Changes in milk fatty acid (FA) profile were examined over eight weeks in eight multiparous Holstein dairy cows grazing on two pastures with differing forage diversity, a diverse pasture (>15 species) and a monoculture pasture. Cows grazed on each pasture for four consecutive weeks. Weekly milk samples were collected from each cow and the milk composition and fatty acid profile determined. Pasture samples were taken on a weekly basis and analyzed for chemical composition and fatty acid profile. When compared to the monoculture, the diverse pasture had higher proportions of monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), oleic acid (18:1 9c), linoleic acid (18:2 9c, 12c), and total n-6 fatty acids. The monoculture pasture had higher proportions of saturated fatty acids (SFA) and α-linolenic acid (18:3 n-3) when compared to the diverse pasture. Milk, fat, and protein yields were highest during the diverse pasture treatment. The milk fatty acid profile of cows grazing the diverse pasture had higher proportions of SFA while the milk fatty acid profile of cows grazing the monoculture had higher proportions of MUFA and PUFA concomitant with lower proportions of SFA. The proportion of de novo synthesized fatty (4:0 - 14:1) acids and mixed fatty acids (de novo and preformed [16:0 and 16:1]) were higher during the diverse period and the proportion of preformed fatty acids (≥ 18:0) was higher during the monoculture period. Overall, the experiment confirmed the ability to modulate the fatty acid profile based on pasture forage diversity level by demonstrating differing milk fatty acid profiles for the diverse pasture period compared to the monoculture pasture period,
with the milk fatty acid profile being more favorable from the monoculture treatment period.