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Biological and physiological characterization of *in vitro* blood feeding in nymph and adult stages of *Ornithodoros turicata* (Acari: Argasidae) ☆

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ABSTRACT

Biological and physiological aspects of blood feeding in nymph and adult *Ornithodoros turicata* were investigated using an *in vitro* technique combined with electrophysiological recordings and respirometry. The duration of blood feeding through a Parafilm® membrane was similar (19.2–22.6 min) in both developmental stages. The mean (±SD) size of blood meal ingested by nymphs, females, and males was 44.2 ± 17.9, 150.6 ± 48.7, and 74.2 ± 36.9 mg, respectively, representing a 2.5-, 2.8- and 3.0-fold increase from their respective unfed weights. Electrophysiological recordings of the pharyngeal pump during blood feeding revealed that ticks ingested blood at a rate of 6.1–6.4 suctions per second. Mean blood volume ingested per suction was 0.013 μl in females and 0.007 μl in both males and nymphs. Blood meal size (mg) correlated with unfed body weight (mg) ($r^2 = 0.50$, $p < 0.05$) and with blood volume ingested per suction ($r^2 = 0.71$, $p < 0.05$). Unfed ticks exhibited a circadian ventilation rhythm with discontinuous gas exchange pattern during the daytime and continuous pattern during nighttime. Mean standard metabolic rates (SMR, $\dot{V}_{CO_2}$) in unfed nymphs, females and males of 1.4, 3.0 and 0.9 μl h^{-1} increased to 2.0, 5.7 and 2.4 μl h^{-1}, respectively, after a blood meal. SMR correlated positively with blood meal size ($r^2 = 0.89$, $p < 0.05$). Mean coxal fluid weight excreted after a blood meal in nymphs, females, and males was 8.7, 20.0, and 7.7 mg, respectively, which represents 27.0%, 23.4% and 26.7% of their blood meal size. This study revealed biological and physiological characteristics of soft tick blood feeding and metabolism important to tick survival.

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1. Introduction

Ornithodoros ticks (family Argasidae, also known as 'soft ticks') are vectors of *Borrelia* spirochetes that cause tick-borne relapsing fever in the western United States (Schwan et al., 2009), and the only recognized biological vectors of African swine fever virus (ASFV) (Hess, 1981; Vial, 2009; Ravaomanana et al., 2010; Boinas et al., 2011; de Carvalho Ferreira et al., 2014). The relapsing fever tick, *Ornithodoros turicata*, is distributed in the United States, including California, Utah, Arizona, New Mexico, Colorado, Kansas, Oklahoma, Texas and Florida; it is also found in central Mexico (Cooley and Kohls, 1944). *O. turicata* is a vector of *Borrelia turicatae*, a spirochete responsible for tick-borne relapsing fever in humans and dogs (Roscoe and Epperly, 2005; Whitney et al., 2007). This soft tick species is able to transmit ASFV in the laboratory (Butler and Gibbs, 1984; Hess et al., 1987). Life cycle characteristics of *O. turicata*, including the developmental stages as well as ovipositional behavior of adult females, have been previously described by Beck et al. (1986). Although the basic biology and certain aspects of feeding physiology have been reported in *Ornithodoros* tick species, including *O. turicata* (Kaufman et al., 1981; Kaufman and Sauer, 1982), the physiological mechanism of blood feeding and changes in metabolism associated with blood feeding remain to be studied.

Ticks have evolved efficient physiological mechanisms to regulate water balance and gas exchange, critical for survival in adverse environmental conditions (Kestler, 1985; Hadley, 1994). Previous studies in Ixodid ticks demonstrated two distinct gas exchange strategies, described as discontinuous CO_2 release in fasting and

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continuous in engorged ticks (Rechav and Fielden, 1995; Fielden et al., 1999). In Argasid ticks, water balance is maintained by water excretion through coxal organs, which functionally is homologous to a vertebrate filtration-resorption renal system (Kaufman, 2010). Excess water is excreted from coxal glands during and/or shortly after feeding depending on the species. Coxal fluid may contain tick-borne pathogens and provide a pathway for transmission to susceptible hosts (Gaber et al., 1984; Kleiboeker et al., 1998; Lopez et al., 2011). The objective of this study was to characterize biological parameters and physiological mechanisms associated with blood-feeding in nymph and adult stages of *O. turicata*.

2. Material and methods

2.1. Ticks

Fifth instar nymphs and unfed adult *O. turicata* used in this study were from a colony maintained at the Tick Research Laboratory, Texas A&M AgriLife Research, College Station, TX. The colony was established from specimens collected from a natural cavern in Travis County, TX in 1992 and were maintained under 14L:10D photoperiod, 25 ± 3 °C and 80–85% relative humidity. Ticks were brought to the USDA, ARS, Knipling-Bushland U.S. Livestock Insects Research Laboratory in Kerrville TX, where they were maintained at 25 ± 2 °C, 80 ± 5% relative humidity, and 12L:12D photoperiod prior to use.

2.2. In-vitro blood feeding

A previously reported Parafilm® based artificial blood feeding technique (Schwan et al., 1991) was modified to feed *O. turicata* (Fig. 1). Briefly, the feeding unit consisted of a clear plastic tube (diameter = 32 mm, length = 40 mm), one end of which was sealed with a piece of stretched Parafilm® (American National Can, Neenah, WI), and a 6-well cell culture plate (Corning Incorporated, Corning, NY). Cattle blood collected from healthy one-year old calves was mechanically defibrinated immediately before being stored at 4 °C. Culture plate wells were filled with 3 ml of cattle blood and placed in a water-filled large glass Petri dish maintained at 38 ± 1 °C over a heating plate. One feeding tube was placed into each culture plate well such that the membrane was slightly submerged in blood (1–2 mm below the blood level) and ticks were individually placed to allow feeding through the membrane.

2.3. Measurements of volumes of blood meal intake and coxal fluid secretion

Each tick was weighed before, and immediately after feeding to obtain weight of blood ingested. Blood feeding duration was determined by recording the time from tick attachment to withdrawal from the membrane. Fed ticks were placed individually in glass vials, maintained under conditions described above, and weighed again at 24 h to measure weight loss as an estimate of coxal fluid secretion (McCoy et al., 2010).

2.4. Electrophysiological recording of feeding activity

A fine silver wire electrode (0.076 mm diameter; A-M systems Inc., Carlsborg, WA) was attached to the anterior dorsum of the tick using a small drop of nail enamel. A second silver wire electrode was placed in the blood well. The electrodes were connected to an AC amplifier (A-M systems Inc. Carlsborg, WA). Upon tick attachment to the membrane, an electric circuit was formed

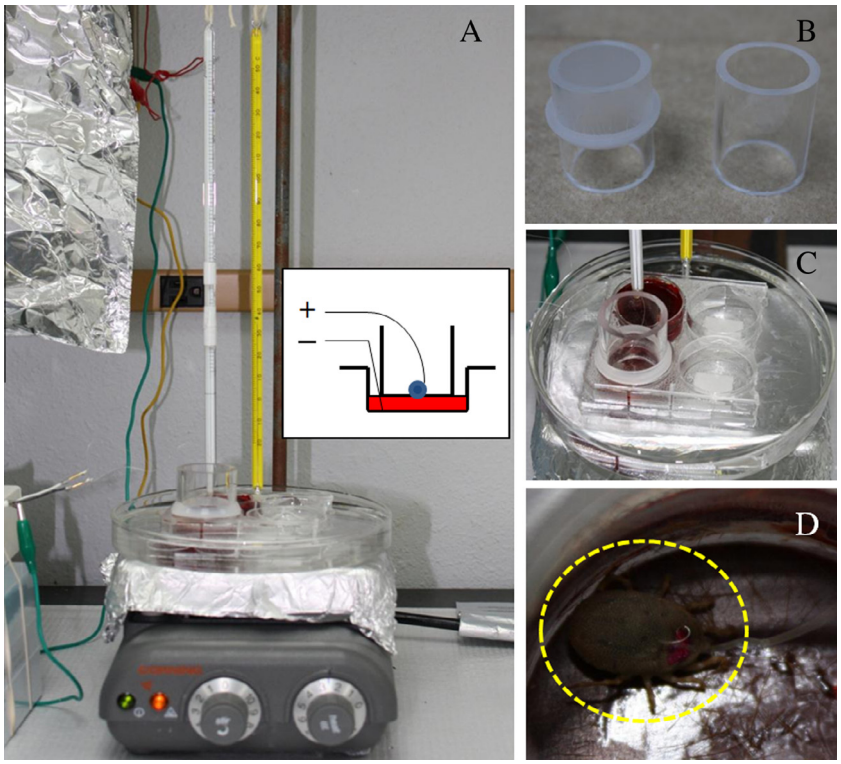

Fig. 1. (A) The *in vitro* membrane feeding system, including a heating plate, a large glass Petri dish with water, a cell culture plate, and a feeding unit. Two thermometers were used to measure temperature in the water bath (yellow) and blood in the well (white). The insert shows the recording electrode glued to the back of the tick, and reference electrode placed in the blood. (B) An empty feeding unit (right) and a second one fitted with membrane (left). (C) Top view of the feeding unit placed in blood in a well on the cell culture plate soaked in warm water bath. (D) A nymph engorging on the membrane in the feeding unit. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)