Interpretive summary

Telling dairy goat's dietary oil apart

Martínez Marín et al.

Fatty acid (FA) analysis of milk fat samples were used to classify milk fats according to the diet consumed through linear discriminant analysis. Milk samples were obtained from dairy goats fed a control diet added with none or one of three plant oils: high oleic sunflower oil, regular sunflower oil and linseed oil. Out of 84 variables (82 FA and two FA ratios) used, 20 proved to be useful predictors. Only one of 112 milk samples was misclassified.

Short communication: Linear Discriminant Analysis and Type of Oil Added to Dairy Goat Diets

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ABSTRACT

Gas chromatography fatty acid (**FA**) analysis of one hundred and twelve milk fat samples from dairy goats fed a basal diet with no added oil or the same diet added one of three vegetable oils (high oleic sunflower oil –**HOSFO-**, regular sunflower oil –**RSFO-** or linseed oil –**LO-**) were used to identify the type of diet consumed through linear discriminant analysis (**LDA**). Twenty variables (19 FA and one FA ratio) were selected as valid predictors out of 84 variables tested. The Mahalanobis squared distance was minimum between HOSFO and RSFO groups and maximum between control and LO groups. Crossvalidation showed that only one observation from RSFO group was misclassified into HOSFO group. We concluded that LDA is useful to classify milk fat samples from dairy goats according to the particular vegetable oil, among the three studied added to their basal diet.

Key Words: discriminant analysis, fatty acid, goat milk, vegetable oils

SHORT COMMUNICATION

Discriminant analysis is a multivariate statistical technique that can be used to build a predictive model of group discrimination based on observed predictor variables and to classify each observation into one of the groups. Linear discriminant analysis (**LDA**) has been used successfully to differentiate milk and cheese from different species based on their mineral content (Martín-Hernández et al., 1992) as well as to detect milk fat adulteration (Ulberth, 1994; Gutiérrez et al., 2009).

It is well established that supplementation of goat diets with fat sources rich in unsaturated fatty acids (**FA**) substantially modifies the milk fat FA profile (Chilliard et al., 2007). Accordingly, enabling the researchers to obtain information about the type of dietary lipids consumed by the animals from milk fat FA data would be of great interest. The aim of this work was to investigate the use of LDA to identify the type of plant oil consumed by goats from data of their milk fat FA profile determined by gas chromatography (**GC**).

Milk fat FA GC analysis (up to 82 FA and two FA ratios per sample, **Table 1**) involving 112 goat milk samples from 16 goats (Martínez Marín et al., 2011), 12 goats (Martínez Marín et al., in press) and 12 goats (Martínez Marín et al., unpublished results) were used to perform the discriminant analysis. The goats were all fed the same basal diet. All the analysis were grouped into four classes (28 analysis in each class): no added fat basal diet (**Control**), c9-18:1 rich diet (high oleic sunflower oil, **HOSFO**), c9c12-18:2 rich diet (regular sunflower oil, **RSFO**) and 18:3n-3 rich diet (linseed oil, **LO**). Of the 28 analysis corresponding to each of the oil added classes (HOSFO, RSFO and LO) 22 analysis corresponded to milk from goats supplemented with 48 g of oil per day, and 6 corresponded to milk from goats supplemented with 32 (three goats) or 66 (three goats) g of oil per day.

SAS 9.1.3 (SAS, 2004) was used to perform the statistical analysis. First, PROC STEPDISC was used to select the FA and ratios that would be included as predictor variables in the model. Probability to enter and stay in the model was set at 0.10 and 0.15, respectively. Following this, PROC DISCRIM was used to determine the coefficients for the optimal subset of FA and ratios included in the linear discriminant functions (**LDF**). In this procedure, the option CROSSVALIDATE was included to assess the robustness of the LDF obtained.

Nineteen FA and one ratio were selected as predictor variables (**Table 2**). Canonical discriminant functions 1 and 2 explained up to 89.2% of total variation between groups (**Figure 1**). The four FA with greater discriminating ability were 19:0, c9-17:1, t11t15-18:2 and 18:0 iso, and the ratio linoleic acid to α-linolenic acid (**LA/ALA**) in function 1, c9-18:1, t11-18:1, c9c12-18:2 and t9c12-18:2 in function 2, and c9-17:1, 18:0 iso, t5-18:1 and t9c12-18:2 in function 3. Pooled within canonical structure (**Table 2**) showed that none of the predictor variables had an absolute correlation value greater than 0.41 with any function. The higher correlation values between different FA and function 1 corresponded to 19:0, c8-16:1, t11-18:1, t9c12-18:2, t11c13-18:2, c9t11t15-18:3 and to the ratio LA/ALA. According to class means (**Table 2**) this function discriminated clearly the LO group. Some of the above cited milk FA (e.g. t11-18:1, t11c13-18:2 and c9t11t15-18:3) are known to be related to the intake of α-linolenic acid rich diets by dairy ruminants (Collomb et al., 2004; Chilliard et al., 2007; Gómez-Cortés et al., 2009). The best correlation values between different FA and function 2 corresponded to 20:0, c7-16:1, c9-17:1, t5-18:1, t11-18:1 and c9-18:1, and to the ratio LA/ALA. According to class means this function discriminated clearly the control group from groups HOSFO and RSFO. Known FA which decrease with oil treatments like those of microbial origin showed higher negative correlations values with function 2 (e.g. c9-17:1). On the contrary FA from direct or indirect dietary origin like t11-18:1, oleic acid or the LA/ALA ratio showed a higher than average positive correlation value with this function. The higher correlation values between different FA and function 3 corresponded to c9c12-18:2, 20:4n-6, t11-18:1, t5-18:1, c7-16:1 and c9-18:1, and to the ratio LA/ALA. According to class means this function discriminated well HOSFO from RSFO groups. The ratio LA/ALA and some FA with known direct or indirect origin in linoleic acid rich diets (e.g. c9c12-18:2, t11-18:1) were negatively correlated to function 3, while c9-18:1 was positively correlated, what suggests that HOSFO diets supplied more preformed c9-18:1 and/or stearic acid to the mammary gland.

Fisher´s linear discriminant functions are shown in **Table 3**. The Mahalanobis squared distance was minimum between HOSFO and RSFO groups (26.99) and maximum between control and LO groups (146.26). The F-test of the distances was highly significant in all cases ($P<0.0001$). Only one observation from RSFO group was misclassified into HOSFO group, both in original and cross-validated classification matrices, resulting in 98.9% of original grouped cases classified correctly.

The discriminant analysis allowed us to identify 20 variables as useful predictors, out of the 84 variables used. The LDA was useful to classify milk fat samples according to the particular vegetable oil, among the three studied added to a basal diet from a number of FA quantified in milk fat.

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Table 1. Fatty acids (mean±sd) and univariate test of equality between group means of the diet classes used in the study.

	Diets[1]				*P*
	Control	HOSFO	RSFO	LO	
SFA					
4:0	2.469±0.194	2.601±0.301	2.643±0.414	2.624±0.320	0.230
5:0	0.022±0.012	0.025±0.011	0.021±0.010	0.026±0.019	0.459
6:0	2.761±0.265	2.855±0.286	2.910±0.430	2.996±0.383	0.150
7:0	0.041±0.023	0.044±0.021	0.037±0.016	0.048±0.035	0.457
8:0	3.029±0.383	3.106±0.366	3.183±0.611	3.355±0.443	0.118
4-methyloctanoate	0.044±0.025	0.043±0.026	0.037±0.020	0.046±0.028	0.588
9:0	0.088±0.051	0.090±0.041	0.075±0.031	0.095±0.061	0.511
10:0	11.328±0.991	10.244±1.088	10.263±1.765	10.764±1.280	0.016
methyldecanoate	0.071±0.041	0.058±0.034	0.051±0.026	0.063±0.037	0.229
12:0	5.218±0.950	3.926±0.554	4.073±0.622	4.109±0.806	<0.001
methyldodecanoate	0.024±0.012	0.023±0.014	0.021±0.009	0.023±0.011	0.739
13:0 iso	0.020±0.008	0.018±0.007	0.018±0.005	0.016±0.005	0.200
13:0 anteiso	0.063±0.032	0.038±0.013	0.043±0.015	0.042±0.020	<0.001
14:0 iso	0.050±0.010	0.046±0.009	0.047±0.014	0.043±0.012	0.260
14:0	10.387±0.866	8.635±0.646	8.813±0.914	8.560±1.014	<0.001
methyltetradecanoate	0.063±0.033	0.048±0.023	0.042±0.016	0.050±0.026	0.044
15:0 iso	0.136±0.020	0.122±0.020	0.130±0.029	0.119±0.017	0.049
15:0 anteiso	0.278±0.056	0.255±0.069	0.250±0.072	0.244±0.056	0.287
15:0	0.833±0.231	0.724±0.156	0.676±0.143	0.713±0.210	0.029
16:0 iso	0.145±0.035	0.143±0.095	0.125±0.034	0.121±0.038	0.361
16:0	32.562±2.834	24.944±3.588	24.31±3.199	24.270±2.831	<0.001
17:0	0.399±0.056	0.377±0.072	0.340±0.076	0.358±0.081	0.034
18:0 iso	0.035±0.015	0.030±0.016	0.037±0.018	0.027±0.011	0.077
10-keto-18:0	0.039±0.018	0.312±0.248	0.133±0.129	0.108±0.060	<0.001
18:0	5.778±1.240	10.171±2.212	9.120±1.878	8.389±1.708	<0.001
19:0	0.016±0.015	0.017±0.012	0.018±0.015	0.047±0.017	<0.001
20:0	0.111±0.018	0.151±0.024	0.139±0.020	0.112±0.015	<0.001
21:0	0.026±0.005	0.027±0.005	0.026±0.008	0.024±0.007	0.635
22:0	0.051±0.012	0.082±0.030	0.081±0.022	0.047±0.009	<0.001
MUFA					
c9-10:1/12:0 iso/11:0	0.392±0.134	0.324±0.085	0.326±0.065	0.354±0.099	0.062
c9-12:1/13:0	0.223±0.100	0.158±0.046	0.159±0.043	0.171±0.061	0.003
c9-14:1	0.216±0.101	0.130±0.051	0.158±0.078	0.146±0.073	0.002
c9-15:1	0.054±0.016	0.045±0.014	0.047±0.013	0.047±0.012	0.126
t8-16:1	0.049±0.012	0.077±0.033	0.078±0.035	0.073±0.021	0.001
t9-16:1/17:0 iso	0.336±0.067	0.361±0.092	0.557±0.185	0.600±0.149	<0.001
c7-16:1	0.241±0.035	0.292±0.047	0.257±0.050	0.271±0.045	0.001
c8-16:1	0.011±0.003	0.010±0.002	0.014±0.003	0.030±0.008	<0.001
c9-16:1/17:0 anteiso	1.321±0.321	0.926±0.149	0.913±0.235	0.884±0.191	<0.001
c13-16:1	0.282±0.110	0.154±0.055	0.167±0.071	0.184±0.091	<0.001
c9-17:1	0.212±0.046	0.167±0.062	0.138±0.033	0.144±0.051	<0.001
t4-18:1	0.013±0.007	0.037±0.020	0.025±0.013	0.022±0.011	<0.001

t5-18:1	0.014±0.006	0.036±0.020	0.023±0.013	0.020±0.010	<0.001
t6/t7/t8-18:1	0.169±0.048	0.448±0.173	0.364±0.121	0.324±0.091	<0.001
t9-18:1	0.195±0.045	0.349±0.101	0.372±0.093	0.347±0.089	<0.001
t10-18:1	0.330±0.164	0.584±0.387	0.901±0.843	0.413±0.171	0.001
t11-18:1	1.011±0.425	1.767±0.756	3.597±1.832	3.732±1.584	<0.001
t12-18:1	0.182±0.053	0.372±0.221	0.328±0.104	0.412±0.144	<0.001
c9-18:1	14.545±1.651	20.477±3.549	17.757±3.319	16.19±4.419	<0.001
t15/c11-18:1	0.306±0.064	0.346±0.101	0.360±0.128	0.571±0.224	<0.001
c12-18:1	0.139±0.051	0.101±0.039	0.287±0.229	0.477±0.340	<0.001
c13-18:1	0.038±0.009	0.042±0.011	0.051±0.011	0.058±0.018	<0.001
t16/c14-18:1	0.175±0.036	0.228±0.048	0.287±0.064	0.441±0.108	<0.001
c15-18:1	0.055±0.010	0.059±0.012	0.076±0.017	0.324±0.171	<0.001
c16-18:1	0.020±0.004	0.026±0.005	0.030±0.014	0.034±0.011	<0.001
c11-20:1	0.046±0.010	0.067±0.017	0.063±0.017	0.050±0.013	<0.001
PUFA					
t11t15-18:2	0.040±0.014	0.044±0.015	0.038±0.014	0.063±0.038	0.001
t9t12/c9t13/t8c12-18:2	0.165±0.034	0.162±0.046	0.216±0.048	0.348±0.112	<0.001
t8c13-18:2	0.061±0.010	0.065±0.016	0.071±0.020	0.143±0.046	<0.001
c9t12-18:2	0.030±0.008	0.032±0.011	0.034±0.014	0.043±0.017	0.006
t9c12-18:2	0.031±0.009	0.027±0.006	0.039±0.013	0.049±0.020	<0.001
t11c15-18:2	0.037±0.012	0.056±0.023	0.065±0.038	0.936±0.561	<0.001
c9c12-18:2	1.725±0.272	1.411±0.329	2.203±0.838	1.684±0.532	<0.001
Other 18:2	0.063±0.025	0.059±0.016	0.050±0.015	0.086±0.031	<0.001
c9t11-18:2	0.616±0.246	0.839±0.347	1.679±0.837	1.660±0.638	<0.001
t9c11-18:2	0.013±0.007	0.017±0.008	0.021±0.013	0.016±0.007	0.016
t10c12-18:2	0.007±0.004	0.008±0.004	0.009±0.005	0.008±0.004	0.410
t11c13-18:2	0.011±0.006	0.011±0.006	0.012±0.006	0.023±0.011	<0.001
t12t14-18:2	0.007±0.003	0.007±0.004	0.009±0.006	0.017±0.008	<0.001
t11t13-18:2	0.006±0.003	0.007±0.004	0.007±0.004	0.015±0.008	<0.001
t9t11-18:2	0.013±0.006	0.017±0.008	0.022±0.014	0.019±0.009	0.016
16:2	0.009±0.005	0.011±0.005	0.009±0.005	0.051±0.034	<0.001
18:3n-6	0.026±0.008	0.024±0.010	0.024±0.007	0.043±0.022	<0.001
18:3n-3	0.163±0.037	0.128±0.028	0.142±0.067	0.614±0.291	<0.001
c9t11t15-18:3	0.007±0.003	0.007±0.003	0.010±0.009	0.044±0.020	<0.001
c9t11c15-18:3	0.037±0.009	0.036±0.007	0.033±0.008	0.104±0.068	<0.001
20:2n-6	0.010±0.005	0.010±0.005	0.012±0.006	0.011±0.004	0.369
20:3n-3	0.006±0.004	0.008±0.005	0.008±0.005	0.009±0.005	0.306
20:4n-6	0.136±0.024	0.117±0.020	0.145±0.037	0.111±0.020	<0.001
20:5n-3	0.025±0.006	0.023±0.006	0.026±0.010	0.039±0.007	<0.001
22:4n-6	0.026±0.007	0.076±0.049	0.043±0.027	0.030±0.011	<0.001
22:5n-3	0.040±0.009	0.035±0.008	0.038±0.015	0.045±0.010	0.024
22:6n-3	0.021±0.009	0.019±0.008	0.026±0.020	0.018±0.009	0.127
RATIOS					
t10-18:1/t11-18:1	0.349±0.161	0.346±0.252	0.251±0.188	0.119±0.037	<0.001
LA/ALA	10.972±2.280	11.138±1.720	16.577±5.434	2.988±0.797	<0.001

[1] Control: basal diet without added oil; HOSFO, RSFO and LO: basal diet enriched with high oleic sunflower oil, regular sunflower oil, or linseed oil, respectively.

Table 2. Total-sample standardized canonical coefficients and pooled within canonical structure

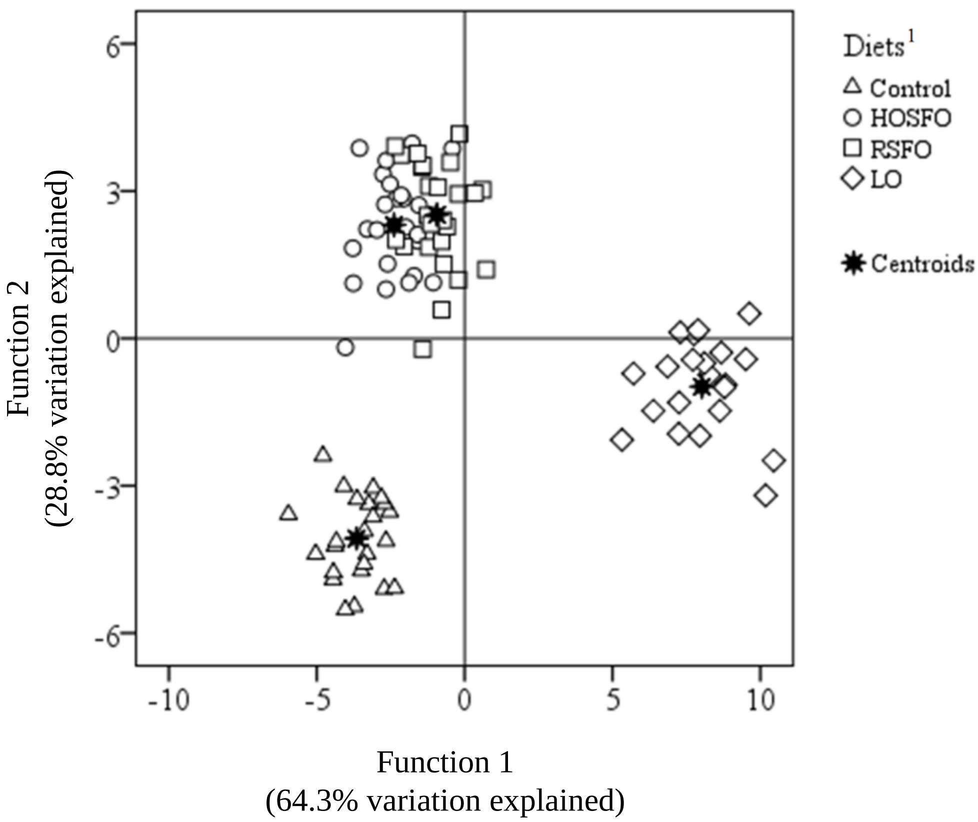
	Standardized canonical coefficients			Canonical structure		
	Function			Function		
	1	2	3	1	2	3
4:0	-0.180	0.722	0.547	0.025	0.067	-0.016
14:0 iso	-0.011	-0.049	-0.727	-0.042	-0.029	-0.031
16:0 iso	-0.574	-0.365	0.472	-0.033	-0.022	0.054
18:0 iso	0.965	0.320	-1.133	-0.042	-0.001	-0.111
19:0	1.076	-0.104	0.190	0.191	-0.045	0.041
20:0	-0.043	0.608	0.460	-0.076	0.288	0.113
c7-16:1	-0.203	-0.442	0.288	0.021	0.104	0.168
c8-16:1	0.762	0.056	-0.096	0.390	-0.077	-0.069
c9-17:1	-1.046	-0.829	1.278	-0.080	-0.166	0.087
t5-18:1	-0.232	-0.262	1.024	-0.018	0.183	0.205
t11-18:1	0.634	1.249	-0.498	0.151	0.164	-0.236
c9-18:1	0.668	2.029	-0.123	-0.023	0.211	0.165
t11t15-18:2	-0.973	-0.398	0.743	0.092	-0.026	0.074
t9c12-18:2	-0.257	0.945	-0.918	0.133	-0.013	-0.139
c9c12-18:2	-0.464	-1.011	0.191	0.000	0.035	-0.291
t11c13-18:2	0.475	-0.470	-0.275	0.147	-0.030	0.026
c9t11t15-18:3	0.743	-0.645	0.429	0.317	-0.073	0.023
20:3n-3	0.026	0.595	0.503	0.034	0.042	0.032
20:4n-6	0.357	-0.483	-0.118	-0.068	0.004	-0.237
LA/ALA	-0.772	0.184	-0.315	-0.255	0.235	-0.408
Eigenvalues	20.34	7.84	3.43			
Canonical correlation	0.976	0.942	0.880			
% variance explained	64.35	24.80	10.85			
Class means						
Control	-3.654	-4.074	-0.253			
HOSFO	-2.390	2.312	2.505			
RSFO	-0.937	2.518	-2.478			
LO	8.022	-0.984	0.377			

Table 3. Coefficients of Fisher´s linear discriminant functions for classifying milk fat samples

	Diets[1]			
	Control	HOSFO	RSFO	LO
Constant	-197.712	-293.826	-252.267	-256.248
4:0	87.435	105.961	97.035	88.928
14:0 iso	-871.073	-1075.907	-760.097	-935.903
16:0 iso	4.470	-26.318	-83.582	-127.807
18:0 iso	-441.419	-432.658	24.476	299.977
19:0	-176.505	-94.471	-54.180	656.837
20:0	871.176	1131.872	1017.772	956.473
c7-16:1	78.035	27.031	-13.538	-1.278
c8-16:1	-64.094	170.676	526.629	1941.017
c9-17:1	481.334	417.845	252.023	194.111
t5-18:1	213.186	277.982	-135.904	-2.993
t11-18:1	4.535	10.310	13.166	13.078
c9-18:1	2.390	6.414	7.012	6.572
t11t15-18:2	370.627	291.897	54.289	-183.546
t9c12-18:2	-155.147	90.123	429.152	-206.191
c9c12-18:2	-15.865	-27.826	-31.195	-31.328
t11c13-18:2	-1267.342	-1695.760	-1429.482	-736.150
c9t11t15-18:3	-245.105	-430.183	-540.298	398.369
20:3n-3	2509.141	3604.659	3112.623	3024.257
20:4n-6	-149.711	-261.963	-223.797	-50.861
LA/ALA	1.461	1.250	1.402	-1.260

[1] Control: basal diet without added oil; HOSFO, RSFO and LO: basal diet enriched with high oleic sunflower oil, regular sunflower oil, or linseed oil, respectively.

Figure 1. Canonical discriminant plot of the first two canonical variables



[1] Control: basal diet without added oil. HOSFO, RSFO and LO: basal diet enriched with high oleic sunflower oil, regular sunflower oil, or linseed oil, respectively.