

## Metabolome analysis reveals potential mechanisms of mannan oligosaccharides to improve health, growth performance, and fatty acids deposition in *Hu* lambs

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### Supplementary materials S1

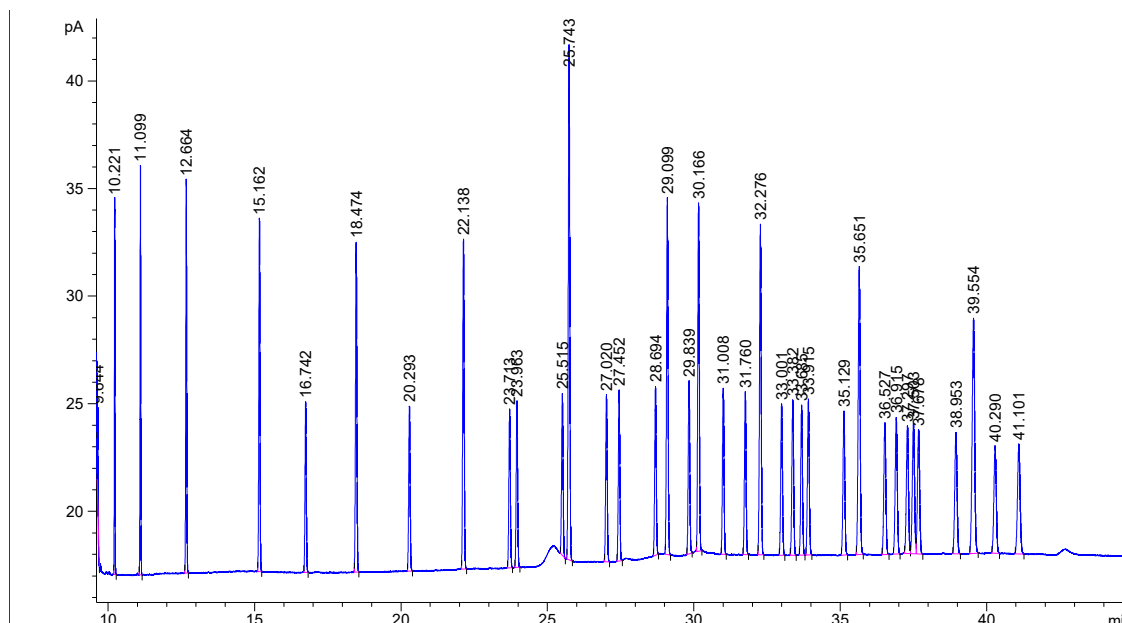
The fatty acid methyl ester synthesis for muscle and adipose samples and fatty acid composition and percent analysis by gas chromatography system[1].

Samples were cut into 1.5-mm rectangular strips with a razor blade. Short cutting times minimized smearing of the fat on the blade. Then, 1.0 g of wet samples were placed into 15-mL tubes, and 0.7 mL of 10 M KOH and 5.3 mL of absolute methanol were added. The tube was incubated in a 55°C water bath for 1.5 h with vigorous hand-shaking for 5 s every 20 min to properly permeate, dissolve, and hydrolyze the sample. After cooling below room temperature in a cold tap water bath, 0.58 mL of 12 M H<sub>2</sub>SO<sub>4</sub> in water was added. The tubes were mixed by inversion and with precipitated K<sub>2</sub>SO<sub>4</sub> present were incubated again in a 55°C water bath for 1.5 h with hand-shaking for 5 s every 20 min. After fatty acid methyl ester synthesis, the tubes were cooled in a cold tap water bath. Three milliliters of hexane was added, and the tubes were vortex-mixed for 5 min on a multitube vortex. The tubes were centrifuged at 3,000 r/min for 5 min in a tabletop centrifuge, and the hexane layer, containing the fatty acid methyl ester, were placed into GC vials.

The fatty acid composition of the fatty acid methyl ester was determined by capillary GC on a SP-2560 (Sigma-Aldrich, Inc., St. Louis, MO, USA), 100 m × 0.25 mm × 0.20 μm capillary column installed on an Agilent 6890 gas chromatograph equipped with an Agilent chemstations, a flame ionization detector, and a split injection (Agilent Technologies, Inc., Santa Clara, CA, USA). The initial oven temperature was 140°C, held for 5 min, subsequently increased to 200°C at a rate of 2°C min<sup>-1</sup>, held for 5 min, subsequently increased to 230°C at a rate of 6°C min<sup>-1</sup>, and then held for 20 min. Nitrogen was used as the carrier gas at a flow rate of 1.2 mL·min<sup>-1</sup>. The injector was set at 220°C and the detector was set at 250°C. The split ratio was 100:1. Fatty acids were identified by comparing their retention times with the fatty acid methyl standards.

The fatty acid methyl ester samples were analysed in triplicated, and the mean was accepted when relative deviation was under 5% among three repeats, or repeated the measurement. Fatty acid percentages were computed as follows[2]:

$$\text{FA}\% = \frac{\text{individual fatty acid area}}{\text{sum total area of fatty acids}} \times 100$$



**Figure S1. Chromatogram of 37 fatty acid methyl ester standards**

According to the chronological order, the 37 standard fatty acid methyl ester were: butyric (C4:0); caproic (C6:0); Octanoic (C8:0); Capric (C10:0); hendecanoic (C11:0); lauric (C12:0); tridecanoic (C13:0); myristic (C14:0); myristoleic (C14:1n5); pentadecanoic (C15:0); pentadecenoic (C15:1n5); palmitic (C16:0); palmitoleic (C16:1n7); heptadecanoic (C17:0); heptadecenoic (C17:1n7); stearic (C18:0); elaidic (C18:1n9t); oleic (C18:1n9c); linolelaidic (C18:2n6t); linoleic (C18:2n6c); arachidic (C20:0);  $\gamma$ -linolenic (C18:3n6); eicosenoic acid (C20:1n11c);  $\alpha$ -linolenic (C18:3n3); heneicosanoic (C21:0); eicosarboxylic acid (C20:2n11c); behenic (C22:0); eicosatrienoic (C20:3n6); erucic (C22:1n9); eicosatrienoic (C20:3n3); tricosanic (C23:0); arachidonic (C20:4n6); docosadienoic (C22:2n6); lignoceric (24:0); eicosapentaenoic (C20:5n3); nervonic (C24:1n9); docosahexaenoic (C22:6n3).

## References

- 1 O'Fallon, J. V.; Busboom, J. R.; Nelson, M. L.; Gaskins, C. T. A Direct Method for Fatty Acid Methyl Ester Synthesis: Application to Wet Meat Tissues, Oils, and Feedstuffs. *J. Anim. Sci.* **2007**, *85*, 1511–1521.
- 2 Pewan, S. B.; Otto, J. R.; Kinobe, R. T.; Adegboye, O. A.; Malau-Aduli, A. E. O.; Lamb, M. Eating Quality and Human Health-Promoting Omega-3 Long-Chain Polyunsaturated Fatty Acid Profiles of Tattykeel Australian White Sheep: Linebreeding and Gender Effects. *Antioxid.-Basel.* **2020**, *9*, 1118.