2.92
10		-16.04	-13.36	- 2.89	-11.59	-10.30	7.20	- 6.87
15		-25.20	-21.77	-11.60	-11.36	12.84	17.10	- 6.67
20		-28.47	-30.27	-18.57	-14.43	4.71	-12.97	-16.67
Hemicellulose								
5		- 0.69	- 3.05	-12.37	-32.47	5.27	6.14	- 6.20
10		9.10	- 5.50	-17.85	-19.17	-14.26	1.13	- 7.76
15		- 3.29	-16.73	-22.57	-23.47	16.44	- 5.61	- 8.11
20		-12.87	- 1.42	-19.87	-17.41	-16.17	16.21	- 8.59

Table 2 b. ANOVA of Table 2a

Item	S	L	C	SxL	SxC	LxC	SxLxC
NDF							
d.f	2	3	1	6	2	3	6
Prob.	.0001	.0001	.0001	.0001	.0001	.0001	.0001
ADF							
d.f.	2	3	1	6	2	3	6
Prob.	.0001	.0001	.003	.0001	.0690	.0001	.0001
Cellulose							
d.f.	2	3	1	6	2	3	6
Prob.	.0001	.0001	.220	.0001	NS	.024	.0001
Hemicellulose							
d.f.	2	3	1	6	2	3	6
Prob.	.0001	NS	NS	.115	.039	.0001	.014

S=fat source L= fat level C= calcium

effect on these digestibilities. Moreover, limited positive effect was recorded in the IVDCWC due to tallow supplement. The greater depression in IVDCWC by oil compared with HVO and tallow supplements might be due to

that vegetable oil is mostly composed of polyunsaturated long chain fatty acids which have greater inhibitory effect on microbial growth than the saturated fatty acids like in case of tallow supplement (Henderson, 1973) because saturated fatty acids react more readily with cations to form insoluble soap then smaller proportion of free fatty acid molecules are available to inhibit microbial growth and metabolism (Jenkins and Palmquist, 1982).

Cellulose and consequently ADF was the most affected component by oil supplement. The adverse drastic effect was less evident on NDF or DM digestion (Table 3a). This could emphasize that bil has a larger inhibitory action on cellulolytic microbes not on all rumen microflora (Henderson 1973 ; Kowalczyk et al. 1977).

Table 3a. Effect of fat source, level and calcium supplements on in vitro change in dry and organic matter digestion

Level,% source	Oil		HVO		Tallow		Level mean
Ca,%	0	1	0	1	0	1	effect
Dry matter							
5	- 1.67	- 1.89	- 6.55	- 3.83	- 2.05	- 4.43	- 3.40
10	- 9.38	- 8.98	- 9.02	-12.06	-14.83	- 1.03	- 9.22
15	-15.01	-15.87	-12.33	- 9.27	- 4.55	-10.06	-10.06
20	-20.73	-20.57	-12.26	-11.19	-13.63	-18.04	-18.04
Organic matter							
5	- 2.37	- 6.56	- 7.73	- 6.05	- 2.74	- 1.45	- 3.57
10	- 8.28	- 9.59	- 9.03	-12.17	-12.95	0.35	- 8.61
15	-14.30	-17.91	-15.65	-13.00	1.26	- 0.53	-10.02
20	-21.12	-21.53	-25.60	-19.65	-11.55	-11.95	-18.57

Effect of fat supplementary level on IVDCWC

Increasing fat-supplementation level decreased (P=0.0001), in general, the in vitro digestibilities of NDF, ADF and cellulose but not hemicellulose. The decrease in the digestibility had not exceed 7% digestion unit till the supplementary level of 15%. The maximum decrease in the in vitro digestion was observed when 20% fat was applied. Czerkawski and Clapperton (1984) found that the effect of fatty acids on microbial growth depends on the concentration of acids, often

resulting in stimulation of growth at low concentrations and then in depression at higher concentrations.

Effect of calcium addition on IVDCWC of fat supplemented hay

Calcium addition alleviated the decrease in the digestibility of NDF (P=0.0001), ADF (P=0.003). However, cellulose and hemicellulose digestibilities had not significantly affected by calcium addition.

Table 3 b. ANOVA of Table 3a

Item	S	L	C	SxL	SxC	LxC	SxLxC
Dry matter							
d.f	2	3	1	6	2	3	6
Prob.	.0001	.0001	.245	.002	NS	NS	.011
Organic matter							
d.f.	2	3	1	6	2	3	6
Prob.	.0001	.0001	.172	.0001	.0001	.0001	.0001

S=fat source L= fat level C= calcium

Effect of fat source x supplementary level interaction on IVDCWC

The decrease in IVDCWC except hemicellulose of berseem hay due to increasing fat-supplementary level was not similar among the three sources of fat. Increasing suppementary level of oil or HVO resulted in greater decrease in IVDCWC in comparison with tallow supplement. Therefore, it might be suggested that tallow could be used more efficiently than vegetable oil or HVO when high fat diets is fed (Doreau et al., 1991; Jenkins and Jenny 1992).

Regression equations was predicted to quantify the relationship between the change in in vitro digestibility (Y) and the level of fat supplement (x) regadless fat source and for each source are presented in Table 4 (a & b). Higher negative correlation was observed between nutrient digestibility and supplementary level in case of oil supplement than HVO. This relationship was not evident in case of tallow supplement. Cellulose was the most affected fiber fraction by increasing oil or HVO supplementary levels.

Table 4a. Regression equations to calculate the change in in vivo nutrient digestibilities (Y) as affected by fat-supplementary level (x)

Item	Oil	HVO	Tallow	All sources
Dry matter	3.88-1.25x	-1.06-1.03x	-0.95-0.50x	0.81-0.93x
Organic matter	1.73-1.16x	-0.88-1.02x	3.37-0.61x	1.41-0.93x
NDF	-0.61-0.91x	-7.27-0.56x	2.82+0.09x	-1.28-.046x
ADF	-4.70-1.08x	-0.57-0.71x	5.47+0.20x	0.71-0.53x
Cellulose	2.41-1.64x	3.57-0.98	-0.30+0.16x	1.99-0.82x
Hemicellulose	2.60-0.49x	22.36+0.14x	2.41-0.10x	-5.78-0.15x

Table 4 b. Sample Size (n), Correlation coefficient (r) and propability of regression (P) of Table 4a

Item	Oil	HVO	Tallow	All sources
Dry matter				
n	136	136	136	408
r	-.64	-.45	-.27	-.45
P	.00001	.00001	.0020	.00001
Organic matter				
n	32	32	32	96
r	-.96	-.92	-.54	-.67
P	.0001	.0001	.0016	.00001
NDF				
n	32	32	32	96
r	-.67	-.53	.07	-.24
P	.0001	.0016	.6980	.0164
ADF				
n	32	32	32	96
r	-.66	-.38	.1001	-.20
P	.0001	.0340	.5855	.05288
Cellulose				
n	32	32	32	96
r	-.78	-.62	-.08	-.34
P	.0001	.0001	.0768	.0008
Hemicellulose				
n	32	32	32	96
r	.27	.06	.04	.05
P	.1378	.7607	.0831	.6096

Effect of fat-source x calcium interaction on IVDCWC

The role of added calcium in alliviation the depression in nutrient digestibilities depended on fat sourc as indicated from the significant fat-source x supplementary level interaction (Table 2b & 3 b). The

positive response of tallow to calcium addition could be related to that saturated fatty acids react more readily with cations to form insoluble soap (Jenkins and Palmquist 1982). This result might indicate that the simple addition of calcium (mixing calcium carbonate with fat supplemented feed) could be effective with tallow but more compilicated methods of calcium addition could be tried with oil or HVO like calcium salt formation (Palmquist 1984; El-Hag and Miller, 1972).

Effect of fat level x calcium addition interaction on IVDCWC

The efficiency of calcium addition in alleviation the decrease in IVDCWC decreased as supplementary level of fat increased. The variable response of nutrient digestibilities of fat supplemented hay with different levels to calcium addition could be explained on basis of lipid solubility. The actual proportions of added lipids to be soluble or insoluble will be influenced by the concentrations of salt in the media. If concentrations of fat will be too high, the salt in the media will be sufficient to form a certain amount of insoluble soap, the rest will be in soluble form which has more inhibitory effect on digestion of the incubated nutrient specially on the higher levels of fat supplement. Levels ranged from 5 to 10% could be recommended from viewpoint of digestibility in the present study.

Data in Table 3a showed the change in DM and OM digestibilities as a difference from each control values, their analyses of variance are shown in Table 3 (b). Less adverse effect of fat supplementation on IVDMOD and IVOMD than that occurred in IVDCWC was observed in case of tallow supplement. Increasing fat level decreased ($P=0.0001$) in general, IVDMD and IVOMD. Calcium addition had no significant effect on IVDMD and IVOMD. Increasing supplementary level was more effective to decrease IVDMD and IVOMD in case of oil and HVO than tallow. No different response of IVDMD to calcium addition interacted with fat sources. However, significant ($P=0.0001$) response of IVOMD was recorded to calcium addition to be higher in case of tallow and HVO than oil. Supplementary level x calcium addition interaction was insignifiant on IVDMD but significant

(P=0.0001) for IVOMD. Calcium was more effective to alleviate the adverse effect of increasing fat supplementary level on IVOMD when levels below 20% were used.

The results of the present study was used to verify the four theories summerized by Devendra and Lewis (1974) to explain the depressed fiber digestion by fatty acid supplement. The first theory is about the physical coating of fiber by fat. If this is true, the in vitro digestibility of NDF should show the same high degree of depression as shown by cellulose and ADF. However, cellulsoe and ADF were severely decreased in comparison with NDF by fat supplement specially with oil (Table 2a). The second theory is that fat supplement has a toxic effect modifying the rumen microbial population and the third theory is the inhibition of microbial activity from surface-active effects of fatty acids on microbial cell membranes.

Regarding the second and third theories while there are no reasons come from the present study to reject them, the two theories did not give the mechanism by which it occures. Moreover, no explanation was given for the specific adverse effect on cellulolytic activity by lipid supplement. The fourth theory is that fat supplement reduces cation availability by formation insoluble complex of cation with long chain fatty acids. The positive effect of calcium addition when low levels of supplementary fat was applied could partially support this theory. However, the negative or no response to calcium addition with the high level of fat supplement will be questionable. Therefore, it might be suggested that the inhibitory effect of fatty acids is a specific effect on cellulytic microbes but the mechanism by which it occurs needs more investigations.

REFERENCES

A.O.A.C., 1975. Association of Official Analytical Chemists. Offficial methods of analysis, 12 th ed. Washington, DC. Official.

Bock B.J., Harman, D.L., Brandt, R.T. and Schneider, J.E., 1991. Fat source and calcium level effects on finishing steer performance, digestion and metabolism. J. Anim Sci. 69:2211-2224.

Czerkawski, J.W. and J.L. Clapperton, 1984. Fats as energy yielding compounds in the ruminant diet. In: Fats in Animal Nutrition. J. Wiseman (Ed.) pp 249-263. Butterworth, London.

Devendra, C. and D. Lewis, 1974. The interaction between dietary lipids and fiber in the sheep. Anim. Prod. 13: 67-76.

Doreau, M., F. Legay and D. Bauchart, 1991. Effect of source and level of supplemental fat on total and ruminal organic matter and nitrogen digestion in dairy cows. J. Dairy Sci. 74:2232-2242.

El-Hag, M.G. and T.B. Miller, 1972. Evaluation of wisky by-products. VI. The reduction in digestibility of malt distiller's grains by fatty acids and the interaction with calcium and other reversal agents. J. Sci., Fed. Agric. 23:247-258.

Firkins, J.L., W.P. Weiss, M.L. Eastridge and S.L. Hull, 1990. Effects of feeding fungal cultutre extract and animal vegetable fat on degradation of hemicellulose and on ruminal bacterial growth in heifers. J. Dairy Sci. 73:1812-1822.

Goering, H.K. and P.J. Van Soest, 1970. Forage fiber analysis. Agric. Handbook, 379. U.S.Dept. Agric. Washington, DC.

Henderson, G., 1973. The effect of fatty acids on pure cultures of rumen bacteria. J. Agric. Sci. (Camb.) 81:107.

Ikwuegbu, O.A. and J.D. Sutton, 1982. The effect of arying the amount of linseed oil supplementation on rumen metabolism in sheep. Brit. J. Nutr. 48: 365-375.

Jenkins, T.C. and B.F. Jenny, 1992. Nutrient digestion and lactation performance of dairy cows fed combinations of prilled fat and canola oil. J. Dairy Sci. 75:796-803.

Jenkins, T.C. and D.L. Palmquist, 1982. Effect of added fat and calcium on in vitro formation of insoluble fatty acids soaps and cell wall digestibility. J. Anim. Sci. 55:957-963.

Kowalczyk, J., E.R. Orskov, J.J. Robinson and C.S. Stewart, 1977. Effect of fat supplementation on voluntary food intake and rumen metabolism in sheep. Brit. J. Nutr. 37:251-257.

Palmquist, D.L., 1984. Use of fats in diets for lactating dairy cows: In Fats in Animal Nutrition. J. Wiseman (Ed.) pp 357. Butter worth, London.

SAS, 1986. SAS User's Guide, Statistics. SAS Inst. Cary.NC.

Tilly, J.M.A and R.A. Terry, 1969. A two stage technique for in vitro digestion of forage feeds. J. Brit. Grassland Soc. 18:104.

Van Soest,P.J., 1982. Nutritional Ecology of the Ruminant. O & B Books, Inc. Corvallis, Oregon. USA.

تأثير نوع ومستوى الدهن واضافة الكالسيوم على هضم الالياف معمليا

صباح علام[1] - هدى الحسينى[2] - طه البداوى[1] - فايزة شاهين[2]

١- قسم الانتاج الحيوانى - كلية الزراعة - جامعة القاهرة ٢- معهد بحوث الانتاج الحيوانى - وزارة الزراعة

أجريت عدة تجارب معملية لدراسة تاثير ثلاثة مصادر من الدهون هى زيت عباد الشمس - زيوت نباتية مهدرجة وشحم حيوانى بمستويات إضافة صفر، ٥،١٠،١٥، ٢٠٪، كل مع أو بدون اضافة ١٪ كالسيوم على معامل الهضم للمادة الجافة والعضوية والألياف غير الذائبة فى المحلول المتعادل والألياف غير الذائبه فى المحلول الحامضى والهيميسليولوز و السليولوز لدريس البرسيم . وذلك من خلال إجمالى ٥١٠ عينة بواقع ١٧ مكرر لكل معاملة لحساب معامل هضم المادة الجافة معمليا ، بينما جمع المتبقى من كل معاملة فى عينتين لتقدير معامل هضم المركبات الأخرى .

وقد وجد أن زيادة مستوى الدهن قلل من معاملات هضم المادة الجافة والعضوية ومكونات الجدار الخلوى لدريس البرسيم خاصة عند استخدام الزيت مقارنة بمصادر الدهن الأخرى. كما أن إضافة الكالسيوم لم تؤدى الى تحسين الهضم. وثبت أن السليولوز هو أكثر المركبات تأثرا بإضافة الدهن. وقد خلصت الدراسة الى أن للدهن المضاف تاثيرا متخصصا على ميكروبات الكرش المحللة للسليولوز .