One of the greatest challenges facing producers is heat stress and its adverse effects on the performance and well-being of livestock. Because of this, there has been a long-term interest in identifying cattle that can tolerate heat stress conditions and still exhibit high productivity. A study was performed to evaluate differences in thermoregulatory ability of two breeds with known differences in heat tolerance to determine reliable physiological markers. Angus and Romosinuano steers were initially housed in climatic chambers under non-stress conditions (TN; 21°C) before cyclic heat stress (HS; 26°C night; 36°C day). Romosinuano is a tropical, Criollo beef breed that is native to Colombia, whereas Angus is a heat-sensitive breed originating from Scotland. Rectal temperature and respiration rate were measured six times daily. Sweat rates at shaved sites were recorded on specific days. Blood samples were taken once a week. The Romosinuano breed maintained a lower respiration rate, sweat rate, and rectal temperature than the Angus breed throughout TN. Both breeds increased sweat rate, respiration rate, and rectal temperature during HS, with the Angus breed maintaining the higher levels. This suggests that heat production or metabolism may be lower in the Romosinuano breed, thus requiring less heat loss to maintain heat tolerance than the Angus breed. Blood analyses showed that Angus steers exhibited HS-induced increases in prolactin, creatinine, and cholesterol, with no change for Romosinuano steers. The present study provides additional physiological and endocrine parameters that may aide in the identification of markers of heat sensitivity for beef cattle.