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It is well known that behavioral stress response is related to stress-related hormones. In ruminants, the relationships between rumen fermentation and endocrine system are also suggested. We examined the effect of rumen fermentation characteristics on stress-related hormones and behavior of sheep under different diet conditions. Eight Suffolk lambs were allocated into high concentrate group (HC; 80% of concentrate and 20% of bermudagrass hay, n=4) and roughage group (RH; 100% of bermudagrass hay, n=4). The experiment consisted of 8-10 days of acclamation and 4-days measurement periods, and repeated twice. Blood samples (10 mL) were collected from jugular vein on day1 of each measurement period for plasma growth hormone (GH) and cortisol were measured. On day 2, rumen fluid was collected orally and the concentration of acetic acid, propionic acid, and butyric acid was measured. The open-field test (OFT) was conducted on day 4 of each period, and behavior of lambs were recorded for 20 minutes. In HC, the concentration of butyric acid and GH concentration tended to be higher than in RH (P<0.1). Frequency of escape attempt in OFT was also higher in HC than RH (P<0.05). Correlation analysis showed a positive relationship between sniffing frequency to the novel object and concentration of acetic acid/propionic acid ratio in the rumen (ρ =0.545, P<0.05), and negative relationship between environmental exploration frequency and blood cortisol concentration (ρ =-0.528, P<0.05). From these results, it was suggested that concentration of volatile fatty acids in the rumen affected behavioral stress response of sheep through changes in plasma hormone concentration.