*animal* journal

How does barley supplementation in lambs grazing alfalfa affect meat sensory quality and authentication?

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**Supplementary Material 1:** Detailed description of critical methodologies

*Lamb perirenal and subcutaneous dorsal fat skatole and indole concentrations*

Perirenal and subcutaneous dorsal fat samples were taken for all lambs at 24h post mortem. The subcutaneous dorsal fat was taken from the posterior end of the loin. Fat samples were wrapped in aluminium foil, vacuum-packed in sealable polyamide bags, snap-frozen, and stored at -20°C until analysis. Fat skatole and indole concentrations were measured by high pressure liquid chromatography (HPLC) according to the method described by Batorek et al. (2012), as follows: fat samples were liquefied in a microwave oven for 2 × 1 min at 350 W. The liquefied lipids were centrifuged for 20 min at 11,200 g at 20°C. After centrifugation, the fat was heated to 50°C and 0.5 ± 0.01 g of water-free liquid fat was transferred into 2.5 mL Eppendorf tubes with 1 mL methanol containing 0.050 mg/L 2 of 3-methylindole (internal standard). After stirring for 30 s, the tubes were incubated for 5 min at 30°C in an ultrasonic water bath, put on ice for 20 min, and centrifuged for 20 min at 11,200 g at 4°C. For skatole and indole determinations, 20 μL of supernatant was injected into an HPLC column, and fluorescence was detected (excitation at 285 nm and emission at 340 nm) using a HP1200 system (Agilent Technologies, Waldbronn, Germany). Concentrations were expressed in µg per gram of lipid fraction from adipose tissue. Limit of detection was 0.03 µg/g liquid fat.

*Results of quality control test*

A quality control test of the reference method for measuring fat skatole and indole concentration was performed by analysing in duplicate fat skatole and indole concentration for 50 fat samples in the same day. Fat samples were collected from 50 animals, 41 of them having low fat skatole and indole concentrations and the other 9 having high fat skatole and indole concentrations. Fat samples from the same animal were extracted twice and submitted to the HPLC procedures. At a mean level of 0.06 and 0.05 µg/g liquid fat, coefficients of variation were 4.6 and 9.9% respectively for fat skatole and fat indole concentrations (n= 41 samples). At a mean level of 0.59 and 0.11 µg/g liquid fat, coefficients of variation were 2.6 and 5.4% respectively for fat skatole and fat indole concentrations (n= 9 samples).

**Supplementary Material 2:** Critical methodologies: Codes of statistical models

*Codes of statistical models regarding data for lamb performance, carcass characteristics, fat and longissimus thoracis and lumborum (LTL) muscle colour, absolute value of the mean integral (AVMI), and skatole and indole concentrations*

**data** a1;

title 'AQ718';

input ;

datalines;

**proc** **means** mean min max cv std stderr; by treatment; var dependent variables;

**Run**;

**proc** **sort**; by treatment;

**proc** **univariate** normal plot data=a1;by treatment; var dependent variables;

**Run**;

**proc** **print**;

**Run**;

**proc** **means** mean min max cv std stderr;

var dependent variables;

**Run**;

**proc** **reg** data=a1 lineprinter; model dependent variables=treatment;

output out XY r=dependent variables Resid; plot r.\*p.;

title 'AQ718';

**Run**;

**proc** **univariate** normal plot data=a1; var dependent variables ;

**Run**;

**proc** **reg** data=a1

model dependent variables =treatment;

output out=a2 r=resid p=p;

title1 h=**1** 'Residual Plot';

title **2** h=**1** 'Residuals against Fitted';

plot r.\*p.;

**run**;

**proc** **mixed** data=a1;

class treatment;

model dependent variables = treatment;

random slaughtersession;

lsmeans treatment / adjust= tukey alpha=**0.05**;

ods output diffs=ppp lsmeans=mmm;

**run**;

%include 'C:\pdmix800.sas';

%***pdmix800***(ppp,mmm,alpha=**0.05**,sort=yes);

%***pdmix800***(ppp,mmm,alpha=**0.05**,sort=yes, SLICE=treatment);

**run**;

*Codes of statistical models regarding data for lamb meat sensory evaluation*

**data** a1;

title 'AQ718';

input ;

datalines;

**proc** **means** mean min max cv std stderr;

var dependent variables;

**proc** **means** mean min max cv std stderr; by treatment; var dependent variables;

**Run**;

**proc** **sort**; by treatment;

**proc** **univariate** normal plot data=a1;by treatment; var dependent variables;

**Run**;

**proc** **reg** data=a1 lineprinter; model dependent variables=treatment;

output out XY r=dependent variables Resid; plot r.\*p.;

title 'AQ718';

**Run**;

**proc** **mixed** data=a1;

class panelsession panellist treatment;

model dependent variables = treatment panelsession;

random panellist;

lsmeans treatment / adjust= tukey alpha=**0.05**;

ods output diffs=ppp lsmeans=mmm;

**run**;

%include 'C:\pdmix800.sas';

%***pdmix800***(ppp,mmm,alpha=**0.05**,sort=yes);

%***pdmix800***(ppp,mmm,alpha=**0.05**,sort=yes, SLICE=treatment);

**run**;

*Statistics for analysing the frequency distribution of the number of anthelmintic drenches per lamb (Chi-square test).*

|  |  |  |  |
| --- | --- | --- | --- |
|  | 0 | 1 or 2 drenches | Total |
| AG | 7 | 5 | 12 |
| AGS | 3 | 9 | 12 |
| Total | 10 | 14 | 24 |

SF: stall feeding; AG: alfalfa (*Medicago sativa*) grazing; AGS: alfalfa (*Medicago sativa*) grazing + supplementation with barley

Χ² = 2.94, d.f. = 1; *P* < 0.05.

*Statistics for analysing the frequency distribution of lambs according to the perirenal fat skatole concentration (Chi-square test).*

|  |  |  |  |
| --- | --- | --- | --- |
|  | <0.16 µg/g | ≥0.16µg/g | Total |
| SF | 11 | 1 | 12 |
| AG | 3 | 9 | 12 |
| AGS | 2 | 10 | 12 |
| Total | 16 | 20 | 36 |

SF: stall feeding; AG: alfalfa (*Medicago sativa*) grazing; AGS: alfalfa (*Medicago sativa*) grazing + supplementation with barley

Χ² = 16.44, d.f. = 2; *P* < 0.001.

*Statistics for analysing the frequency distribution of lambs according to the dorsal fat skatole concentration (Chi-square test).*

|  |  |  |  |
| --- | --- | --- | --- |
|  | <0.16 µg/g | ≥0.16µg/g | Total |
| SF | 11 | 1 | 12 |
| AG | 5 | 7 | 12 |
| AGS | 5 | 7 | 12 |
| Total | 21 | 15 | 36 |

SF: stall feeding; AG: alfalfa (*Medicago sativa*) grazing; AGS: alfalfa (*Medicago sativa*) grazing + supplementation with barley

Χ² = 8.23, d.f. = 2; *P* < 0.02.