## ABSTRACT

## Development of Lc-BHLH and C1-MYB double gene transformed alfalfa: Effect of double gene transformation on ruminal degradability of protein and carbohydrate and methane production

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The transcription factors Lc-bHLH and C1-MYB associated with regulation of flavonoid pathway were transformed into alfalfa with the intention of stimulating proanthocyanidin (aka condensed tannin) that would reduce the protein degradation and digestive disorders in ruminants. The objective of the current study is to evaluate the Lc and C1 gene transformed alfalfa (Lc1C1 and Lc3C1) compared to non-transgenic (NT) alfalfa in relation to (1) the degradable protein and carbohydrate fractions and (2) methane gas production by rumen microorganisms. The extractable anthocyanidin contents in double gene alfalfa averaged about  $185\pm74 \ \mu g/g$  DM. The Lc1C1 genotype has shown a significantly lower (P<0.05) ruminally degradable true protein content (8%) than both Lc3C1 (12%) and NT (10%) genotypes. The total ruminally degradable carbohydrate contents tended to be lower (P=0.08) in double gene transformed alfalfa compared to NT. There was no significant difference in methane gas production among genotypes but total gas production was tended to be lower (P=0.07) in LcC1 genotypes. In conclusion, double gene transformation resulted in anthocyanidin accumulation in aerial parts of the plants and Lc1C1 gene combination has reduced the degradable protein content.