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Effects of Sunflower Cake Integration on Ration Digestibility and Metabolic Profile in Equine Tourism Horses

Research Article

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ABSTRACT

The scope for using sunflower cake in ration formulation was tested in 6 horses employed for equine tourism. Three experimental diets providing the same amount of proteins and energy as the control diet were formulated using rising doses of sunflower cake (SD_3: 0.3 kg; SD_6: 0.6 kg and SD_{10}: 1.0 kg). Digestibility evaluation using internal markers showed that rising sunflower cake doses reduced digestibility of nutrients. Statistical analysis using the diet effect demonstrated a significant reduction in dry matter (DM) (P=0.0006), ether extract (EE) (P=0.0001), crude fibre (CP) (P=0.0279), neutral detergent fiber (NDF) (P=0.0247) and acid detergent fiber (ADF) (P=0.0269) digestibility. Comparison of the two internal markers acid insoluble ash (AIA) and acid detergent lignin (ADL) showed better digestibility values for all measures using ADL. Also glucose and urea showed statistical differences due to diet effect.

KEY WORDS digestibility, horse, sunflower cake.

INTRODUCTION

Over the past six years the land devoted to sunflower crops has increased worldwide from 24725000 ha in 2008/09 to 25225000 ha in 2012/13, while increased yield per hectare has raised total production from 34753000 t / ha to 35568000 t / ha over the same time period. This increase may be attributable to the greater interest in the oil production for energy. In the EU and in Russia-Ukraine, production rose from 6909000 t and 14370000 t to 7060000 t and 16350000 t, respectively; it declined in Asia (China, India and Turkey) from 3750000 t in 2008/9 to 3450000 t in 2012/13 and remained stable in the USA and Argentina (4258000 t). Over the past few years sunflower seed has been employed in Europe to produce fuel oil (for direct use) or biodiesel (after transesterification).

By-products of these processes are sunflower cake and / or sunflower meal. Whereas the dietary value of sunflower meal has been extensively documented, less is known about sunflower cake, a valuable protein source that can be used to feed income-generating animals. The use of sunflower seed cake from whole seed, partially and fully dehulled seed can also be used in the preparation of foodstuffs for human consumption (Srilatha and Krishnakumari, 2003). Some studies have investigated its nutritional profile to assess its effects on production performances of broiler, pig, beef cattle, sheep and horse (Mattii *et al*. 2009; Mlay *et al*. 2006; Talha and Yagoub, 2008; Trombetta and Mattii, 2005; Trombetta *et al*. 2007; Yagoub and Talha; 2009). This work examines the effect of rising amounts of sunflower cake on ration digestibility in horses used in equine tourism.

MATERIALS AND METHODS

Subjects were 6 horses 4 geldings and 2 mares, mean age 7.8 ± 1.7 years, mean live weight 484.2 ± 22.7 kg employed in a farm (Randis, Piano d'Arta Terme, north-eastern Italy) for horseback trekking. During the study they did standard work and were housed in individual boxes with wood chips. They were fed twice daily a ration control diet (CD) consisting of hay (7 kg) and compound feed (4 kg). The experimental plan, approved by the Ethics Committee of the School of Veterinary Medicine of the University of Bologna (Italy), covered 2 months and envisaged the following rations:

CD: standard ration.

SD_3, SD_6, SD_{10}: part of the standard ration (feed and hay) was replaced with rising amounts of sunflower cake (0.3 kg; 0.6 kg; 1.0 kg respectively) taking care that this did not affect the nutrient supply (Tables 1, 2 and 3).

Table 1 Composition of daily ration (kg)

Ingredients	Rations[1]			
	CD	SD_3	SD_6	SD_{10}
Hay	7.0	7.0	6.5	6.0
Feed	4.0	3.4	3.5	3.6
Sunflower cake	-	0.3	0.6	1.0
Total	11.0	10.7	10.6	10.6

CD: standard ration.
SD_3, SD_6 and SD_{10}: part of the standard ration (feed and hay) was replaced with rising amounts of sunflower cake (0.3 kg; 0.6 kg and 1.0 kg respectively).
SD: standard deviation.

Table 2 Chemical composition (Mean±SD) of ration components (% DM)

Chemical composition	Ration components		
	Hay	Feed	Sunflower cake
Moisture	11.6±2.4	11.2±0.3	8.0±0.6
CP	10.4±0.3	13.1±0.5	22.3±0.2
EE	2.0±0.3	3.0±0.3	21.5±2.5
CF	29.9±1.5	10.4±0.3	21.8±1.4
NDF	59.7±2.8	26.0±0.3	35.7±3.8
ADF	37.1±1.0	14.5±0.9	26.2±2.9
ADL	6.1±0.5	3.9±0.3	8.7±1.5
Ash	6.7±0.4	7.6±0.3	4.6±0.3
Calcium	0.9±0.2	1.5±0.09	0.46±0.1
Phosphorus	0.26±0.06	0.48±0.03	0.61±0.06
GE MJ/kg	18.48	17.75	24.40

CP: crude protein; EE: ether extract; CF: crude fibre; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin and GE: gross energy.

According to the farm owners all rations met the nutritional requirements of their horses (Kohnke, 1989; Martin-Rosset, 1990). The owners checked that each ration was consumed entirely.

Each diet change from CD to SD_3, from SD_3 to SD_6 and from SD_6 to SD_{10} included a 10-day habituation period. Then faeces samples were collected twice daily for 5 days. Every day the morning faece of each subject, stored at 4 ˚C, pooled with the evening samples were weighed and frozen. The 120 samples collected at the end of the experiment were lyophilized.

Table 3 Daily nutrients supply provided by the rations

Nutrients[1]	Rations[2]			
	CD	SD_3	SD_6	SD_{10}
DM g	9740	9483	9406	9421
CP g	1109	1101	1128	1176
EE g	230	274	327	358
CF g	2220	2224	2162	2119
NDF g	4618	4578	4436	4326
ADF g	2811	2806	2727	2672
ADL g	516	412	520	528
Ash g	685	657	647	678
GE MJ	177.4	174.7	174.8	177.2

[1] DM: dry matter; CP: crude protein; EE: ether extract; CF: crude fibre; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; GE: gross energy and SD: standard deviation.
[2] CD: standard ration.
SD_3, SD_6 and SD_{10}: part of the standard ration (feed and hay) was replaced with rising amounts of sunflower cake (0.3 kg; 0.6 kg and 1.0 kg respectively).

Each pooled faeces sample and samples of each ration were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fibre (CF), fibrous fraction [NDF, ADF and acid detergent lignin (ADL)], ash and acid insoluble ash (AIA), which were determined according to ASPA guidelines (Martillotti *et al.* 1987); gross energy (GE) was measured with an adiabatic calorimetric bomb Parr 1261. On the final day of each period, blood was also collected into heparinized vacutainer tubes.

Plasma obtained by immediate centrifugation was stored at -20 ˚C and analyzed for glucose, cholesterol, total protein, urea and albumin with an Olympus® Autoanalyzer.

The results of faeces and feed analyses were used to calculate the apparent digestive utilization coefficient (DUC_a) using AIA and ADL as internal markers and applying the formula:

$$DUC_a\ (\%) = ((C_f - C_a) / (C_f)) \times 100$$

Where:
C_f and C_a: AIA and ADL concentrations in faeces and feed, respectively.

The DUC_a data were subjected to analysis of variance (ANOVA) for the Diet effect, the Marker effect, and the Diet × Marker effect by the JMP statistical package (SAS, 2009) using the equation:

$$Y_i = \mu + \alpha_i + \beta_j + (\alpha_i \times \beta_j) + \varepsilon_{ij}$$

Where:
μ: mean.
α_i: diet effect (i=CD, SD_3, SD_6 and SD_{10}).
β_j: marker effect (j=AIA and ADL).
$\alpha_i \times \beta_j$: interaction effect.

ε_{ij}: residual error.

Blood parameters were analyzed by ANOVA one way considering only the diet effect.

RESULTS AND DISCUSSION

Chemical analysis of ration components (Table 2) indicated that hay and CD feed provided a limited CP supply and that the protein, CF and fibrous fraction content of sunflower cake were in line with the values reported for non-decorticated cake.

In contrast the EE was very high compared with literature data (Piccioni, 1989), a difference that may be related to the oilseed extraction technique.

The daily nutrients provided by the four rations, reported in Table 3, show that all diets were balanced and that they met the requirements of active horses weighing 475-525 kg (Kohnke, 1989; Martin-Rosset, 1990). The DUC_a of each nutrient, determined considering the Diet effect, is listed in Table 4.

Significant differences were found for DM digestibility (P=0.006), EE (P=0.0001), CF (P=0.0279) and fibrous fractions NDF and ADF (P=0.0247 and P=0.0269). In particular DM digestibility was greater for the CD feed and declined significantly as the sunflower cake dose increased. EE digestibility exhibited significant differences, declining to very low values in SD_6 and then rising again in SD_{10}, albeit without reverting to the values found in CD and SD_3. As regards CF, NDF and ADF, their digestibility declined significantly with rising sunflower cake dose. The energy DUC_a showed similar means for CD and SD_3, whereas lower but not significantly different values were found for SD_6 and SD_{10}.

Considering the diet effect, the mean DUC_a values were lower than those described by Trombetta *et al.* (2007) in a study of quarter horses where the feed was integrated with 300 g and 600 g sunflower cake.

The protein DUC_a values were similar to those reported by Miraglia *et al.* (1999) in two tests, whereas the EE DUC_a in diet DG_{10} was similar to the one described by Miraglia *et al.* (1999) in horses fed “hay and concentrate 2” (a value obtained using ADL as an internal marker) and to that reported by Kienzle *et al.* (2002) in horses fed straw. The digestibility of CF and fibrous fractions was lower than the values reported by Miraglia *et al.* (1999). The DUC_a of nutrients, determined using AIA as a marker, are reported in Table 5. Statistical analysis highlighted significant differences for DM (P=0.0001), CP (P=0.0047), EE (P=0.0001), NDF (P=0.0057) and ADF (P=0.0066). The best coefficients were again found for the CD feed, as the rising amount of sunflower cake adversely affected digestibility; these data contrast with those reported by Trombetta *et al.* (2007) in a study where integration with 300 g sunflower cake enhanced digestibility. In particular, DM values were better than those reported by De Marco *et al.* (2012) in horses fed soybean and sunflower meal. The poor EE digestibility obtained in the present study using AIA is similar to the one described by Miraglia *et al.* (1999) using ADL. When the DUC_a of nutrients was examined using ADL as an internal marker, statistical analysis disclosed significant differences in all diets for all nutrients except PG (Table 6). The DUC_a values were higher that those obtained with AIA; moreover, with the exception of DM, they were significantly better for diets CD and SD_3 than for diets SD_6 and SD_{10}. Diet SD_3 was superior to the other diets only for CF and the fibrous fractions, a finding that is partly in line with Trombetta *et al.* (2007). The DUC_a values obtained using ADL as an internal marker, which are in line with Varloud *et al.* (2004) but at variance with Miraglia *et al.* (1999) and Bergero *et al.* (2003), would indicate that ADL is a more suitable marker of digestibility than AIA, since the coefficients calculated for EE, CF, NDF and ADF are better than those obtained using AIA according to Varloud *et al.* (2004). Table 7 reports the DUC_a values of GE, DE and the energy DUC_a.

Table 4 Apparent digestive utilization coefficient (DUC_a) of nutrients in relation to the diet effect

	Rations[1]					
Samples (n)	CD	SD_3	SD_6	SD_{10}	Standard error	P
	60	60	60	60		
DUC_a of nutrients %[2]	Mean	Mean	Mean	Mean		
DM DUC_a	89.12^A	87.55^B	87.27^B	86.95^B	0.394	0.0006
OM DUC_a	60.19	60.44	58.24	58.62	1.394	0.6005
CP DUC_a	70.05	67.99	66.30	67.89	1.199	0.1821
EE DUC_a	36.65^A	28.14^A	8.82^C	11.31^B	3.105	0.0001
CF DUC_a	30.70^a	28.44^{ab}	20.82^{bc}	17.69^c	3.500	0.0279
NDF DUC_a	40.01^a	36.41^{ab}	33.16^b	31.71^b	2.070	0.0247
ADF DUC_a	25.39^a	21.74^{ab}	16.18^b	14.67^b	2.816	0.0269
Energy DUC_a	57.07	57.31	55.23	55.59	1.321	0.6008

[1] CD: standard ration.
SD_3, SD_6 and SD_{10}: part of the standard ration (feed and hay) was replaced with rising amounts of sunflower cake (0.3 kg; 0.6 kg and 1.0 kg respectively).
[2] DM: dry matter; CP: crude protein; EE: ether extract; CF: crude fibre; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; GE: gross energy and SD: standard deviation.
[a, b] the means within the same row with different letter, are significantly different (P<0.05).
[A, B] the means within the same row with different letter, are significantly different (P<0.05).

Table 5 Apparent digestive utilization coefficient (DUC_a) of nutrients in relation to the acid insoluble ash marker effect

Samples (n)	Rations[1]				Standard error	P
	CD	SD_3	SD_6	SD_{10}		
	30	30	30	30		
DUC_a of nutrients %[2]	Mean	Mean	Mean	Mean		
DM DUC_a	88.17^A	85.12^B	84.97^B	84.76^B	0.534	0.0001
OM DUC_a	54.05	52.93	48.94	52.45	0.534	0.2610
CP DUC_a	67.44^A	61.71^B	59.85^B	61.79^B	1.536	0.0047
EE DUC_a	31.39^A	13.60^B	-9.35^C	-8.13^C	3.728	0.0001
CF DUC_a	28.63	20.54	10.48	8.06	6.632	0.1130
NDF DUC_a	34.47^A	24.15^B	21.15^B	21.37^B	2.990	0.0057
ADF DUC_a	18.44^A	4.92^B	0.113^B	0.197^B	2.990	0.0066

[1] CD: standard ration.
SD_3, SD_6 and SD_{10}: part of the standard ration (feed and hay) was replaced with rising amounts of sunflower cake (0.3 kg; 0.6 kg and 1.0 kg respectively).
[2] DM: dry matter; CP: crude protein; EE: ether extract; CF: crude fibre; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; GE: gross energy and SD: standard deviation.
[A, B] the means within the same row with different letter, are significantly different (P<0.05).

Table 6 Apparent digestive utilization coefficient (DUC_a) of nutrients in relation to the acid detergent lignin marker effect

Samples (n)	Rations[1]				Standard error	P
	CD	SD_3	SD_6	SD_{10}		
	30	30	30	30		
DUC_a of nutrients %[2]	Mean	Mean	Mean	Mean		
DM DUC_a	90.07^a	89.99^a	89.57^{ab}	89.14^b	0.230	0.0187
OM DUC_a	66.32^{ab}	67.94^a	67.54^a	64.79^b	0.735	0.0134
CP DUC_a	72.65	74.27	72.75	73.99	1.159	0.6717
EE DUC_a	41.42^A	42.68^A	26.99^B	39.75^B	3.000	0.0002
CF DUC_a	32.78^B	36.63^A	31.26^B	27.33^B	1.084	0.0001
NDF DUC_a	45.55^B	48.67^A	45.18^B	42.04^B	1.024	0.0002
ADF DUC_a	32.34^B	38.56^A	32.25^B	29.14^C	1.019	0.0001

[1] CD: standard ration.
SD_3, SD_6 and SD_{10}: part of the standard ration (feed and hay) was replaced with rising amounts of sunflower cake (0.3 kg; 0.6 kg and 1.0 kg respectively).
[2] DM: dry matter; CP: crude protein; EE: ether extract; CF: crude fibre; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; GE: gross energy and SD: standard deviation.
[a, b] the means within the same row with different letter, are significantly different (P<0.05).
[A, B] the means within the same row with different letter, are significantly different (P<0.05).

Table 7 Gross energy (GE), digestible energy (DE) and digestive utilization coefficient of energy (DUC_a) in relation to the acid insoluble ash (AIA) or acid detergent lignin (ADL) marker effect

Samples (n)	Rations[1]				Standard error	P
	CD	SD_3	SD_6	SD_{10}		
	30	30	30	30		
	Mean	Mean	Mean	Mean		
GE MJ/kg DM	18.18	18.37	18.52	18.71	-	-
DE_{AIA} MJ/kg DM	9.31	9.22	8.60	9.31	0.332	0.3609
DE_{ADL} MJ/kg DM	11.43^c	11.83^{ab}	11.86^a	11.49^{bc}	0.128	0.0327
DUC_{a-AIA} %	51.26	50.20	46.42	49.75	1.799	0.2621
DUC_{a-ADL} %	62.89^{ab}	64.42^a	64.04^a	61.43^b	0.697	0.0134

[1] CD: standard ration.
SD_3, SD_6 and SD_{10}: part of the standard ration (feed and hay) was replaced with rising amounts of sunflower cake (0.3 kg; 0.6 kg and 1.0 kg respectively).
[a, b] the means within the same row with different letter, are significantly different (P<0.05).

The GE values were similar in all diets, whereas DE showed improved and significantly different values for DE_{ADL} in diets SD_3 and SD_6 than in diets CD and SD_{10}. The DUC_a calculated using ADL as a marker was significantly better (P=0.0134) for diets SD_3 and SD_6; with AIA the highest DUC_a was obtained for CD, although the difference was not significant.

Statistical analysis of the blood parameters (Table 8) evidenced significant differences due to the Diet effect for glucose and urea.

In particular glucose increased significantly in diet SD_3 while in diets SD_6 and SD_{10} it reverted to levels similar to those of CD. Urea also increased significantly in diet SD_3, whereas in diets SD_6 and SD_{10} values were similar to that of CD. The trend of total protein was similar to the one of urea: it showed high values in subjects receiving SD_3 whereas in diet SD_{10} it fell to values lower than that found in CD. Only cholesterol showed a gradual increase, similar to the one found by Trombetta *et al.* (2007) in quarter horses fed rising doses of sunflower cake.

Table 8 Values of the blood parameters in relation to the diet effect

Samples (n)	Diet[1]				Standard error	P
	CD	SD_3	SD_6	SD_{10}		
	6	6	6	6		
Blood parameters	Mean	Mean	Mean	Mean		
Glucose (mmol/L)	5.8^B	9.5^A	5.6^B	5.1^B	0.522	0.0001
Cholesterol (mmol/L)	1.9	2.3	2.8	2.3	0.293	0.2942
Total protein (g/L)	78.4	100.5	93.0	67.0	0.949	0.0896
Urea (mmol/L)	6.5^a	8.8^b	7.0^{ab}	6.6^{ab}	0.568	0.0300
Albumin (g/L)	32.2	41.0	39.2	32.6	0.318	0.1302

[1] CD: standard ration.
SD_3, SD_6 and SD_{10}: part of the standard ration (feed and hay) was replaced with rising amounts of sunflower cake (0.3 kg; 0.6 kg and 1.0 kg respectively).
SD: standard deviation.
a, b: the means within the same row with different letter, are significantly different (P<0.05).
A, B: the means within the same row with different letter, are significantly different (P<0.05).

The variation of glucose and urea in the diet D_3 is due to the inclusion of sunflower meal in the diet, the subsequent return of these parameters in the diets D_6, D_{10} may indicate a metabolic adaptation of horses to the ration so integrated.

CONCLUSION

The results obtained in this study prompt a number of considerations:
A) Even though each experimental diet supplied the same amount of protein and energy as the standard diet, rising amounts of sunflower cake reduced nutrient digestibility, confirming earlier data obtained with a sunflower cake integration of 600 g (Trombetta *et al.* 2007).
B) As regards the internal markers, AIA recovery from faeces seems to have been suboptimal, resulting in generally low and / or negative DUC_a values for some parameters compared with those obtained using ADL. These data are supported by Varloud *et al.* (2004), who reported that ADL is better suited than AIA to assess digestibility, especially in diets containing NDF ≥ 52.8% (Schaafstra *et al.* 2012). Another factor that may have adversely affected fibre digestibility is the amount of fat supplied by sunflower cake (+55.6% in SD_{10} *vs.* CD). In this connection De Almeida and de Godoi (2011) highlighted the negative effect of oil integrations on fibre digestibility; they related this effect, observed in some studies, to a number of possible factors including mode of ration administration; forage / concentrate ratio; ration transit velocity and inhibition of large intestine microflora by oil. In addition chlorogenic acid, which exerts both favourable (antioxidant, increase of resistance to fatigue) and unfavourable effects (anti-nutritional factor with a negative influence on the digestive utilization of dietary nutrients), has recently been detected in sunflower panel (Galassi, 2013). In conclusion, it may be stated that high doses (600-1000 g) of sunflower cake in horse rations adversely affect the digestibility of the various dietary components, confirming earlier data described in horses receiving 600 g sunflower cake integration reported by our group (Trombetta *et al.* 2007).

However the present findings suggest that further research is required to learn how this by-product of short supply-chain processes can be used in animal breeding and to establish whether the decorticated product has a better effect on horse ration digestibility than non-decorticated cake.

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