Response of anti-nutrients compounds and methane production to bio-inoculation of arbuscular mycorrhizal fungi and *Rhizobia* bacteria on three herbaceous forage legumes

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1. BACKGROUND

Forage legumes are potential used as an alternative fodder resource for nitrogen supplement of ruminant diet.

Nevertheless, Tropical region is characterized by severe animal feed shortages, particularly during the dry season.

Forage legumes interactions with bio-inoculation

Even so, improved high quality forages with high crude protein usual contained and contaminated with high anti-nutrient compounds which reduces forage intake and digestibility.

Improved forage quality due to bio-fertilization could increase forage legume species nutritive value, decreases the amount of anti-nutrients available on forages and enrich dry matter intake, forage digestibility and reduce methane gas production.

2. Materials and Methods

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Study Sites and layout

- The research was carried out at the University of Fort Hare Research Farm.
- The farm is located at latitude 32°46′ S and longitude 26°50′ E at an elevation of approximately 535 m above sea level (m.a.s.l.).
- It has an average annual rainfall of about 575 mm, The highest and minimum temperatures are 24.6 and 11.1 °C, respectively, with an average temperature of 17.8 °C (Mpongwana *et al.*, 2023a).
- The land was fallow for 4 years before planting in 2017.
- According to the International Union of Soil Sciences (IUSS) Working Group World Reference Base (2022), the soil at the farm is alluvial in origin and is classified as *Eutric Cambisols* (ochric).
- Further details on the soil physicochemical properties of the experimental site before the commencement of a field trial are presented in a study by Mpongwana *et al.* (2023a).

Research design and treatments

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- The study was carried out as a 3 x 2 x 2 factorial experiment arranged in a randomized complete block design (RCBD) with 12 treatments that were replicated four times.
- The field area was 59 x 22 m, and measurements were gathered from 48 plots, each measuring 4 × 4 m.
- The plots and blocks were separated by 1 and 2 m, respectively.
- The treatments comprised three forage legumes [cowpea (black-eyed pea cultivar), lablab (Rongai), and mucuna (*Utilis*)]; two AMF (with and without inoculation); and two *Rhizobium* (with or without inoculation).
- The rates for inoculating seeds with Mycoroot™ products were calculated using the optimal recommended rate of 45 kg P ha^{-1} (Moila *et al.*, 2020).
- The plots were fertilized with a single superphosphate rate of 40 kg P ha^{-1} with 10.5% phosphate (0:46:0%; N: P_2O_5:K_2O).
- The forage legumes were planted at a depth of 4– 6 cm with an inter- and intra-row spacing of 0.9 x 0.3 m and a seeding rate of 50 kg. ha^{-1}, making a plant density of 37 037 plants ha^{-1}.

Treatments

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Treatments	
T1	Cowpea Control
T2	Cowpea + Rhizobium
T3	Cowpea + AM fungi
T4	Cowpea + AM fungi + Rhizobium
T5	Lablab Control
T6	Lablab + Rhizobium
T7	Lablab + AM fungi
T8	Lablab + AM fungi + Rhizobium
T9	Mucuna Control
T10	Mucuna + Rhizobium
T11	Mucuna + AM fungi
T12	Mucuna + AM fungi + Rhizobium

Forage anti-nutrients analysis

- The harvested forage (at 120 days) was measured from each plot, and fresh weights were oven-dried at 60 ^{o}C for 48 h to obtain dry mass.
- The samples were then ground using a Wiley mill to pass through a 1 mm sieve screen for chemical analysis and kept in plastic bags at room temperature before laboratory analysis (Matizha *et al.*, 2001).
- The dry matter content (DMC) was determined by oven drying at 105 ^{o}C for 24 hours [Association of Official Analytical Chemists (AOAC), 2005].
- The samples were analyzed in triplicate for total condensed tannins, total polyphenols, hydrolysable tannins, and saponins content.
- Total condensed tannins and hydrolysable tannins were determined using the methods as reported by Polshettiwar *et al*. (2007).
- The total polyphenols were estimated spectrophotometrically using the Folin-Ciocalteu method as described by Makkar (2005) and Samatha *et al*. (2012).
- The hydrolysable tannins were estimated using Gallotannins method described by Porter *et al*. (1986).

- The saponins content in the plant extracts was determined using the method described by Obadoni and Ochuko (2001).
- The *in vitro* gas production was determined according the procedure described by Menke and Steingass (1988) and Fievez *et al*. (2005).
- Animal as rumen inoculum donors- The methane gas production by each herbaceous legume was estimated immediately after withdrawal from the incubator through dispensing 4 ml of 10N sodium hydroxide (NaOH) into each incubated sample at the end of 48 hours of incubation periods.
- The content of NaOH was introduced using 5 ml capacity syringe as reported by Fieves *et al*. (2005).
- The remaining volume of gas was recorded as methane according to method of Fieves *et al*. (2005) through calibration reading.
- Other In vitro parameters that were measured are, IVDMD, NGV, ME, OMD, SCFA, IVRD (48hrs)

3. Statistical analysis

- Data on anti-nutrients and *in vitro* digestibility were analyzed using ANOVA by the general linear model procedure of SAS (SAS, 2012) version 9.4. Means were separated using Least Significant Difference (LSD) at 5% significant level. The statistical model shown below was used:
- $Y_{ijkl} = \mu + B_h + L_i + M_j + R_k + (LM)_{ij} + (LR)_{ik} + (MR)_{jk} + (LMR)_{ijk} + eijkl$
- Where: Y_{ijkl} = is the dependent variable (e.g. anti-nutrients and *methane production*)
- μ = overall mean; B_h = h^{th} block effect (h = 1, 2, 3, 4); L_i = i^{th} effect of legume species (i = 1, 2, 3); M_j = j^{th} effect of AM fungi (j = 1, 2); P =effect of *Rhizobium* inoculum (k = 1, 2); LM_{ij} = ij^{th} interaction between legume species and AM fungi: LR_{ik} = ik^{th} interaction between legume species and *Rhizobium* inoculum: MR_{jk} = jk^{th} interaction between AM fungi and *Rhizobium* inoculum: LMR_{ikj} = ijk^{th} interaction effect of legumes species, AM fungi and *Rhizobium* inoculum; eijkl = residual error.

Figure 1: Total condensed tannins

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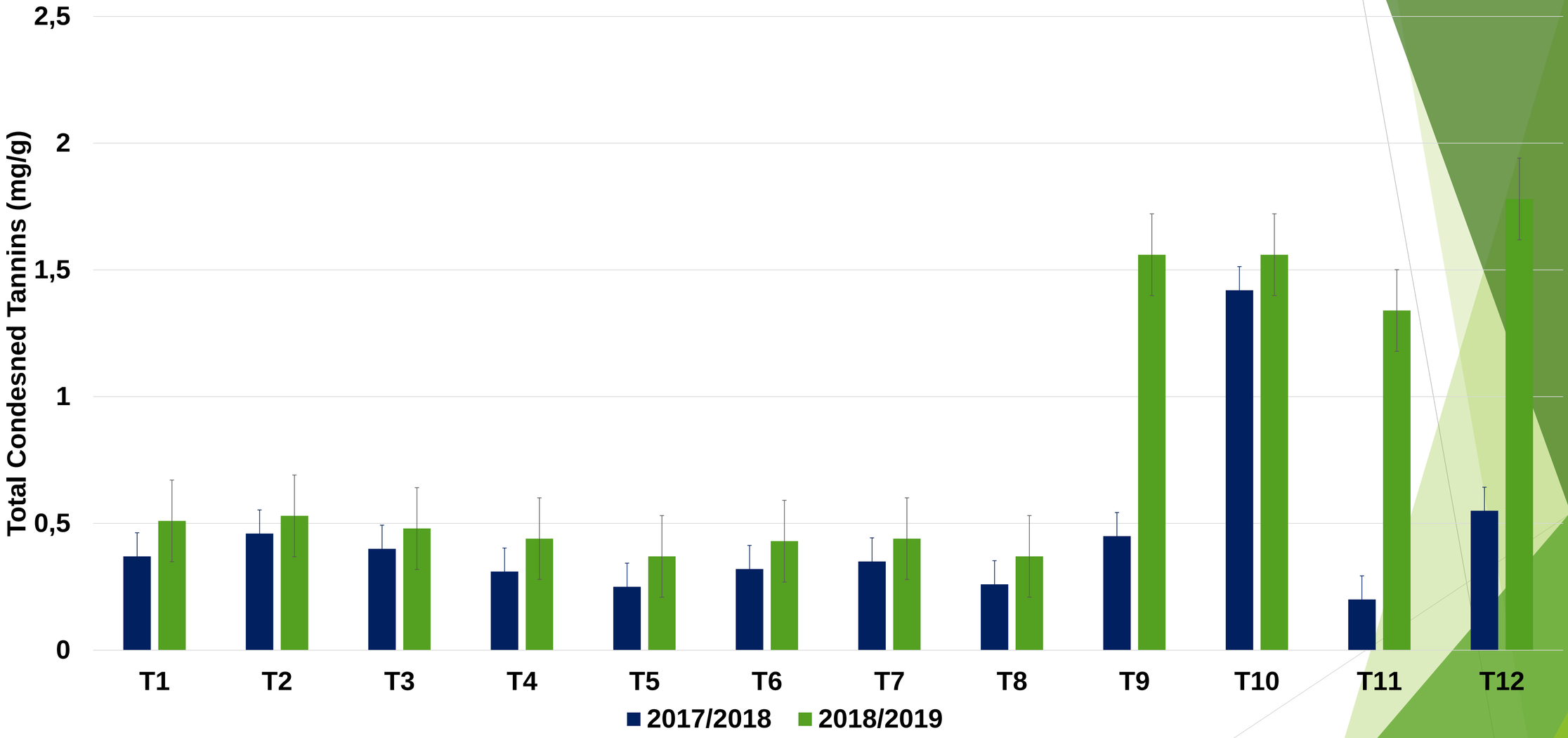

Figure 2: Total polyphenols (mg/g)

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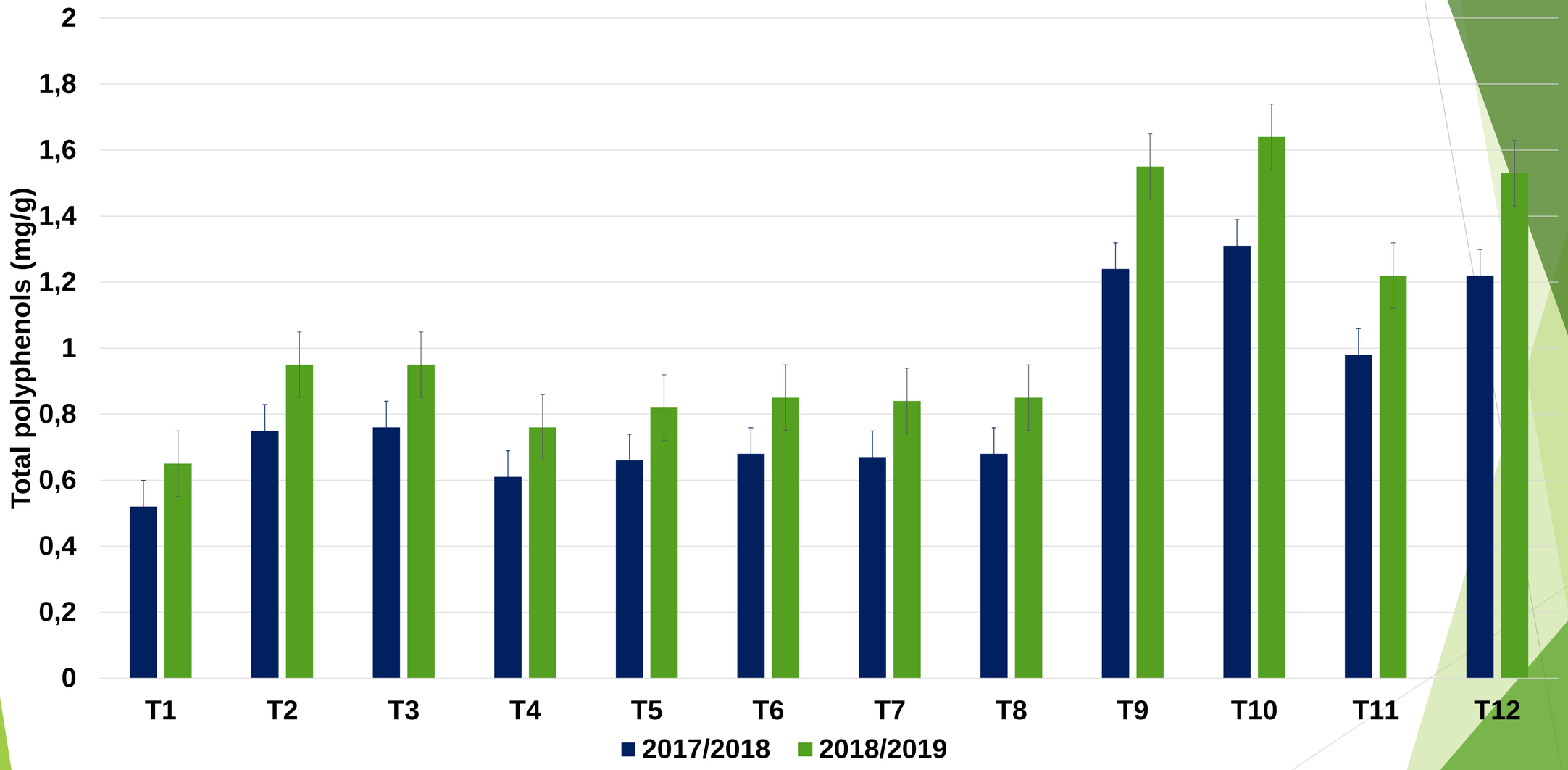

Figure 3:Hydrolysable tannins (mg/g)

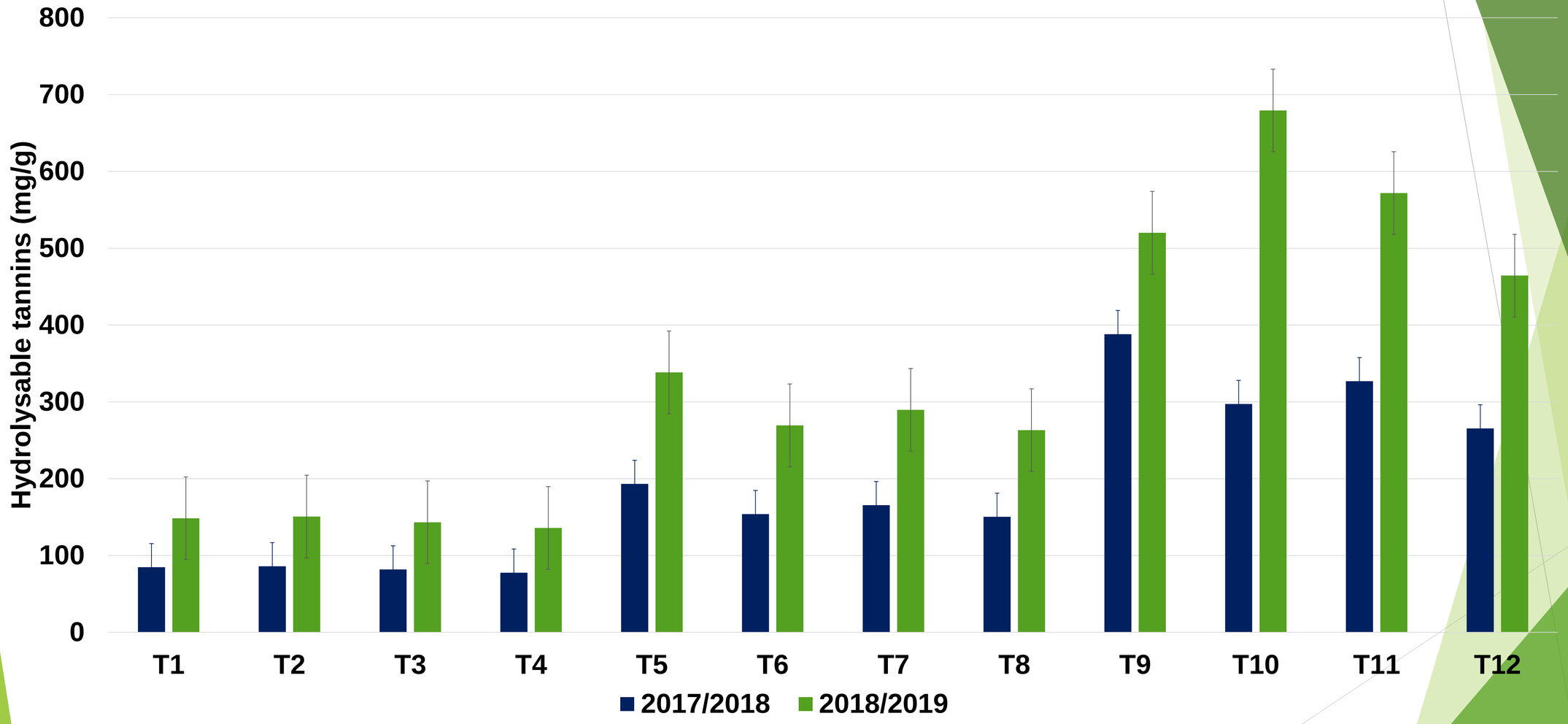

Figure 5: Saponins (%)

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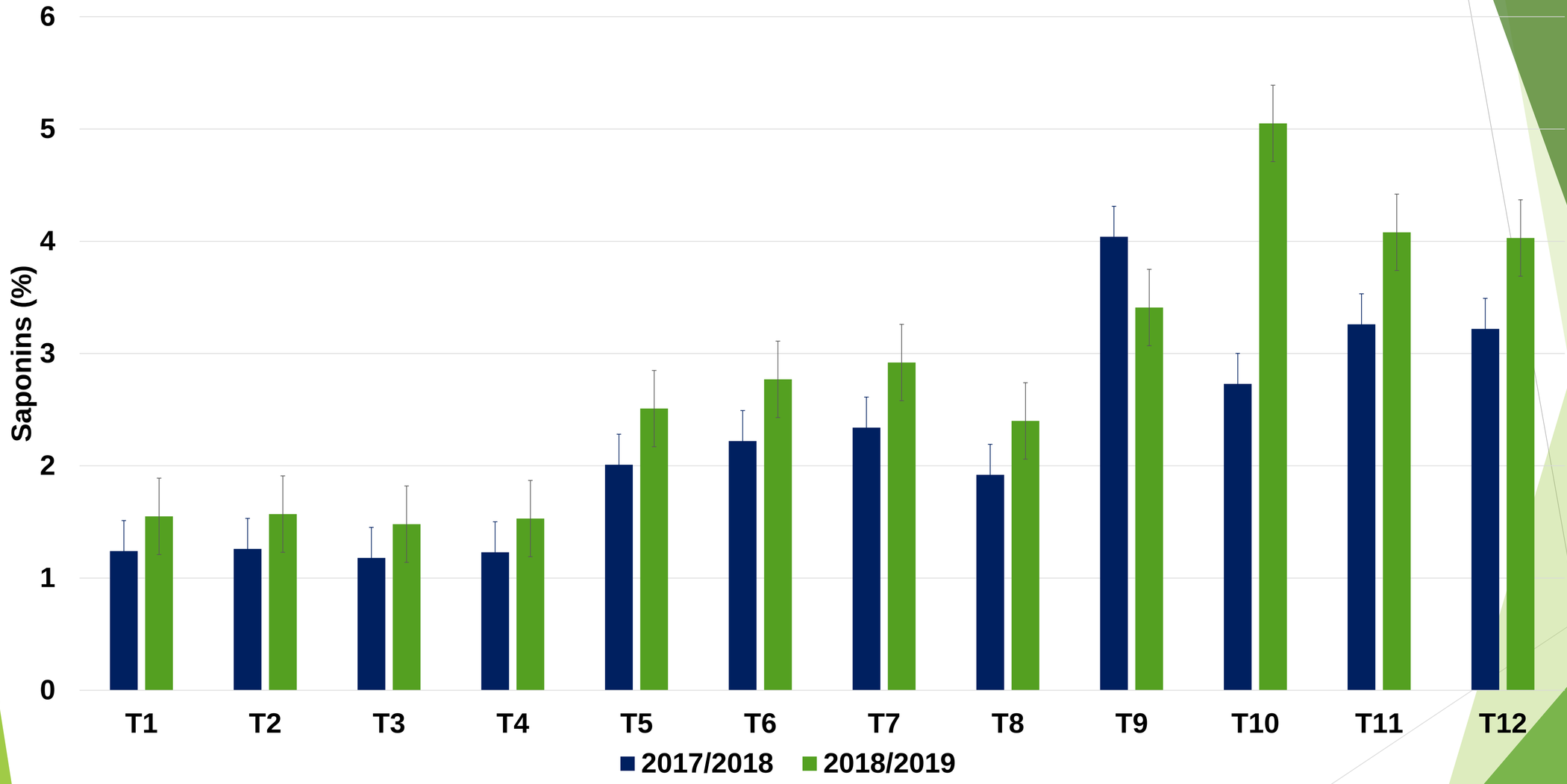

Figure 5: Methane Production (ml/200 mg)

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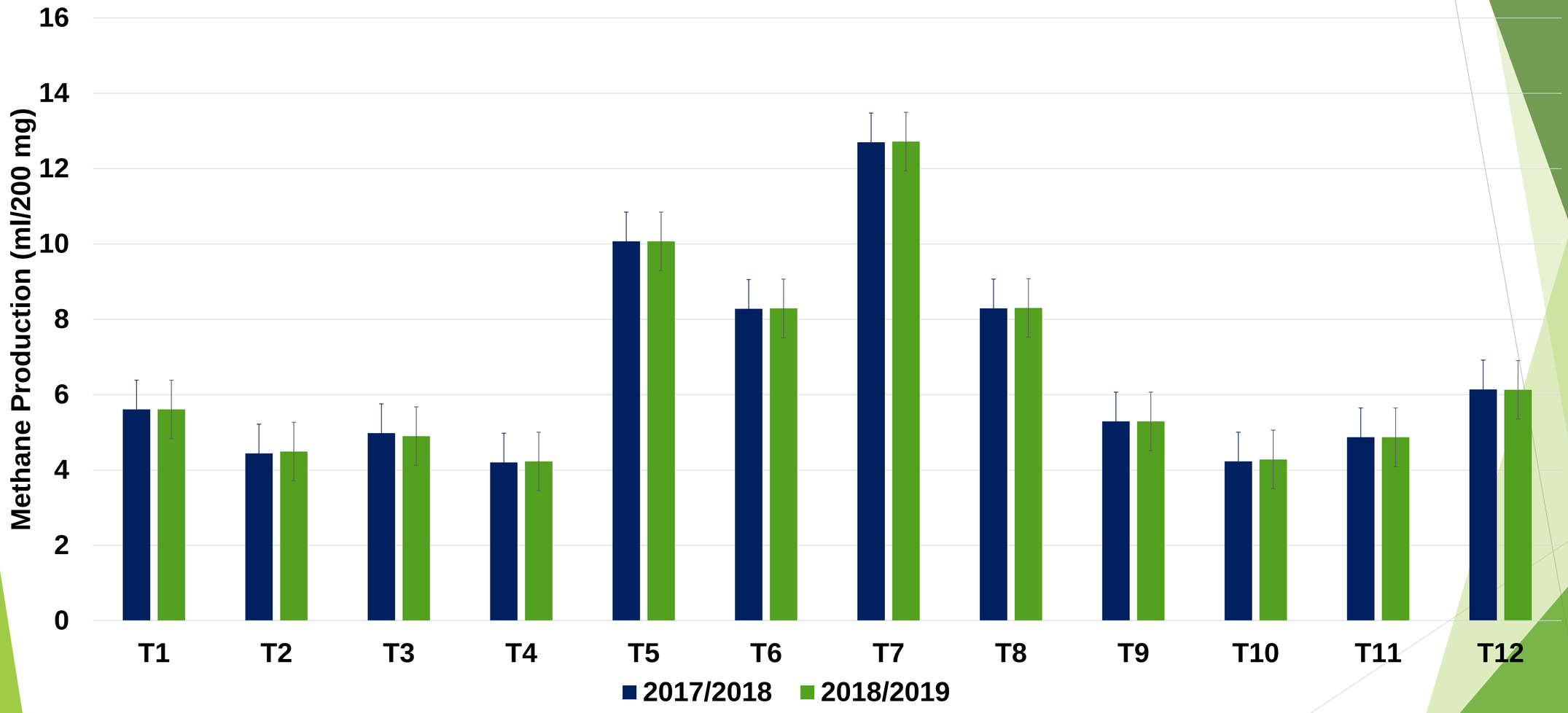

5. Discussion

- Results indicated that T9 and T12 interaction had highest levels of total condensed tannins, total polyphenols, hydrolysable tannins, and saponins over two seasons.
- The results also showed that bio-inoculation reduced methane production gas.
- Lablab control (T5) and T7 (lablab + AM fungi) had highest methane gas production over two season.

6. Conclusion

- The results showed that dual inoculation has the ability reduce methane gas production
- and improve forage quality as well as low level of anti-nutrient compounds so these forages can be included to improve ruminant dietary feed.