PhD School on Agriculture, Environment and Bioenergy

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(XXXVI cycle, 2020-23)

Project draft

1. Field of interest

Animal nutrition and feeding (AGR/18)

2. Project title

Tannins enrichment of the diet for increasing the sustainability of dairy goat production

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4. Relevance of the topic and state of the art

Livestock contributes 14.5% of anthropogenic Greenhouse Gasses (GHG) emissions and about 2/3 of these are caused by ruminants. One of the most important GHG is methane (CH_4). Due to enteric fermentations, the 44% of GHG emitted by ruminants is CH_4. Moreover, ruminants contribute to the release of nitrogen in the environment in the form of ammonia (NH_3) and nitrous oxide (N_2O), for 29% of total GHG emitted (Gerber *et al*., 2013). Both methane and nitrogen emissions are not only related to environmental problem, but are also associated to energy and organic matter losses that undermine efficiency and productivity (Niderkorn *et al*., 2020).

One of the possible strategies to reduce emissions and to improve efficiency of ruminant breeding could lie in the addition of tannins to the diet. Tannins are polyphenolic substances with variable complexity and usually classified into two groups: Condensed Tannins (CT) and Hydrolysable Tannins (HT). CTs are polymers of flavonoids and have high molecular weight; HTs are polyesters of gallic acid and various individual sugars and have low molecular weight (Jayanegara *et al*., 2011). As reported by Mueller-Harvey et al. (2019), despite the widespread occurrence of CTs, but also HTs, in the plant kingdom, there are still large gaps in our knowledge.

Depending most of all on their concentrations in the diet but also on animal species and other factors, tannins could have both adverse and positive effect on ruminants (Makkar, 2003; Zeller, 2019). One of the positive effect of tannins is the reduction of CH_4 emitted from ruminal fermentation. This effect has been seen in several *in vitro* trials but there are some aspects that need to be explored like the concentration of tannins added in ruminal fluids, their source and type. In this sense, Jayanegara et al. (2010) have found a greater and more constant effect of HT from chestnut and sumach respect CT from mimosa and quebracho. Like observed by Jayanegara et al. (2011) it is necessary to investigate even if the reduction of emissions is linked to a reduction of digestibility of the organic matter. Moreover, it is important to consider the animal species. Bovines emitted more methane than small ruminants as measured on a degraded organic matter basis and, as reported by Bueno et al. (2015), tannins have greater effects in large ruminants than in small ruminants.

Consumption of CT and HT by ruminants can reduce urinary nitrogen excretion and increase faecal nitrogen excretion without negatively affecting animal performance (Mueller-Harvey *et al*., 2019). This shift can improve soil nitrogen status, reduce N_2O emissions and nitrogen leachate into groundwater.

Negative effects include reduced palatability of the diet, which leads to a reduction of the dry matter intake, and some antinutritive and toxic effect associated with high concentrations of tannins in the diet. However, it is necessary to consider the species of ruminants. Indeed, as reported by Aboagye and Beauchemin (2019) goats are less susceptible to the reduced palatability due to higher production of tannin-binding salivary proteins relative to sheep and cattle.

5. Layout of the project

5.1. Materials & Methods

The research activities will be organised at different level exploiting the labs facilities of the Department of Agricultural and Environmental Sciences – Production, Landscape, Agroenergy (DiSAA) (Milan) and of the experimental farm Cascina Baciocca (Cornaredo, MI). *In vitro* trials, simulating the rumen digestion process using rumen fluid from cows and goats as donor animals, will be performed. The flock of Alpine dairy goats of Cascina Baciocca will be used to conduct the *in vivo* experiments, one of which by means of the indirect calorimetry system (four respiration chambers) equipped to determine the respiratory exchange (O_2, CO_2 and CH_4) of the animals.
In particular, the *in vitro* trials will be performed to evaluate: i) the nutritive value of forages and concentrate naturally rich in CT or HT, and of a total mixed ration (TMR) enriched with CT or HT extracts (mainly from Quebracho and Chestnut); ii) the effects of CT and HT on the end products (volatile fatty acids and $N\text{-}NH_3$) of rumen digestion process; iii) the effects of CT and HT on the rumen fermentation kinetics of total gas and methane production; iv) the effects of CT and HT in relation to the difference species (bovine and goat) used as source of rumen fluid inocula.
In these tests, polyethylene glycol (PEG) will be added in order to evaluate tannins effect. Moreover, microbial community before and after incubations will be analysed.
Regarding the *in vivo* trials, as first, an experiment will be conducted in the experimental farm “Cascina Baciocca” on twenty four Alpine goats divided into four different pens to evaluate the palatability of concentrates enriched with two different levels of Quebracho and Chestnut extracts. After a limited period of adaptation to new feeds, goats' feed preferences will be analysed considering the type and dosage of tannins. During the trial milk yields will be recorded and samples for composition analysis will be taken. Moreover, the feeds enriched with tannins will be compared with the same with the addition of (PEG), a tannin binding substance, in order to evaluate the tannins effect.
The *in vivo* trial on lactating goats, using the respiration chambers in the experimental farm “Cascina Baciocca”, will evaluate the effects of diets with high tannin content, comparing the source of tannins (forage *vs* extracts) and the type (CTs *vs* HTs). A replicated Latin square design with four treatments will be applied. Goats will be individually placed in metabolic cages and then in respiratory chambers. Feed intake, diet digestibility and milk production will be assessed. Milk samples will be tested for its characteristics, including the fatty acids composition. Faeces and urine production will be recorded, sampled and analysed in order to determine the *in vivo* digestibility and the N balance. The determination of individual heat production, estimated indirectly by the respiratory exchanges, will allow to determine the energy balance. Rumen fluid will be collected and sampled with an esophageal probe. Enteric methane production will be determined and related to rumen and faeces microbiome.

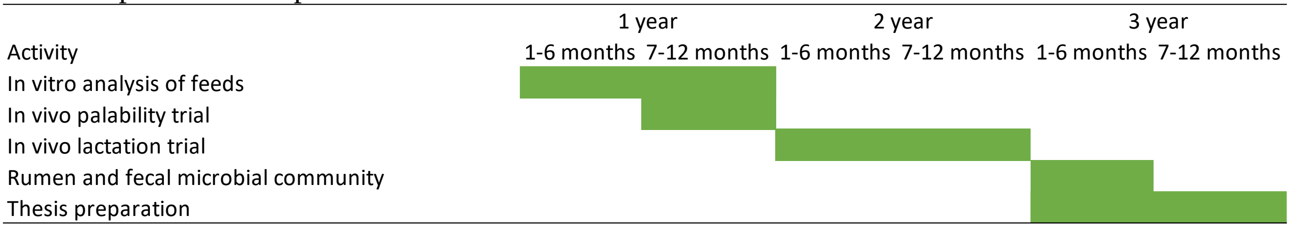
5.2 Schedule and major steps

The major steps that should be achieved during the project are:

- In vitro analysis: Sampling for chemical and in vitro analyses. The collected samples (forages and concentrate naturally rich in CT or HT, and of a TMR enriched with CT or HT extracts) will be chemically and *in vitro* analyzed. Year 1.
- In vivo experiment 1: an experiment will be conducted on Alpine goats to evaluate the palatability of concentrates enriched with two different levels of Quebracho and Chestnut extracts. Year 1.

- In vivo experiment 2: an experiment on lactating goats, using the respiration chambers will evaluate the effects of diets with high tannins content, comparing the source of the tannins (forage *vs* extracts) and the type (CTs *vs* HTs). Year: 2.
- Rumen and faeces microbiota analysis and relationship with enteric methane production. Year: 3.

Detailed plan of the experimental activities



6. Available funds

Programma di Sviluppo Rurale 2014-2020, Operazione 16.1.01 "Valutazione di un sistema integrato per il controllo delle parassitosi gastrointestinali degli allevamenti caprini: implicazioni ambientali, agro-zootecniche e sanitarie" (CASCO): € 574.445,55 (total value of the project)

7. Literature

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