**The effect of harvest time of timothy grass on in-vitro and in-sacco digestibility in horses**

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**Shot summary:** In-sacco and in-vitro digestibility of timothy harvested at different maturities were investigated. The digestibility decreased as the plants matured, and the there was a good correlation between the two methods.

**Ethical animal research:** The study was conducted in accordance with Norwegian legislation and ethical guidelines.

**Source of funding:** The Swedish-Norwegian Foundation for Equine Research

**Competing interests:** None.

**Poster:** Yes/No (state what you prefer)

**Oral presentation:** Yes/No (state what you prefer)

**The effect of harvest time of timothy grass on in-vitro and in-sacco digestibility in horses**

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**Introduction:** Plant maturity at harvest of grass affects the energy and chemical content of forage as well as the digestibility. The objective of the present study was to investigate how plant maturity affects digestibility in horses using in-vitro and in-sacco methods. The hypothesis was that increased plant maturity of timothy grass alters the nutrient composition and lowers the digestibility, and that this can be quantified using in-vitro and in-sacco methods.

**Materials and methods:** Timothy grass was harvested from the Norwegian University of Life Sciences, Vollebekk research farm eight times from the 22nd of May until the 3rd of July 2021. The in-sacco digestibility was estimated using cecum cannulated horses. Four bags were fastened with rubber bands to a 78 cm long nylon tube and incubated in the cecum for 0, 2, 4, 8, 16 and 24 h, then washed, dried for 48 h and weighed to determine dry matter (DM) loss after incubation. Inoculum was collected from three cecum cannulated horses and used in the ANKOM RF in-vitro gas production test (IVGPT). One gram of feed, 66 ml of buffer solution and 33 ml of cecum inoculum was added to 250 ml in-vitro bottles and incubated for 48 h.

**Results:** Chemical analysis showed a decrease in crude protein (CP) and an increase in neutral detergent fiber (NDFom) from early to late harvest, as evidence of progressive maturity in the grass. Further, water-soluble carbohydrates (WSC) content was high, low and thereafter high in early, middle, and late plant maturity, respectively. Fructan content was highest in late maturity. The in-sacco digestibility of DM ranged from 89.5 to 52.9 % (P<0.001) and in-vitro DM digestibility ranged from 82.4 to 53.4 (P<0.001). The two methods correlated well (r=0.85, P<0.001).

**Conclusion:** The digestibility decreased as the grass matured, and the there was a good correlation between the two methods.

**Key words:** fructan, gas production, hay, methods