

# PhD School on Agriculture, Environment and Bioenergy

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(XXXVI cycle, 2020-23)

## Project draft

### 1. Field of interest

*Indicare il/i settore/i scientifico disciplinari: AGR/18*

### 2. Project title

**Tannin enrichment of the diet to increase the sustainability of dairy goat production.**

**3. Tutor** (membro del Collegio dei Docenti): **prof. G. Matteo Crovetto**

**- Eventually: co-tutor/s: prof. Luca Rapetti**

### 4. Relevance of the topic and state of the art:

Livestock contributes 14.5% of anthropogenic Greenhouse Gases (GHG) emissions and about 2/3 of these are caused by ruminants. One of the most important GHG is methane (CH<sub>4</sub>). Due to enteric fermentations, 44% of the GHG emitted by ruminants is CH<sub>4</sub>. Moreover, ruminants contribute to the release of nitrogen in the environment in the form of ammonia (NH<sub>3</sub>) and nitrous oxide (N<sub>2</sub>O), for 29% of total GHG emitted (Gerber *et al.*, 2013). Both methane emission and nitrogen excretion are not only source of environmental problems, 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 the environmental problems previously described and to improve the efficiency of cattle could be the addition of tannins to the diet. Tannins are polyphenolic substances with variable complexity and are 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 and HTs in 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<sub>4</sub> 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<sub>2</sub>O 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 (draft)**

### **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  $N-NH_3$ ) 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 boxes 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 tannins content, comparing the source of the tannins (forage *vs* extracts) and the type (CTs *vs* HTs). A replicated Latin square design with four treatments will be applied. Goats will be placed in the metabolic cages and then in the 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 and samples of them will be 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 studied and related to the rumen and faeces microbiome.

### **5.2. Schedule and major steps (3 years): mezza pagina max**

## **6. Available funds (source and amount):**

**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: max 10 citazioni

- 1) Aboagye I.A., Beauchemin K.A. 2019. Potential of Molecular Weight and Structure of Tannins to Reduce Methane Emissions from Ruminants: A Review. *Animals*, 2019, 9, 856. doi: 10.3390/ani9110856.
- 2) Bueno I.C.S., Brandi R.A., Franzolin R., Benetel G., Fagundes G.M, Abdalla A.L., Louvandini H., Muir J.P. 2015. In vitro methane production and tolerance to condensed tannins in five ruminant species. *Animal Feed Science and Technology*, Volume 205, 2015, Pages 1-9. ISSN 0377-8401. doi:10.1016/j.anifeedsci.2015.03.008
- 3) Gerber P.J., Steinfeld H., Henderson B., Mottet A., Opio C., Dijkman J., Falcucci A., Tempio G. 2013. Tackling Climate Change through Livestock: A Global Assessment of Emissions and Mitigation Opportunities. FAO: Rome, Italy, 2013.
- 4) Jayanegara A., Goel G., Makkar H.P.S., Becker K. 2010. Reduction in methane emissions from ruminants by plant secondary metabolites: effects of polyphenols and saponins. Odongo N.E., Garcia M., Viljoen G.J. Sustainable Improvement of Animal Production and Health, FAO, Rome, Italy, 2010, pp. 151-157. È un articolo di un libro della FAO, non so se è citato correttamente
- 5) Jayanegara A., Leiber F., Kreuzer M. 2011. Meta-analysis of the relationship between dietary tannin level and methane formation in ruminants from in vivo and in vitro experiments. *Journal of Animal Physiology and Animal Nutrition*. 96: 365-375. doi:10.1111/j.1439-0396.2011.01172.x
- 6) Makkar H.P.S. 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. *Small Ruminant Research*, Volume 49, Issue 3, 2003, Pages 241-256. ISSN 0921-4488. doi:10.1016/S0921-4488(03)00142-1
- 7) Mueller-Harvey I., Bee G., Dohme-Meier F., Hoste H., Karonen M., Kölliker R., Lüscher A., Niderkorn V, Pellikaan W.F., Salminen J., Skøt L., Smith L.M.J, Thamsborg S.M., Totterdell P., Wilkinson I., Williams A.R., Azuhnwi B.N., Baert N., Brinkhaus A.G., Copani G., Desrues O., Drake C., Engström M., Fryganas C., Girard M., Huyen N.T., Kempf K., Malisch C., Mora-Ortiz M., Quijada J., Ramsay A., Ropiak H.M., Waghorn G.C. 2019. Benefits of Condensed Tannins in Forage Legumes Fed to Ruminants: Importance of Structure, Concentration, and Diet Composition. *Crop Sci*. 59:861-885. doi:10.2135/cropsci2017.06.0369
- 8) Niderkorn V, Barbier E., Macheboeuf D., Torrent A., Mueller-Harvey I., Hoste H. 2020. In vitro rumen fermentation of diets with different types of condensed tannins derived from sainfoin (*Onobrychis viciifolia* Scop.) pellets and hazelnut (*Corylus avellana* L.) pericarps. *Animal Feed Science and Technology*, Volume 259, 2020. ISSN 0377-8401. doi:10.1016/j.anifeedsci.2019.114357.
- 9) Zeller W.E. 2019. Activity, Purification, and Analysis of Condensed Tannins: Current State of Affairs and Future Endeavors. *Crop Sci*. 59:886-904. doi:10.2135/cropsci2018.05.0323