Tannin-rich sainfoin pellet supplementation reduces fat volatile indoles content and delays digestive parasitism in lambs grazing alfalfa

D. Rivaroli, A. Prunier, K. Meteau, I.N. do Prado and S. Prache

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

*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 Performance 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 Watt. 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

Feeding regimes: SI (stall-feeding concentrate and grass hay indoors); AF (alfalfa grazing); AS (alfalfa grazing + supplementation with sainfoin pellets)

*Codes of statistical models regarding data for animal average daily gain, carcass characteristics and fat colour coordinates*

**data** a1;

title 'AQ717';

input treatment slaughtersession variables;

cards;

**;**

**proc** **print**;

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

class treatment slaughtersession;

model variables = treatment/solution;

random slaughtersession;

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

**run**;

*Codes of statistical models regarding data for longissimus thoracis et lumborum muscle colour coordinates*

**data** a1;

title 'AQ717';

input treatment slaughtersession day variables;

cards;

**;**

**proc** **print**;

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

class treatment slaughtersession day;

model variables = treatment slaughtersession day treatment\*day/solution;

repeated day;

random slaughtersession;

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

**run**;

*Codes of statistical models regarding data for sensory evaluation*

**data** a1;

title 'AQ717';

input panelsession panellist treatment variables;

cards;

**;**

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

class panelsession panellist treatment;

model variables = treatment panelsession/solution;

random panellist;

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

**run**;

*Codes of statistical models regarding the comparison between AF and AS lambs in fat volatile indoles concentration*

**data** a1;

title 'AQ717';

input treatment slaughtersession site variables;

cards;

**;**

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

class treatment slaughtersession site;

model variables = treatment site treatment\*site/solution;

repeated site;

random slaughtersession;

**run**;

*Statistics for analysing the treatment effect on perirenal fat skatole concentration (Kruskall-Wallis test). WT is the rank for T feeding regime*

WSI = 12.5; WAF = 38.26; WAS = 31.5

H= 26.35, degrees of freedom = 2, *P* < 0.001

*Statistics for analysing the treatment effect on dorsal fat skatole concentration (Kruskall-Wallis test).*

WSI = 9.5; WAF = 39.53; WAS = 33.12

H= 36.84, degrees of freedom = 2, *P* < 0.001

*Statistics for analysing the treatment effect on perirenal fat indole concentration (Kruskall-Wallis test).*

WSI = 10.86; WAF = 42.58; WAS = 28.26

H= 37.63, degrees of freedom = 2, *P* < 0.001

*Statistics for analysing the treatment effect on dorsal fat indole concentration (Kruskall-Wallis test).*

WSI = 9.58; WAF = 43.08; WAS = 29.06

H= 42.16, degrees of freedom = 2, *P* < 0.001

*Statistics for comparing the faecal egg count between AF and AS lambs on D41 and D56 (Wilcoxon test).*

Value for εWAF =

D41: 2.34 (*P* < 0.05)

D56: = 3.02 (*P* < 0.01)

*Statistics for comparing the faecal egg count between AF and AS lambs on D69, D83 and D97 (t test).*

D69: t = 1.52, NS

D83: t = 1.18, NS

D97: t = 1.08, NS

*Statistics for comparing the faecal oocyst count between AF and AS lambs on D56, D69 and D97 (Wilcoxon test).*

Value for εWAF =

D56: 3.52 (*P* < 0.001)

D69: 0.808 (NS)

D97: -1.253 (NS)

*Statistics for comparing the faecal oocyst count between AF and AS lambs on D83 (t test).*

t = 1.30, NS

*Statistics for comparing lamb liveweight between AF and AS lambs (t test).*

D43: t = 0.89, NS

D58: t = 0.95, NS

D71: t = 2.01, NS

D85: t = 1.58, NS

D92: t = 1.91, NS

*Statistics for comparing the number of individual anthelmintic drenches between AF and AS lambs.*

Mean (standard deviation) for AF feeding regime: 1.632 (0.597)

Mean (standard deviation) for AS feeding regime: 0.941 (0.556)

t = 3.595, degrees of freedom = 34, *P* < 0.001

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

|  |  |  |  |
| --- | --- | --- | --- |
|  | 0 or 1 drench | 2 or 3 drenches | Total |
| AF | 8 | 11 | 19 |
| AS | 15 | 2 | 17 |
| Total | 23 | 13 | 36 |

Χ² = 7.74, degree of freedom = 1; *P* < 0.01.