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ORIGINAL ARTICLE

Physiological Entomology: How Insects Work - Linking Genotype to Phenotype

The interaction between *Onthophagus binodis* and cattle dung pH: Impacts on reproduction and offspring phenology

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Abstract

The environment surrounding invertebrates can influence the physiology of larval offspring. Dung beetles provide several significant ecological functions, including dung breakdown, fly control and nutrient cycling. Cattle diet influences the chemical and physical constituents of dung, of which pH is considered critical. Few studies have assessed this, though a pH of 6.3 is the lowest threshold for dung beetle reproduction. We investigated the effects of an introduced and widespread dung beetle (*Onthophagus binodis)* on cattle dung pH (7.3, 6.0 and 5.0) and pH on *O. binodis* reproduction, offspring phenotypic traits and development time. Dung beetle presence increased the Δ pH (more alkaline) within dung pads after 96 h. Dung beetles produced broods in dung with a pH of 5.0, though in fewer numbers compared with the other pH treatments. Larval development was delayed in pH 5.0 with an average of 50 days compared with 44 days in dung with pH 6, 7, and the control (7.3). Smaller broods (ellipsoid volume [mm^3]) were produced in dung with a pH of 5.0 compared with pH 6.0 and 7.0, and offspring emerging from broods produced from dung with a pH of 6.0 were larger compared with the other pH treatments. Our results show that dung pH is important for brood production and progeny phenotypic traits of *O. binodis*, an agricultural ecosystem engineer and that there is no experimental evidence to support the suggestion that dung pH influences the provisioning of broods in this species.

KEYWORDS
cattle dung, dung beetle, pH, reproduction

INTRODUCTION

The environment surrounding invertebrates can influence the physiology of larval offspring. Abiotic factors known to influence the physiology of invertebrates include temperature, rainfall, soil moisture and pH, with pH impacting the availability of nutrients within soil environments (Neina, 2019; Nicol et al., 2008; Pietri & Brookes, 2008). The influence of pH on terrestrial invertebrates has primarily focused on soil pH influence, with a limited understanding of the impact on coprophagous insects (Dadour & Cook, 1996; Meyer et al., 1978; Morgan & Schmidt, 1966). Notably, the diet of livestock can influence the physiology of dung beetles (Dadour & Cook, 1996: Heddle, Hemmings, Burns, & Andrew, 2023). Increasing the pH of grain-fed cattle dung increased the pupal weight of *Haematobia irritans* L. 1758 (Diptera: Muscidae) (Morgan & Schmidt, 1966). The inverse occurs for mortality as a decrease in dung pH results in an increase in mortality of *Musca autumnalis* DeGeer 1776 (Meyer et al., 1978).

The fodder for livestock varies depending on location, with fodder quality fluctuating across seasons with changing soil, rainfall and temperature patterns, and pasture management programmes (Cobon et al., 2020; Issah et al., 2021; Perera et al., 2020; Schoenian, 2020; Willoughby, 1959). Pasture-grazed livestock may require supplementary feeding, particularly during summer when there is limited rainfall and herds are maintained above paddock carrying capacity (Moore et al., 2009) or during periods of drought or lush growth (Moore et al., 2009). Common supplements include hay, silage and grains (oats, barley, wheat, lupins, etc.), distributed directly onto the soil or via field bins (Cheng et al., 1998; Greenwood et al., 2018). Furthermore, grain feeding is used intensively in feedlots to increase the body fat and improve the growth rates of livestock (Dadour & Cook, 1996; Greenwood et al., 2018).

The effect of supplementary feeding on the physiochemical qualities of livestock dung, and its subsequent colonisation by dung beetles, is poorly understood (Dadour & Cook, 1996). The proportion of grain within the diet of livestock influences many factors of the chemistry of dung, including pH, moisture, starch, nitrogen and carbon concentrations, organic matter and neutral detergent fibre (Ali et al., 2021; Gittings & Giller, 1998; Wheeler & Noller, 1977). The foraging diet of cattle influences the pH of cattle dung (Dadour & Cook, 1996; Meyer et al., 1978; Morgan & Schmidt, 1966), with grass-based diets of cattle resulting in dung pH of 6.92 (Meyer et al., 1978) to 7.02 (Dadour & Cook, 1996), alfalfa hay diets resulting in dung pH between 6.9 and 7.4 (Meyer et al., 1978) and grain feeding reducing cattle dung to 6.3 (Dadour & Cook, 1996) and 5.58 (Meyer et al., 1978). The magnitude of these effects is influenced by seasonal changes in pasture quality and livestock physiology, as seen in New South Wales, where the pH of cattle dung varied from 6.75 in November to 7.67 in April (Kaur et al., 2021).

The single study on dung beetle reproduction on grain-fed cattle dung by Dadour and Cook (1996) demonstrated that *Onthophagus binodis* produced more brood balls on dung derived from cattle grazing pasture compared with grain-fed cattle dung. Both the survival of the F1 progeny and the size of the progeny were also greater on pasture-fed cattle dung (Dadour & Cook, 1996). Dadour and Cook (1996) hypothesized that cattle dung with a pH below 6.3 would not allow dung beetle reproduction. Some species of dung-dwelling invertebrates are able to survive in acidic dung, with the horn fly (*H. irritans*) being capable of reproducing at pH 5.5 (Morgan & Schmidt, 1966). Additionally, face fly (*M. autumnalis*) was able to survive in dung with a pH of 5.58; however, the mortality rate increased with increasing proportions of ground ear corn, which decreased the pH of cattle dung (Meyer et al., 1978).

Dung beetle activity also affects the chemical composition of dung over time (Holter & Scholtz, 2007). Dung beetles are known to feed on microbial biomass within the dung pad (Holter, 2016; Holter & Scholtz, 2007), the process of which causes shredding of the dung pad, which reduces pest fly reproduction (Ridsdill-Smith et al., 1987; Ridsdill-Smith & Hayles, 1990; Ridsdill-Smith & Matthiessen, 1988; Tyndale-Biscoe & Vogt, 1996). Furthermore, dung beetle processed dung provides a higher C:N ratio diet to the larvae within the brood than that in raw dung (Byrne et al., 2013; Holter, 2016; Holter et al., 2002; Holter & Scholtz, 2007; Shukla et al., 2016).

Ecological and pest issues may ensue from the low pH of cattle dung. Increased dung acidity may deter dung beetles and other important dung fauna, resulting in dung accumulation and a subsequent decline in pasture coverage due to smothering (Doube, 2018). Dung beetles provide significant ecosystem services, including the breakdown of dung, fly control and nutrient cycling (Doube, 2008; Heddle et al., 2021; Nichols et al., 2008). Prior to the successful establishment of 26 introduced dung beetle species in Australia (Bornemissza, 1976), the cattle dung pads would remain on the surface with only periodic breakdown by native dung beetles (Bornemissza, 1976; Doube et al., 1991; Tyndale-Biscoe, 1994; Tyndale-Biscoe, 1996). If dung is frequently below pH 6.2 (the hypothetical tolerance of dung beetles (Dadour & Cook, 1996)), this provides a problematic niche for flies, with a known tolerance to pH 5.6 (Meyer et al., 1978), to reproduce without competition.

In this study, we manipulated the pH of cattle dung to focus on one component of dung chemistry, to test the hypothesis that in cattle dung with a pH of 6.3 or less, dung beetles cannot successfully reproduce (Dadour & Cook, 1996). Specifically, we investigated dung beetle reproduction and survival through artificial pH manipulation of dung, as well as the reciprocal effects of dung beetle activity on dung pH. We predict that both reproductive outputs and the survival of F1 progeny in broods will decline in dung of pH 6 and lower and that cattle dung pH will negatively influence the phenotypic traits and development time of progeny. Further, this study investigated whether dung beetles influence the rate of change of pH inside dung pads.

METHODS

Dung collection

Dung was collected from a property north of Ebor, NSW (−30.32, 152.39), where cattle are rotationally grazed on pasture comprised of a mixture of native and introduced grass species. The property is in the high rainfall basalt zone on the Ebor Plateau, which is approximately 1200 m above sea level with an annual rainfall of 1200 mm. Dung was collected on 18 January 2021, which was 2 months post-cattle drenching. Dung was homogenised upon returning to the laboratory, packaged and stored at −4°C. When required, dung was defrosted at room temperature and homogenised with a paint mixer. Sub-samples (∼100 g each) of fresh dung were taken to measure the starting pH and moisture content of the original dung. Moisture content was measured by weighing and drying the samples in an oven at 60°C for 7 days. The dung pH was measured directly on five homogenised sub-samples with a pH electrode (HANNA, pHep4: HI98127). Fresh cattle dung had a pH of 7.36 (±0.06 SE) and a moisture content of 86.1% (±0.70).

Dung beetle culture

O. binodis individuals were collected from a property 10 km west of Armidale NSW from March to April 2022. Due to low populations of other species, trials were limited to this species (Heddle, Hemmings, & Andrew, 2023). *O. binodis* was collected through live trapping, which consisted of multiple traps with a 1 kg dung pad bait placed upon a pot with 50% sand and 50% vermiculite (Figure S1).

Traps were set up at 8 AM and collected 24 h later. Dung and sand/vermiculite were sieved for live beetles on collection.

Cultures of *O. binodis* were reared and maintained in a temperature-controlled glasshouse (day 25 ± 2°C, night 20 ± 2°C, 16L:8D) with artificial lighting (PHILIPS© TLD 36W/840). These conditions were maintained for the cultures and experiments. *O. binodis* was separated by sex and kept in different 5 L containers, which were partly filled with a 50:50 sand and vermiculite mix and moistened to 2% w/w. Beetles were fed the same dung (as above) every Tuesday and Friday. Culture boxes were emptied on a fortnightly basis with the removal of dead beetles and used dung, with live beetles being placed into fresh containers.

Experiment 1: Dung beetle impact on dung pH

To test how the presence of dung beetles influenced the pH of cattle dung pads, we manipulated the dung pH using 1.0 M hydrochloric acid (HL015-2.5L-P, CAS No. 7647-01-0). Initially, hydrochloric acid was tested against several other products—cream of tartar, vinegar and acetic acid—to determine how much of each chemical was required to adjust dung pH to 5.0 without creating an unusable dung. Hydrochloric acid was the simplest and most consistent chemical to attain the required range of pH values. Two pH treatments were selected (pH 5.0, pH 6.0 and control pH 7.3), to which treatments had the dung beetle species *O. binodis* applied or withheld. For every 1000 g of cattle dung, 141 mL of total liquid (based on 141 mL HCl to attain pH 5.0) was added to the dung pad, which resulted in equivalent moisture content across the treatments. A total of 10 replicates of each pH treatment by dung beetle presence/absence were run for the duration of the experiment. The experiment was set up as a randomised complete block design using Agricolae (de Mendiburu & de Mendiburu, 2019). Artificially manipulated dung pads (250 g) were placed into an enclosure consisting of a 3L cylindrical container 2/3 filled with a 50:50 sand/vermiculite mixture, which was moistened to 2% w/w with RO water. Treatments with dung beetles had one pair of *O. binodis* added to the enclosure at 0 h and were retained for the full 96 h.

Dung pH was measured at five points internal by removing the surface layer of dung and a further five points across the surface of the dung pad prior to the introduction of dung beetles to the enclosure (0 h) and at 3, 6, 9, 12, 24, 36, 48, 60, 72 and 96 hours after introduction. At the end of the 96-h period, dung pads were measured and removed from enclosures and containers emptied to determine the presence/absence of broods in beetle present treatments. We determined the overall delta pH (Δ pH) for each replicate by calculating the difference between the pH at 96 and 0 h and averaging this across all replicates.

Experiment 2: Impact of pH on dung beetle reproduction

To test how dung pH influences dung beetle reproduction, we followed the above methodology to manipulate dung pH for three treatments (pH 5, 6 and 7) and untreated control (pH 7.3). A total of 10 replicates of each pH treatment were conducted for a minimum of 21 days, with any adult beetle deaths recorded and dead adult individuals replaced the following week (day 7 or 14). Beetle weight and length, excluding head and pronotum width, were measured before introduction to the enclosure. pH-adjusted dung pads (200 g) were placed into an enclosure consisting of a 3 L cylindrical container 2/3 filled with a 50:50 sand/vermiculite mixture, which was moistened to 2% w/w with RO water. Dung was replaced every 3–4 days with fresh dung added at the same time. Of note, pH-5-treated dung from both experiments became covered by a fungus after a few days, which was identified to the class of Zygomycetes (T. Elliot & T. Lebel personal comms., Figure S2). This fungus was found on all pH-5-treated dung pads and did not appear to influence the response of dung beetles as it primarily colonised the pad surface.

At the end of each 7-day period, each container was emptied and sifted to remove beetles and whole broods. Incomplete broods were excluded from brood calculations. Remaining beetles were placed into fresh containers of the same treatment as they had previously occupied. In the event males died, the death was noted, and a new male was added to the replicate, and the replicate continued. When a female beetle died, this replicate was reset to day 0 and run for a further 21 days. This was to ensure pH influence on brood size and progeny could be traced back to the original female.

To determine if the pH had an influence on brood size, broods were randomly split into two groups, with the first group being destructively measured. All broods were cleaned of sand and vermiculite, and the fresh weight of individual broods was taken using a 120 g scale. Using callipers (precise to 0.01 mm; Kingcrome © K11105), the equatorial and polar diameter was recorded and then halved to get the radius. Then, using the equation 4/3.π.a2.c (a = equatorial radius; c = polar radius), we determine the volume of each individual brood. After measuring the fresh broods, these broods were labelled and placed into an oven at 60°C for 7 days to determine the dry weight. In the event that only 1–2 broods were produced, these broods were measured before being placed into the second group (below).

To determine the development (days), progeny size (mm) and brood mortality, the remaining broods were placed in a bed of vermiculite, which were placed into an incubator set at 23°C with 70% humidity (Thermoline, TRH-300-SD). The development days for individual beetles were recorded and averaged by taking the initial start date and the changeover date (days 7 and 14) to provide the 'average' start date. The number of days between the emergence date and the average start date was used to calculate the average days to emergence. Finally, upon emergence, beetles were measured to determine the influence of pH on the progeny beetle size. The pronotum width and the length of the pronotum and elytra were measured by the emerging progeny to determine the beetle size (Radtke & Williamson, 2005). To determine brood mortality, leftover broods were carefully opened and inspected for the presence of an egg, head capsule, pupae or dead adult. The number of broods that died out of the total broods produced in individual replicates determined the life stage mortality.

Statistical analysis

All statistical analyses were implemented in R studio 3.4.3 (R Core Team, 2022), using the framework of generalised linear mixed-effects models or two-way analysis of variance (ANOVA). All data are presented as evidence-based practices (Muff et al., 2022).

For experiment one, we tested the Δ pH for normality using Shapiro-Wilk tests (Royston, 1992) and identified that the data were not normally distributed. We ran two-way ANOVAs using a type 3 chi-squared test to determine the influence of surface or internal measurement. As it was significant, we subsequently subsetted the data by surface or internal measurement for further analysis. Surface and internal Δ pH was not normally distributed, resulting in a two-way ANOVA using rank transformations (Conover & Iman, 1981). Tukey's Honest Significant Difference post hoc tests were conducted to determine significant differences for Δ pH (Abdi & Williams, 2010).

For the second experiment, generalised linear mixed-effect models were fitted with a Gaussian family and were fitted with the functions 'glmmTMB' from the package glmmTMB (Magnusson et al., 2017). These models were simulated with a refit as False, plot = True and $n = 1500$. For generalised linear mixed models, we checked for under and overdispersion with the functions 'simulateResiduals' and 'testResiduals' from the DHARMa package (Hartig & Hartig, 2017). An analysis of the deviance table was run using a type 3 Chi-Squared test. Models were adjusted using Bonferroni in the function 'emmeans' from the package lsmeans (Lenth & Lenth, 2018). Post hoc comparisons were made with 'emmeans' using the 'pairs' function. The model predicted values were determined using 'emmeans' (package lsmeans (Lenth & Lenth, 2018)), with final values showing 95% confidence intervals and letters used to notify where significant differences were observed. All models use the Gaussian family, with the exception of the dry weight of broods, a Gaussian family with a log-link function was used, and data was reverse-transformed for graphing purposes. For F1 progeny mortality, a Poisson distribution best fits with a log link on angular transformed data. Logistic transformations, which can be preferred over angular transformations (Warton & Hui, 2011), were also considered for proportions but led to the same conclusions. We broke down the mortality into the life stage in which the brood died (i.e., egg, larval or beetle) and ran a general linear model as above.

RESULTS

Experiment 1: Dung beetle impact on dung pH

There was very strong evidence for a difference in Δ pH between surface and dung pad samples ($F = 609.13$, df = 1, $p < 0.0001$). On the surface of the dung pad, there was no difference in Δ pH between the treatment without dung beetles compared with dung beetles ($p = 0.99$). There was very strong evidence that with beetles, Δ pH was higher (less acidic) at the surface of the dung pad compared to the internal dung ($p < 0.0001$), and internal Δ pH with beetles was higher compared to internal Δ pH without beetles ($p < 0.0001$) (Figure S3). Similarly, without dung beetles, Δ pH on the surface was also higher compared to internal Δ pH ($p < 0.0001$).

Natural variation in the dung pad surface pH was observed, with no influence resultant for treatments with/without dung beetles (Figure S3). On the surface of the dung pads, there was very strong evidence to suggest differences between pH treatments for Δ pH ($F = 63.96$, df = 2, $p < 0.0001$). There was no evidence for an influence by dung beetle presence ($F = 0.19$, df = 1, $p = 0.66$) nor an interaction between pH treatment and dung beetle presence for Δ pH ($F = 0.20$, df = 2, $p = 0.82$). Rapid pH increases (acidity decrease) occurred in the first 6 h on the surface of dung pads, though the change after 6 h was minimal. The median Δ pH values with and without dung beetles on the surface were 2.3, 2.3 and 1.6 for pH 5, pH 6 and the control, respectively, with dung beetles. Without dung beetles, the Δ pH was 2.2, 2.3 and 1.5 in pH 5, pH 6 and the control, respectively (Figure S3).

Analysis of the internal Δ pH revealed strong evidence for an interaction between dung beetle presence and pH treatment ($F = 9.76$, df = 2, $p = 0.0002$). Very strong evidence of differences in Δ pH between treatments with and without dung beetles was identified in pH 5 ($p = 0.0004$), pH 6 ($p < 0.0001$), and in the control ($p < 0.0001$). Median Δ pH values for treatments with dung beetles were 1.2, 1.4 and 1.2 in pH 5, pH 6 and the control, respectively. Comparatively, median Δ pH values for treatments without dung beetles were lower at 0.7, 0.9 and 0.2 in pH 5, pH 6 and the control, respectively.

Experiment 2: The impact of pH on dung beetle reproduction

Brood production

In total, 1073 broods were produced by *O. binodis* from all treatments; 120, 314, 332 and 307 broods were produced in dung with pH 5, pH 6, pH 7 and from control dung pads. There was a weak effect of pH treatment on brood production of *O. binodis* $(\chi^2_{3,1} = 6.74, p = 0.081)$ while no effect was found over time (weeks) $(\chi^2_{2,1} = 2.45, p = 0.29)$, and there was no evidence for an interaction between pH treatment and week $(\chi^2_{6,1} = 7.03, p = 0.32)$ (Figure 1a).

Brood size—Ellipsoid volume and dry weight

To calculate brood ellipsoid volume (mm^3), 143, 65, 147 and 156 broods were used from the control, pH 5, pH 6 and pH 7, respectively. The maximum median brood size was 4200 mm^3 at pH 6 and 7, whereas the smallest was 3700 mm^3 at pH 5 (Figure 1c). For the ellipsoid volume of broods, differences were significant between pH treatments $(\chi^2_{3,1} = 10.64, p = 0.014)$ but no evidence of differences over time (week) $(\chi^2_{2,1} = 2.46, p = 0.29)$ and there was no interaction between pH treatment and week $(\chi^2_{6,1} = 9.43, p = 0.15)$. Post hoc

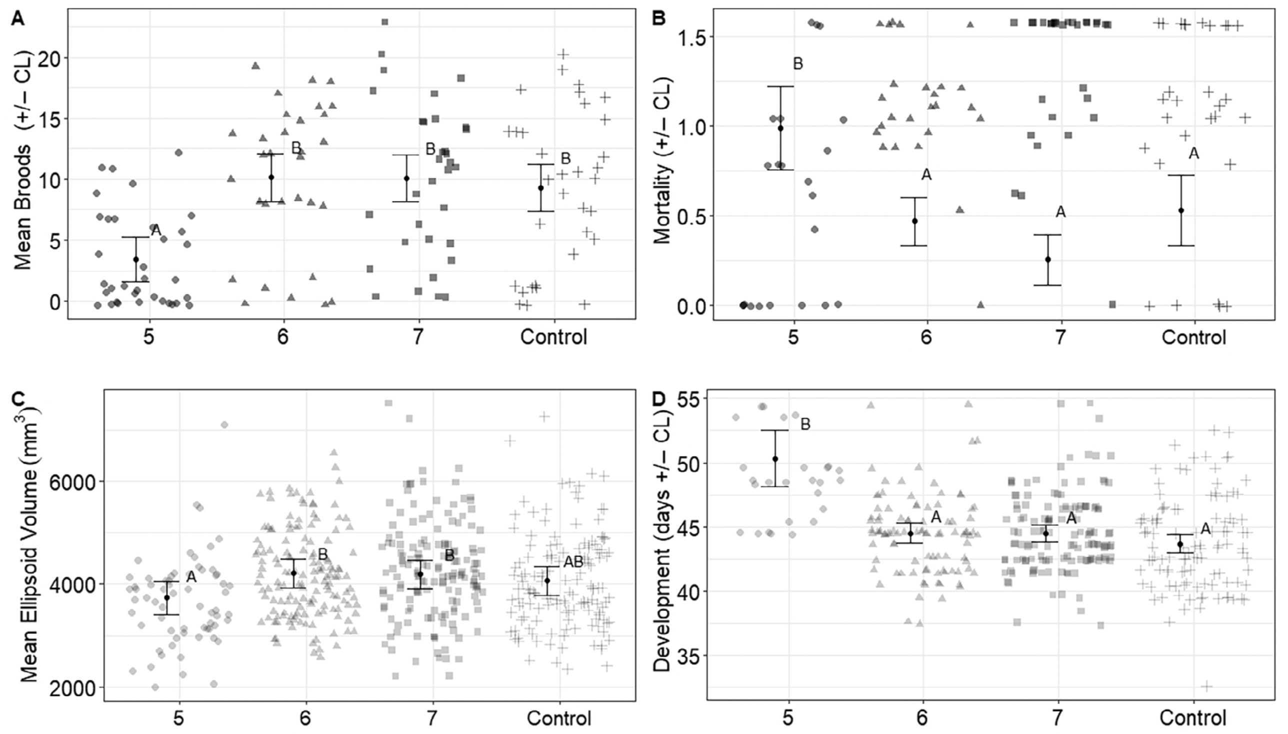

FIGURE 1 (a) Predicted mean broods produced (+/− 95% confidence level [CL]) across the different pH treatments, (b) predicted mean mortality of broods (angular transformed data) (+/− CL), (c) predicted mean ellipsoid volume (mm^3) (+/− CL) and (d) predicted mean development days (+/− CL). Shape indicates raw data of pH treatment with: circle indicating pH 5, triangle indicating pH 6, square indicating pH 7 and the plus (+) indicating control treatments. Significant differences are indicated with letters. For (a), 1073 broods were produced by *O. binodis* from all treatment: 120, 314, 332 and 307 broods were produced in dung with pH 5, pH 6, pH 7 and from control dung pads. For (b), 477 broods were utilised. For (c), 143, 65, 147 and 156 broods were used from the control, pH 5, pH 6 and pH 7, respectively. For (d), 365 broods successfully emerged, of which 7.1%, 26.6%, 36.4% and 29.9% came from pH 5, 6, 7 and the control, respectively.

testing showed very strong evidence for differences between pH 5 and pH 6 ($p = 0.002$) and pH 5 and pH 7 ($p = 0.004$), while there was only weak evidence for differences between pH 5 and control (0.05).

The pH treatment had no effect on the dry weight of the broods ($\chi^2_{3,1} = 0.096, p = 0.99$) (Figure S5). To analyse the effects of pH over time (week) on brood dry weight (g), 486 broods were measured randomly across treatments for F1 emergence. From the control, pH 5, 6 and 7, respectively, 143, 40, 147 and 156 broods were measured. However, the number of broods produced within a treatment had a negative influence on the dry weight of broods ($\chi^2_{19,1} = 73.20, p < 0.0001$).

Brood mortality

Of the 477 broods incubated for F1 development from the original brood production, 420 broods resulted in emerging progeny (88.1% emergence overall). Of the broods that did not survive F1 emergence, three were accidentally killed during 60-day post-experiment checking and were excluded. Of the remaining broods, 9% died at the egg stage and 9.4% at the larval stage. A small number (1.6%) of beetles died after pupation, resulting in failed emergence. The lowest survival percentage was observed at pH 5 (49%), with progressively higher survival at pH 6 (79.3%), control (84.2%) and pH 7 (91.3%).

There was very strong evidence that pH treatment influenced survival of F1 progeny ($\chi^2_{3,1} = 28.41, p < 0.0001$, arcsine transformed) (Figure 1b) with mortality significantly higher between pH 5 and all other treatments: pH 6 ($p = 0.001$), pH 7 ($p < 0.0001$) and control ($p = 0.020$). No evidence was found for differences of mortality between pH 6 and pH 7 ($p = 0.15$), pH 6 and the control (0.96), nor between pH 7 and control ($p = 0.13$). 43.9% of broods survived to the emergence in pH 5.0 dung compared to 78.9%, 89.3% and 80.6% for pH 6.0, pH 7.0 and control treatments, respectively. When overall mortality is broken down by larval stage, there was no evidence for pH treatment influencing mortality ($\chi^2_{3,1} = 0.26, p = 0.97$, angular transformed) whereas there was moderate evidence of differences between life stages ($\chi^2_{2,1} = 7.47, p = 0.024$) (Table S2). The interaction between pH and life stage showed weak evidence of differences ($\chi^2_{6,1} = 10.91, p = 0.091$).

F1 development time

Across all treatments, 420 broods successfully emerged, of which 7.1%, 26.6%, 36.4% and 29.9% came from pH 5, 6, 7 and the control, respectively. pH had a very strong effect on the development time of *O. binodis* ($\chi^2{}_{3,1} = 16.32, p < 0.0001$) (Figure 1d), no evidence was found for differences over time (week) ($\chi^2{}_{2,1} = 2.86, p = 0.24$) and no evidence was found for an interaction between pH treatment and time (week) ($\chi^2{}_{6,1} = 7.26, p = 0.30$). Strong evidence was found to suggest differences in the development of F1 progeny between pH 5 and pH 6 ($p < 0.0001$), pH 5 and pH 7 ($p < 0.0001$) and between pH 5 and control ($p < 0.0001$). There was no difference in F1 development time between pH 6 and pH 7 ($p = 1.000$), pH 6 and the control ($p = 0.24$) and between pH 7 and the control ($p = 0.21$). Mean development time for progeny was 50.2, 44.5, 44.5 and 43.5 days for pH 5, pH 6, pH 7 and the control, respectively (Figure 1d).

F1 progeny size—Length and pronotum width

To determine the influence of pH on the length and pronotum width of *O. binodis* a total of 420 beetles were measured across all treatments (Table S1). There was moderate evidence to suggest an interaction between pH treatment and time (week) on *O. binodis* length ($\chi^2{}_{6,1} = 13.12, p = 0.041$). At week 1, there was moderate evidence to indicate differences between pH 5 and pH 6 ($p = 0.030$), very strong evidence for differences between pH 6 and pH 7 ($p < 0.0001$) and between pH 6 and the control ($p < 0.0001$). There was no evidence to suggest differences between pH treatments within week 2 or 3. Beetles from pH 6 in week 1 were 8.6% larger compared with pH 5 and pH 7 while also being 9.0% larger than the control beetles (Figure 2a). Post hoc testing indicated no difference in weeks 2 and 3 for beetle length across pH treatments (Figure 2a).

Strong evidence was found to suggest the interaction between pH treatment and time (week) ($\chi^2{}_{6,1} = 17.63, p = 0.0072$) was influential on pronotum width (Figure 2b). Sex was tested in the model but was found not to influence the model and was, therefore, removed. Strong evidence was found to indicate differences between week 1 pH 6 and pH 5 ($p = 0.026$), pH 7 ($p = 0.0001$) and control ($p < 0.0001$). No evidence was found for any further differences between weeks and pH treatments. Beetle pronotum width in pH 6 in week 1 was consistently larger than pH 5, pH 7 and the control: 9.4%, 8.8% and 11%, respectively. From week 2, there were no clear differences. At week 3, pronotum width in pH 6 was 26.2% greater than at pH 5.

DISCUSSION

In this study, we demonstrate that *O. binodis* can successfully breed at pH 6.0; further, it can breed at pH 5, albeit at a reduced capacity in egg production and development rate, larval development, and survival of F_1 progeny. Additionally, our study has shown that pH 6.0 appears to be around the optimum pH for *O. binodis* beetle length and

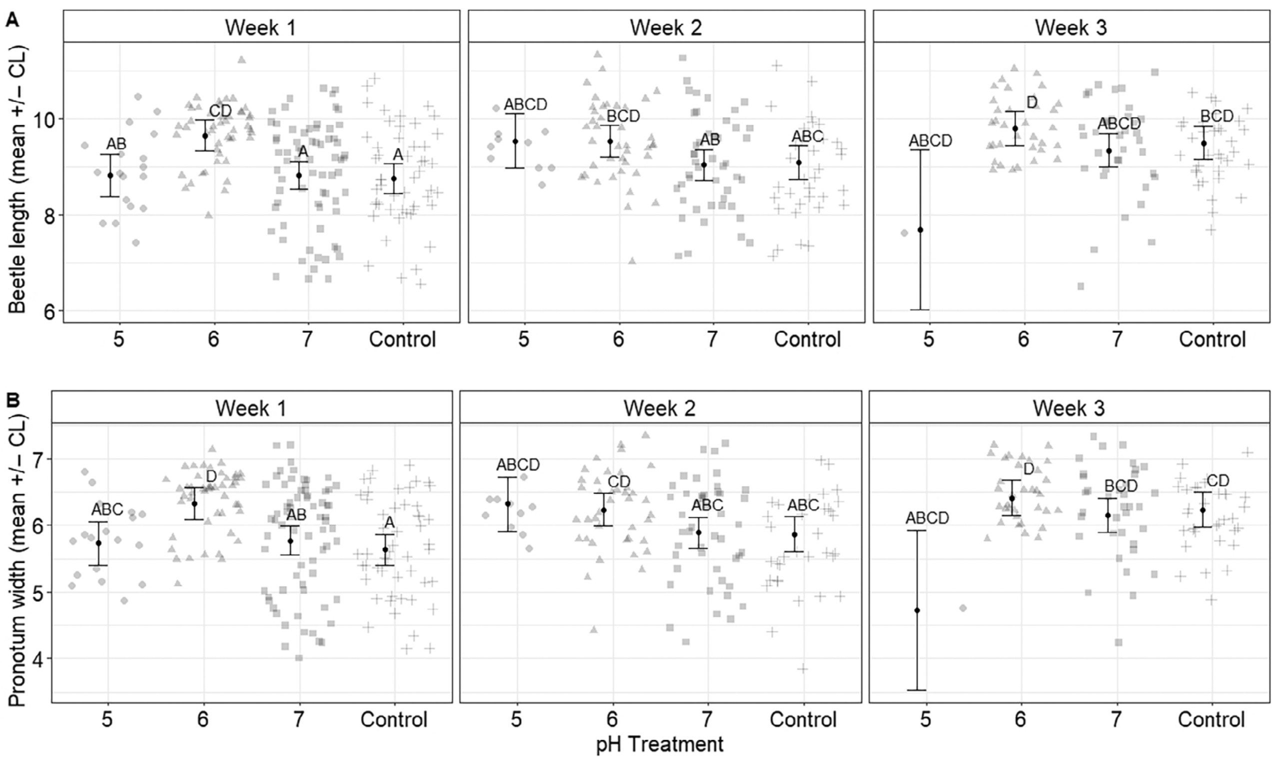

FIGURE 2 (a) Mean beetle length (mean +/− 95% confidence interval) across different pH treatments and (b) mean pronotum width (mean +/− 95% confidence interval) across different pH treatments by 3 weeks, which the trial was conducted. Shape indicates raw data of pH treatment with: Circle indicating pH 5, Triangle indicating pH 6, square indicating pH 7 and the plus (+) indicating control treatments. Significant differences are indicated with letters. A total of 420 beetles were measured from all treatments.

pronotum width, which has previously not been observed. This study focused only on pH, independent of other dung parameters, and provides evidence to reject the hypothesis put forward by Dadour and Cook (1996) that dung beetles cannot breed in dung with a pH below 6.3. Furthermore, our study has shown that the presence of dung beetles, in itself, influences the pH inside the dung pad after 96 h of colonisation.

The nutrient availability within soils is influenced by proximal pH and is likely to affect the nutrient composition and growth of microbes in dung (Neina, 2019; Nicol et al., 2008; Pietri & Brookes, 2008). At pH 5.0 in soil compared with pH 6.0 and 7.0, nitrogen, phosphorus, sulphur, calcium and magnesium availability decreases while iron, manganese, boron, copper and zinc content becomes more readily available (Binkley & Vitousek, 1989; Lucas & Davis, 1961). The nutrient availability may have resulted in longer and wider beetles in pH 6.0 dung and may have resulted in the higher mortality of pH 5 brood balls. It was beyond the scope of this study to determine the microbial and nutritional availability in different dung pH treatments, though it is likely that pH 5.0 treatments did negatively affect the beneficial microbes and symbionts within the reproductive broods (Byrne et al., 2013). Grain-fed dung has a different microbial community due to the different microbes required for breaking down grain in the rumen and intestines of cattle (Pitta et al., 2014; Sim et al., 2022; Van Vliet et al., 2007). How these differences in microbes vary based on diet and the impact on dung beetles should be investigated.

The diet of livestock is important in that it can change the chemical and physical parameters of the dung (Dadour & Cook, 1996). The pH of cattle dung varies between animals fed pastures compared with grain, resulting in a more acidic dung (Dadour & Cook, 1996). Higher nitrogen, phosphorus and water-soluble NH_4^+ occur in dung when cattle are fed with grain over pasture (Hao et al., 2009). Further, the growth stage of pastures can influence the nutritional content of dung, with higher nitrogen dung content from young plant growth stages, and a higher fibrous dung content found in older plant growth stages (Albon & Langvatn, 1992; Demment & Van Soest, 1985; Fryxell, 1991; Hansen et al., 2009; Mårell et al., 2006). High-fibre diets characterised by low nitrogen have a high C:N ratio compared with high-nitrogen, low-fibre diets in cattle (Van Vliet et al., 2007). Conversely, grain-fed cattle results in lower pH dung (Dadour & Cook, 1996) and carbohydrate content (Nortey et al., 2007). Dung beetles were released into Australia to combat two economically significant pest species, the Bush Fly (*Musca vetustissima*, Walker) and the Buffalo Fly (*Haematobia exigua*, de Meijere 1906) (Diptera: Muscidae) (Bornemissza, 1960; Bornemissza, 1970; Bornemissza, 1976). The pH tolerance for these two Diptera species is unknown, although relatives of these species have known pH tolerances. Given dung beetles were able to survive albeit at a reduced capacity to breed down to pH 5, grain-fed dung may produce a fragile ecotone between the functional capacity of dung beetles and flies. It is important to further investigate whether the pH of dung or other factors of dung composition (especially from grain) influence the fly-dung beetle interaction.

If there is a difference between the tolerance of key coprophagous invertebrates (Diptera and Coleoptera) for pH and or other dietary factors, there is the potential for the ecosystem services in agricultural landscapes to be left unfulfilled. As both flies and dung beetles have been shown to tolerate dung pH below 6.0, the competition at this niche boundary is likely to be greater than at higher dung pH levels. Dung beetles may have a competitive advantage, as we demonstrated that dung beetle activity changes the internal pH of dung pads. Equivalent studies are required on flies to determine if fly reproduction has similar buffering capacity. Furthermore, local adaptations to grain-fed cattle, in regions where the practice is more common, may also enable dung beetles to utilise a niche resource and have a localised adaptation.

The chemical and physical property parameters within dung pads, including dung pad moisture, nitrogen/carbon content, ash/organic matter content, carbohydrate content and pH, likely affect dung beetle breeding time and success (Dadour & Cook, 1996; Edwards, 1991; Holter, 2016). Natural pH ranges of cattle dung have been shown to be influenced by the feeding regime, with ranges from 5.58 to 6.24 with grain-based diets (Dadour & Cook, 1996; Meyer et al., 1978;) to 6.75 to 7.67 in pasture-reared cattle (Barth et al., 1994; Dadour & Cook, 1996; Jost et al., 2011; Kaur et al., 2021; Kirchmann & Witter, 1992; Meyer et al., 1978). Given that dung beetles can both reproduce in low-pH dung and change the internal pH of dung pads to be more suitable, the ecosystem services provided by dung beetles may not be reduced in low-pH dung from grain-fed cattle. As both microbial processes and dung beetle activity increase dung pad pH (acidity decrease) (Castro-Ramos et al., 2022), then perhaps the pH is not the limiting factor of ecosystem services provided by dung beetle communities.

The rapid rise of dung pH in the pH 5.0 treatment and moderate rise in pH in the pH 6.0 and 7.0 treatments are likely the result of microbial activity (Kazuhira et al., 1991), and specifically nitrogen processing (Kadlec & Knight, 1996; Saeed & Sun, 2012; Vymazal, 2007). Ammonification (N to NH_3) occurs optimally between pH 6.5 and 8.5, while nitrification occurs optimally at pH 8.0 and is limited below pH 6.0 (Kadlec & Knight, 1996; Saeed & Sun, 2012; Vymazal, 2007). There is no knowledge of how dung beetles influence microbial processing, although decomposition cycles are expected to be inextricably linked and contribute to the internal pH of dung pads. Brood ball production by tunnellers (paracoprids) inhibits ammonia volatilisation and promotes the proliferation of aerobic bacteria, thus enhancing nitrification (Kazuhira et al., 1991). Aeration by tunnels also facilitates nitrogen mineralisation (Bertone et al., 2006). The production of ammonia from urea or organic acids is commonly used to counteract acidity by microbes and raise pH (less acidic), and is rapidly increased under aerobic conditions (Krulwich et al., 2011; Pennacchietti et al., 2018). Furthermore, the nutrient cycling processes of microorganisms are affected by grain-based diets, which create lower pH environments in dung (Aarons et al., 2009; Carpinelli et al., 2020; Maldonado et al., 2019). Nutrients are tied up in lower pH dung pads, as seen with soil nutritional availability and microbial abundance (Beadle, 1966; Brady et al., 2008; Neina, 2019; Pietri &

Brookes, 2008; Penn & Camberato, 2019; Šimek & Cooper, 2002). The microbiota of the dung beetle intestinal tract also plays a role in the processing of dung (Ebert et al., 2021; Shukla et al., 2016; Suárez-Moo et al., 2020). The beetles in this study were all collected from pasture-reared farms rather than grain-based farms. This likely influences the gut microbiota of dung beetles, which may influence the tolerance of different diets and nutrient variations.

The pH of dung is the bi-product of the diet and is associated with differing nutritional contents of the dung. pH binds nutrients within dung pads similar to soil nutritional availability and microbial abundance (Beadle, 1966; Brady et al., 2008; Neina, 2019; Penn & Camberato, 2019; Pietri & Brookes, 2008; Šimek & Cooper, 2002). In our study, grass-fed dung was chemically manipulated using hydrochloric acid to a lower pH, whereas Dadour and Cook (1996) compared grain-derived dung to pasture-derived cattle dung. This may have exacerbated different outcomes between our study and Dadour and Cook (1996), as carbohydrates within the cattle diet will increase the carbohydrate output in dung. Carbohydrates were beyond the scope of this study, though dietary shifts caused by pH will manipulate dung parameters in different ways and might induce a different response by coprophagous insects. The rumen undergoes chemical changes to tolerate the grain in livestock diet (Yang et al., 2001), which could, in turn, result in higher dung carbohydrate concentrations. How different dung beetle species respond to pH and different dung nutrition is poorly understood and warrants further investigation.

Prior to this research, little was known about the influence of pH on brood production and the size of dung beetle broods. We have demonstrated that more broods were produced in pH 6, pH 7 and control dung (pH 7.3) compared with pH 5, but there was no difference in the brood dry weight across all treatments. More broods were produced by *O. binodis* in annual pasture-derived dung, which had a higher pH (7.02) compared with dung derived from grain-fed cattle (6.22 and 6.24) (Dadour & Cook, 1996). This degree of variation in brood production was not observed between the lab-manipulated pH treatments from 6 to 7. *O. binodis* can produce up to 14 broods per female per week depending on dietary and seasonal factors (Ridsdill-Smith, 1986), which suggests pH is not the only factor influencing brood production. Dung beetle reproduction within pasturelands has been shown to be influenced by a range of factors, including pasture quality (Kaur et al., 2021; Ridsdill-Smith, 1986), mammalian species (Gittings & Giller, 1998) and ruminant versus non-ruminant (Edwards, 1991), all of which influence the quality of dung. The effects of dung quality on dung beetles and their reproduction are widely observed (Dadour & Cook, 1996; Kaur et al., 2021; Ridsdill-Smith, 1986). The interaction between grain and the resulting pH of cattle dung and the negative influence on *O. binodis* reproduction is clear (Dadour & Cook, 1996). However, different dung beetle species may be tolerant to different dung qualities, and there are still many aspects of the effects of the dung environment on reproductive success that require investigation.

The size (length and pronotum width) of dung beetle progeny in this study was impacted by pH, with the largest *O. binodis* progeny produced at pH 6. This is contrary to previous findings, with larger *O. binodis* being produced in pasture-derived dung, with a pH of 7, compared with grain-fed dung with a pH of 6.2 (Dadour & Cook, 1996). If pH was negatively influencing the progeny size, it is likely that this would produce a decreasing size trend for the progeny in our study. Dung beetle body size is important in dung removal, and the efficiency of removal increases with increasing body size (Nervo et al., 2014; Piccini et al., 2018). When species are influenced by the diet, either positively or negatively (Heddle et al., 2023), they will have an advantage or disadvantage compared to other species. This in turn can influence the ecosystem function of species and the reproductive effort exhibited by individuals and individual species (Servín-Pastor et al., 2021). Body size is an important factor for ecosystem services (dung removal) (Nervo et al., 2014), thus, any negative influence of dung characteristics, which influence the progeny size, will negatively influence the ecosystem services.

Progeny size has been associated with non-genetic traits such as the dung provision by adults (Cook, 1993; Juliano, 1985). Much of the variation in size within dung beetle populations is attributed to environmental pressure that is applied to the breeding adults (Fox & Czesak, 2000). The provisioning of broods with greater amounts of dung influences the horn morphological traits and brood masses of dung beetles (Cook, 1988; Cook, 1990; Eberhard, 1982; Hunt & Simmons, 2000; Hunt & Simmons, 2004). Larger male beetles are able to provide more dung by working cooperatively with reproductive females compared with smaller beetles of the same species (Cook, 1988; Cook, 1993). We found, however, that there was no influence of dung pH on brood size (dry weight) and, thus, brood provisioning. The larger beetles produced at pH 6 compared with all other treatments may be due to greater nutrient availability or beneficial fungal growth within the broods, optimizing the progeny size. However, the direct link between the provisioning of broods and F_1 progeny was not recorded as broods were destructively sampled. The results do indicate that pH does influence body size and future studies could determine the mechanisms behind this. The research conducted has shown that dung pH does influence the number of broods produced, but not to the degree outlined by Dadour and Cook (1996).

The evidence acquired in this research refutes the broad hypothesis originally put forward that dung beetles cannot reproduce in dung with a pH lower than 6.3 (Dadour & Cook, 1996). There were, however, unexpected effects of the dung pH, mainly in regards to the length of beetles being larger at pH 6 compared with the other pH treatments. The question remains: is it the pH that influences the changes observed by Dadour and Cook (1996), or is it the grain diet and subsequent dung quality influencing the results? Further research is needed to investigate the influence of grain feeding on the reproduction of dung beetles and, further, the impact of other supplementary feed types. Additional studies should investigate the link between cattle diet, dung microbiota and dung beetle production and their gut microbiota. While this study identifies the flaws in the original hypothesis of Dadour and Cook (1996), further work is required to determine the tolerance of a range of dung beetle species in acidic dung.

AUTHOR CONTRIBUTIONS

Thomas Heddle: Conceptualisation, data curation, formal analysis, investigation, methodology, writing—original draft. **Zac Hemmings**: Project administration, methodology, supervision, resources, writing—review & editing. **Nigel R. Andrew**: Funding acquisition, supervision, resources, writing—review & editing. **Adrienne Burns**: Supervision, writing—review & editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data used in the paper are currently archived in the online data base, Figshare (10.6084/m9.figshare.22144700).

ETHICS STATEMENT

The authors declare that this paper is completely original and has not been previously submitted to another journal. The paper reflects the authors' own research and analysis in a truthful and complete manner. The paper properly credits the meaningful contributions of co-authors and co-researchers. The results are appropriately placed in the context of prior and existing research. All sources used are properly disclosed (correct citation).

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REFERENCES

Aarons, S.R., O'connor, C.R., Hosseini, H.M. & Gourley, C.J. (2009) Dung pads increase pasture production, soil nutrients and microbial biomass carbon in grazed dairy systems. *Nutrient Cycling in Agroecosystems*, 84, 81–92. Available from: https://doi.org/10.1007/s10705-008-9228-5

Abdi, H. & Williams, L.J. (2010) Tukey's honestly significant difference (HSD) test. *Encyclopedia of Research Design*, 3, 1–5.

Albon, S. & Langvatn, R. (1992) Plant phenology and the benefits of migration in a temperate ungulate. *Oikos*, 65, 502–513. Available from: https://www.jstor.org/stable/3545568

Ali, A.I., Wassie, S.E., Joergensen, R.G., Korir, D., Goopy, J.P., Butterbach-Bahl, K. et al. (2021) Feed quality and feeding level effects on faecal composition in East African cattle farming systems. *Animals*, 11, 564. Available from: https://doi.org/10.3390/ani11020564

Barth, D., Karrer, M., Heinze-Mutz, E. & Elster, N. (1994) Colonization and degradation of cattle dung: aspects of sampling, fecal composition, and artificially formed pats. *Environmental Entomology*, 23, 571–578. Available from: https://doi.org/10.1093/ee/23.3.571

Beadle, N. (1966) Soil phosphate and its role in molding segments of the Australian flora and vegetation, with special reference to xeromorphy and sclerophylly. *Ecology*, 47, 992–1007. Available from: https://doi.org/10.2307/1935647

Bertone, M.A., Green, J.T., Washburn, S.P., Poore, M.H. & Watson, D.W. (2006) The contribution of tunneling dung beetles to pasture soil nutrition. *Forage & Grazinglands*, 4, 1–12. Available from: https://doi.org/10.1094/fg-2006-0711-02-rs

Binkley, D. & Vitousek, P. (1989) Soil nutrient availability. In: Pearcy, R.W., Ehleringer, J., Mooney, H.A. & Rundel, P.W. (Eds.) *Plant physiological ecology: field methods and instrumentation*. New York, NY: Chapman & Hall, pp. 75–96. Available from: https://doi.org/10.1007/978-94-009-2221-1_5

Bornemissza, G. (1960) Could dung eating insects improve our pastures? *Journal of the Australian Institute of Agricultural Science*, 26, 54–56.

Bornemissza, G. (1970) Insectary studies on the control of dung breeding flies by the activity of the dung beetle, *Onthophagus gazella* F. (Coleoptera: Scarabaeinae). *Australian Journal of Entomology*, 9, 31–41. Available from: https://doi.org/10.1111/j.1440-6055.1970.tb00767.x

Bornemissza, G. (1976) Australian dung beetle project 1965-75. Australian Meat Research Committee Review, 30, 1–30. Available from: http://hdl.handle.net/102.100.100/306301?index=1

Brady, N.C., Weil, R.R. & Weil, R.R. (2008) *The nature and properties of soils*. Upper Saddle River, NJ: Prentice Hall. Available from: https://doi.org/10.1023/a:1016012810895

Byrne, M.J., Watkins, B. & Bouwer, G. (2013) Do dung beetle larvae need microbial symbionts from their parents to feed on dung? *Ecological Entomology*, 38, 250–257. Available from: https://doi.org/10.1111/een.12011

Carpinelli, S., Da Fonseca, A.F., Weirich Neto, P.H., Dias, S.H.B. & Pontes, L.D.S. (2020) Spatial and temporal distribution of cattle dung and nutrient cycling in integrated crop–livestock systems. *Agronomy*, 10, 672. Available from: https://doi.org/10.3390/agronomy10050672

Castro-Ramos, J.J., Solís-Oba, A., Solís-Oba, M., Calderón-Vázquez, C.L., Higuera-Rubio, J.M. & Castro-Rivera, R. (2022) Effect of the initial pH on the anaerobic digestion process of dairy cattle manure. *AMB Express*, 12, 162. Available from: https://doi.org/10.1186/s13568-022-01486-8

Cheng, K.-J., Mcallister, T., Popp, J., Hristov, A., Mir, Z. & Shin, H. (1998) A review of bloat in feedlot cattle. *Journal of Animal Science*, 76, 299–308. Available from: https://doi.org/10.2527/1998.761299x

Cobon, D.H., Stone, G., Carter, J., Mckeon, G., Zhang, B. & Heidemann, H. (2020) Native pastures and beef cattle show a spatially variable response to a changing climate in Queensland, Australia. *European Journal of Agronomy*, 114, 126002. Available from: https://doi.org/10.1016/j.eja.2020.126002

Conover, W.J. & Iman, R.L. (1981) Rank transformations as a bridge between parametric and nonparametric statistics. *The American Statistician*, 35, 124–129. Available from: https://doi.org/10.1080/00031305.1981.10479327

Cook, D. (1988) Sexual selection in dung beetles. 2. Female fecundity as an estimate of male reproductive success in relation to horn size, and alternative behavioral strategies in *Onthophagus binodis* Thunberg (Scarabaeidae, Onthophagini). *Australian Journal of Zoology*, 36, 521–532. Available from: https://doi.org/10.1071/zo9880521

Cook, D. (1990) Differences in courtship, mating and postcopulatory behaviour between male morphs of the dung beetle *Onthophagus binodis* Thunberg (Coleoptera: Scarabaeidae). *Animal Behaviour*, 40, 428–436. Available from: https://doi.org/10.1016/s0003-3472(05)80522-6

Cook, D.F. (1993) Influence of adult body size and the male pronotal horn on phenotypic variation in *Onthophagus binodis* Thunberg (Coleoptera: Scarabaeidae). *Australian Journal of Entomology*, 32, 45–50. Available from: https://doi.org/10.1111/j.1440-6055.1993.tb00541.x

Dadour, I.R. & Cook, D.F. (1996) Survival and reproduction in the Scarabaeine dung beetle *Onthophagus binodis* (Coleoptera: Scarabaeidae) on dung produced by cattle on grain diets in feedlots. *Environmental Entomology*, 25, 1026–1031. Available from: https://doi.org/10.1093/ee/25.5.1026

De Mendiburu, F. & De Mendiburu, M.F. (2019) Package 'agricolae'. R Package, Version, 1, 3.

Demment, M.W. & Van Soest, P.J. (1985) A nutritional explanation for body-size patterns of ruminant and nonruminant herbivores. *The American Naturalist*, 125, 641–672. Available from: https://doi.org/10.1086/284369

Doube, B. (2008) The pasture growth and environmental benefits of dung beetles to the southern Australian cattle industry. *Meat & Livestock Industry, North Sydney, NSW*. https://doi.org/10.13140/rg.2.1.1025.0720

Doube, B., Macqueen, A., Ridsdill-Smith, T. & Weir, T. (1991) Native and introduced dung beetles in Australia. In: Hanski, I. & Cambefort, Y. (Eds.) *Dung beetle ecology*. Princeton, NJ: Princeton University Press. Available from: https://doi.org/10.1515/9781400862092

Doube, B.M. (2018) Ecosystem services provided by dung beetles in Australia. *Basic and Applied Ecology*, 26, 35–49. Available from: https://doi.org/10.1016/j.baae.2017.09.008

Eberhard, W.G. (1982) Beetle horn dimorphism: making the best of a bad lot. *The American Naturalist*, 119, 420–426. Available from: https://doi.org/10.1086/283920

Ebert, K.M., Arnold, W.G., Ebert, P.R. & Merritt, D.J. (2021) Hindgut microbiota reflects different digestive strategies in dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae). *Applied and Environmental Microbiology*, 87, e02100–e02120. Available from: https://doi.org/10.1128/aem.02100-20

Edwards, P. (1991) Seasonal variation in the dung of African grazing mammals, and its consequences for coprophagous insects. *Functional Ecology*, 5, 617–628. Available from: https://doi.org/10.2307/2389480

Fox, C.W. & Czesak, M.E. (2000) Evolutionary ecology of progeny size in arthropods. *Annual Review Entomology*, 45, 341–369. Available from: https://doi.org/10.1146/annurev.ento.45.1.341

Fryxell, J.M. (1991) Forage quality and aggregation by large herbivores. *The American Naturalist*, 138, 478–498. Available from: https://doi.org/10.1086/285227

Gittings, T. & Giller, P.S. (1998) Resource quality and the colonisation and succession of coprophagous dung beetles. *Ecography*, 21, 581–592. Available from: https://doi.org/10.1111/j.1600-0587.1998.tb00550.x

Greenwood, P.L., Gardner, G.E. & Ferguson, D.M. (2018) Current situation and future prospects for the Australian beef industry—a review. *Asian-Australasian Journal of Animal Sciences*, 31, 992. Available from: https://doi.org/10.5713/ajas.18.0090

Hansen, B.B., Aanes, R., Herfindal, I., Sæther, B.-E. & Henriksen, S. (2009) Winter habitat–space use in a large arctic herbivore facing contrasting forage abundance. *Polar Biology*, 32, 971–984. Available from: https://doi.org/10.1007/s00300-009-0597-2

Hao, X., Benke, M.B., Gibb, D.J., Stronks, A., Travis, G. & Mcallister, T.A. (2009) Effects of dried distillers' grains with solubles (wheat-based) in feedlot cattle diets on feces and manure composition. *Journal of Environmental Quality*, 38, 1709–1718. Available from: https://doi.org/10.2134/jeq2008.0252

Hartig, F. & Hartig, M.F. (2017) *Package 'DHARMa'*. Vienna, Austria: R Development Core Team.

Heddle, T., Hemmings, Z. & Andrew, N.R. (2023) A baited time sorting pitfall trap allowing more temporal fidelity of dung beetle (Coleoptera: Scarabaeidae) activity. *The Coleopterists Bulletin*, 77, 1–15. Available from: https://doi.org/10.1649/0010-065X-77.1.1

Heddle, T., Hemmings, Z., Burns, A. & Andrew, N.R. (2023) Pasture diet of cattle contributes to the reproductive success of dung beetles. *Agricultural and Forest Entomology*, 1-13, 38–50. Available from: https://doi.org/10.1111/afe.12590

Heddle, T., Nash, M. & Henry, K. (2021) Indigenous and introduced dung beetles (Coleoptera: Scarabaeidae) of temperate Australia: a review of biology, importance and effect of climate change on population distributions. *General and Applied Entomology*, 49, 1–11. Available from: https://doi.org/10.3316/informit.574579267125431

Holter, P. (2016) Herbivore dung as food for dung beetles: elementary coprology for entomologists. *Ecological Entomology*, 41, 367–377. Available from: https://doi.org/10.1111/een.12316

Holter, P., Scholtz, C. & Wardhaugh, K. (2002) Dung feeding in adult Scarabaeines (tunnellers and endocoprids): even large dung beetles eat small particles. *Ecological Entomology*, 27, 169–176. Available from: https://doi.org/10.1046/j.1365-2311.2002.00399.x

Holter, P. & Scholtz, C.H. (2007) What do dung beetles eat? *Ecological Entomology*, 32, 690–697. Available from: https://doi.org/10.1111/j.1365-2311.2007.00915.x

Hunt, J. & Simmons, L. (2000) Maternal and paternal effects on offspring phenotype in the dung beetle *Onthophagus taurus*. *Evolution*, 54, 936–941. Available from: https://doi.org/10.1111/j.0014-3820.2000.tb00093.x

Hunt, J. & Simmons, L.W. (2004) Optimal maternal investment in the dung beetle *Onthophagus taurus*? *Behavioral Ecology and Sociobiology*, 55, 302–312. Available from: https://doi.org/10.1007/s00265-003-0705-1

Issah, G., Schoenau, J. & Knight, J.D. (2021) Landscape position, sampling time and tillage, but not legume species, affect labile carbon and nitrogen fractions in a four-year-old rejuvenated grazed pasture. *Canadian Journal of Soil Science*, 101, 641–653. Available from: https://doi.org/10.1139/cjss-2021-0052

Jost, D.I., Indorf, C., Joergensen, R.G. & Sundrum, A. (2011) Determination of microbial biomass and fungal and bacterial distribution in cattle faeces. *Soil Biology and Biochemistry*, 43, 1237–1244. Available from: https://doi.org/10.1016/j.soilbio.2011.02.013

Juliano, S.A. (1985) The effects of body size on mating and reproduction in *Brachinus lateralis* (Coleoptera: Carabidae). *Ecological Entomology*, 10, 271–280. Available from: https://doi.org/10.1111/j.1365-2311.1985.tb00724.x

Kadlec, R. & Knight, R. (1996) *Treatment wetlands*. Boca Raton, FL: Lewis Publishers. Available from: https://doi.org/10.1201/9781420012514

Kaur, A.P., Holley, J.M. & Andrew, N.R. (2021) Assessing how changes in temporal resource quality influence the reproductive performance of the dung beetle *Onthophagus binodis*. *Ecological Entomology*, 46, 1315–1323. Available from: https://doi.org/10.1111/een.13078

Kazuhira, Y., Hdeaki, K., Takuro, K. & Toshiharu, A. (1991) Nitrogen mineralization and microbial populations in cow dung, dung balls and underlying soil affected by paracoprid dung beetles. *Soil Biology and Biochemistry*, 23, 649–653. Available from: https://doi.org/10.1016/0038-0717(91)90078-x

Kirchmann, H. & Witter, E. (1992) Composition of fresh, aerobic and anaerobic farm animal dungs. *Bioresource Technology*, 40, 137–142. Available from: https://doi.org/10.1016/0960-8524(92)90199-8

Krulwich, T.A., Sachs, G. & Padan, E. (2011) Molecular aspects of bacterial pH sensing and homeostasis. *Nature Reviews Microbiology*, 9, 330–343. Available from: https://doi.org/10.1038/nrmicro2549

Lenth, R. & Lenth, M.R. (2018) Package 'lsmeans'. *The American Statistician*, 34, 216–221.

Lucas, R.E. & Davis, J. (1961) Relationships between pH values of organic soils and availabilities of 12 plant nutrients. *Soil Science*, 92, 177–182. Available from: https://doi.org/10.1097/00010694-196109000-00005

Magnusson, A., Skaug, H., Nielsen, A., Berg, C., Kristensen, K., Maechler, M. et al. (2017) Package 'glmmtmb'. R Package Version 0.2. 0.

Maldonado, M.B., Aranibar, J.N., Serrano, A.M., Chacoff, N.P. & Vázquez, D.P. (2019) Dung beetles and nutrient cycling in a dryland environment. *Catena*, 179, 66–73. Available from: https://doi.org/10.1016/j.catena.2019.03.035

Mårell, A., Hofgaard, A. & Danell, K. (2006) Nutrient dynamics of reindeer forage species along snowmelt gradients at different ecological scales. *Basic and Applied Ecology*, 7, 13–30. Available from: https://doi.org/10.1016/j.baae.2005.04.005

Meyer, J.A., Christensen, C.M. & Knapp, F.W. (1978) The influence of various levels of ground ear corn and alfalfa hay in the bovine diet on the development of the face fly. *Environmental Entomology*, 7, 829–830. Available from: https://doi.org/10.1093/ee/7.6.829

Moore, A.D., Bell, L.W. & Revell, D.K. (2009) Feed gaps in mixed-farming systems: insights from the grain & graze program. *Animal Production Science*, 49, 736–748. Available from: https://doi.org/10.1071/an09010

Morgan, N.O. & Schmidt, C.D. (1966) The pH tolerance of horn fly larvae. *Journal of Economic Entomology*, 59, 222–223. Available from: https://doi.org/10.1093/jee/59.1.222

Muff, S., Nilsen, E.B., O'hara, R.B. & Nater, C.R. (2022) Rewriting results sections in the language of evidence. *Trends in Ecology & Evolution*, 37, 203–210. Available from: https://doi.org/10.1016/j.tree.2021.10.009

Neina, D. (2019) The role of soil pH in plant nutrition and soil remediation. *Applied and Environmental Soil Science*, 2019, 1–9. Available from: https://doi.org/10.1155/2019/5794869

Nervo, B., Tocco, C., Caprio, E., Palestrini, C. & Rolando, A. (2014) The effects of body mass on dung removal efficiency in dung beetles. *PLoS One*, 9(9), e107699. Available from: https://doi.org/10.1371/journal.pone.0107699

Nichols, E., Spector, S., Louzada, J., Larsen, T., Amezquita, S. & Favila, M.E. (2008) Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. *Biological Conservation*, 141, 1461–1474.

Nicol, G.W., Leininger, S., Schleper, C. & Prosser, J.I. (2008) The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. *Environmental Microbiology*, 10, 2966–2978. Available from: https://doi.org/10.1111/j.1462-2920.2008.01701.x

Nortey, T.N., Patience, J.F., Simmins, P.H., Trottier, N.L. & Zijlstra, R.T. (2007) Effects of individual or combined xylanase and phytase supplementation on energy, amino acid, and phosphorus digestibility and growth performance of grower pigs fed wheat-based diets containing wheat millrun. *Journal of Animal Science*, 85, 432–1443. Available from: https://doi.org/10.2527/jas.2006-613

Penn, C.J. & Camberato, J.J. (2019) A critical review on soil chemical processes that control how soil pH affects phosphorus availability to plants. *Agriculture*, 9, 120. Available from: https://doi.org/10.3390/agriculture9060120

Pennacchietti, E., D'alonzo, C., Freddi, L., Occhialini, A. & De Biase, D. (2018) The glutaminase-dependent acid resistance system: qualitative and quantitative assays and analysis of its distribution in enteric bacteria. *Frontiers in Microbiology*, 9, 2869. Available from: https://doi.org/10.3389/fmicb.2018.02869

Perera, R.S., Cullen, B.R. & Eckard, R.J. (2020) Changing patterns of pasture production in south-eastern Australia from 1960 to 2015. *Crop and Pasture Science*, 71, 70–81. Available from: https://doi.org/10.1071/cp19112

Piccini, I., Nervo, B., Forshage, M., Celi, L., Palestrini, C., Rolando, A. et al. (2018) Dung beetles as drivers of ecosystem multifunctionality: are response and effect traits interwoven? *Science of the Total Environment*, 616, 1440–1448. Available from: https://doi.org/10.1016/j.scitotenv.2017.10.171

Pietri, J.A. & Brookes, P. (2008) Relationships between soil pH and microbial properties in a UK arable soil. *Soil Biology and Biochemistry*, 40, 1856–1861. Available from: https://doi.org/10.1016/j.soilbio.2008.03.020

Pitta, D.W., Parmar, N., Patel, A.K., Indugu, N., Kumar, S., Prajapathi, K.B. et al. (2014) Bacterial diversity dynamics associated with different diets and different primer pairs in the rumen of Kankrej cattle. *PLoS One*, 9, e111710. Available from: https://doi.org/10.1371/journal.pone.0111710

R Core Team. (2022) *A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Available from: https://www.r-project.org/

Radtke, M. & Williamson, G. (2005) Volume and linear measurements as predictors of dung beetle (Coleoptera: Scarabaeidae) biomass. *Annals of the Entomological Society of America*, 98, 548–551. Available from: https://doi.org/10.1603/0013-8746(2005)098%5b0548:valmap%5d2.0.co;2

Ridsdill-Smith, T. (1986) The effect of seasonal changes in cattle dung on egg production by 2 species of dung beetles (Coleoptera, Scarabaeidae) in southwestern Australia. *Bulletin of Entomological Research*, 76, 63–68. Available from: https://doi.org/10.1017/s0007485300015285

Ridsdill-Smith, T. & Hayles, L. (1990) Stages of bush Fly, *Musca vetustissima* (Diptera, Muscidae), killed by Scarabaeine dung beetles (Coleoptera, Scarabaeidae) in unfavourable cattle dung. *Bulletin of Entomological Research*, 80, 473–478. Available from: https://doi.org/10.1017/s0007485300050744

Ridsdill-Smith, T., Hayles, L. & Palmer, M. (1987) Mortality of eggs and larvae of the bush fly, *Musca vetustissima* Walker (Diptera: Muscidae), caused by Scarabaeine dung beetles (Coleoptera: Scarabaeidae) in favourable cattle dung. *Bulletin of Entomological Research*, 77, 731–736. Available from: https://doi.org/10.1017/s0007485300012220

Ridsdill-Smith, T. & Matthiessen, J. (1988) Bush fly, *Musca vetustissima* Walker (Diptera, Muscidae), control in relation to seasonal abundance of Scarabaeine dung beetles (Coleoptera, Scarabaeidae) in southwestern Australia. *Bulletin of Entomological Research*, 78, 633–639. Available from: https://doi.org/10.1017/s0007485300015480

Royston, P. (1992) Approximating the Shapiro-Wilk W-test for non-normality. *Statistics and Computing*, 2, 117–119. Available from: https://doi.org/10.1007/bf01891203

Saeed, T. & Sun, G. (2012) A review on nitrogen and organics removal mechanisms in subsurface flow constructed wetlands: dependency on environmental parameters, operating conditions and supporting media. *Journal of Environmental Management*, 112, 429–448. Available from: https://doi.org/10.1016/j.jenvman.2012.08.011

Schoenian, S. (2020) 193 growth performance of lambs and kids on pasture. *Journal of Animal Science*, 98, 67. Available from: https://doi.org/10.1093/jas/skz397.154

Servín-Pastor, M., Salomão, R.P., Caselín-Cuevas, F., Córdoba-Aguilar, A., Favila, M.E., Jácome-Hernández, A. et al. (2021) Malnutrition and parasitism shape ecosystem services provided by dung beetles. *Ecological Indicators*, 121, 107205. Available from: https://doi.org/10.1016/j.ecolind.2020.107205

Shukla, S.P., Sanders, J.G., Byrne, M.J. & Pierce, N.E. (2016) Gut microbiota of dung beetles correspond to dietary specializations of adults and larvae. *Molecular Ecology*, 25, 6092–6106. Available from: https://doi.org/10.1111/mec.13901

Sim, S., Lee, H., Yoon, S., Seon, H., Park, C. & Kim, M. (2022) The impact of different diets and genders on fecal microbiota in Hanwoo cattle. *Journal of Animal Science and Technology*, 64, 897–910. Available from: https://doi.org/10.5187/jast.2022.e71

Šimek, M. & Cooper, J. (2002) The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years. *European Journal of Soil Science*, 53, 345–354. Available from: https://doi.org/10.1046/j.1365-2389.2002.00461.x

Suárez-Moo, P., Cruz-Rosales, M., Ibarra-Laclette, E., Desgarennes, D., Huerta, C. & Lamelas, A. (2020) Diversity and composition of the gut microbiota in the developmental stages of the dung beetle *Copris incertus* Say (Coleoptera, Scarabaeidae). *Frontiers in Microbiology*, 11, 1698. Available from: https://doi.org/10.3389/fmicb.2020.01698

Tyndale-Biscoe, M. (1994) Dung burial by native and introduced dung beetles (Scarabaeidae). *Australian Journal of Agricultural Research*, 45, 1799–1808. Available from: https://doi.org/10.1071/ar9941799

Tyndale-Biscoe, M. (1996) *Australia's introduced dung beetles: original releases and redistributions*. Canberra: CSIRO Australia. Available from: https://doi.org/10.4225/08/5862acd845526

Tyndale-Biscoe, M. & Vogt, W. (1996) Population status of the bush fly, *Musca vetustissima* (Diptera: Muscidae), and native dung beetles (Coleoptera: Scarabaeinae) in south-eastern Australia in relation to establishment of exotic dung beetles. *Bulletin of Entomological Research*, 86, 183–192. Available from: https://doi.org/10.1017/s0007485300052433

Van Vliet, P., Reijs, J., Bloem, J., Dijkstra, J. & De Goede, R. (2007) Effects of cow diet on the microbial community and organic matter and nitrogen content of feces. *Journal of Dairy Science*, 90, 5146–5158. Available from: https://doi.org/10.3168/jds.2007-0065

Vymazal, J. (2007) Removal of nutrients in various types of constructed wetlands. *Science of the Total Environment*, 380, 48–65. Available from: https://doi.org/10.1016/j.scitotenv.2006.09.014

Warton, D.I. & Hui, F.K. (2011) The arcsine is asinine: the analysis of proportions in ecology. *Ecology*, 92, 3–10. Available from: https://doi.org/10.1890/10-0340.1

Wheeler, W. & Noller, C. (1977) Gastrointestinal tract pH and starch in feces of ruminants. *Journal of Animal Science*, 44, 131–135. Available from: https://doi.org/10.2527/jas1977.441131x

Willoughby, W. (1959) Limitations to animal production imposed by seasonal fluctuations in pasture and by management procedures. *Australian Journal of Agricultural Research*, 10, 248–268. Available from: https://doi.org/10.1071/ar9590248

Yang, W., Beauchemin, K. & Rode, L. (2001) Effects of grain processing, forage to concentrate ratio, and forage particle size on rumen pH and digestion by dairy cows. *Journal of Dairy Science*, 84, 2203–2216. Available from: https://doi.org/10.3168/jds.s0022-0302(01)74667-x

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1. Supporting Information.

Figure S1. Live trap used to capture *O. binodis* in the paddocks around Armidale, NSW.

Figure S2. pH 5 fungus on the surface of the dung pad (a) and under the microscope (b).

Figure S3. Δ pH of dung pads with measurements taken 1) internally and 2) the surface of the dung pad over 96 hours. Dung beetle treatment is with beetles (dark grey) and without beetles (light grey).

Figure S4. mean pH (+/- SE) of dung pads over 96 hours 1) internally and 2) externally across different dung pH treatments. Solid line indicates treatments with dung beetles, dashed line indicates treatments without dung beetles for individual pH treatments. Circles indicates a treatment which commenced at pH 5, triangle indicates a treatment of pH 6 and square with a control treatment.

Figure S5. Predicted mean dry weight (g) of broods with 95% confidence intervals. Raw data is presented as points behind confidence intervals. Significant differences are indicated with letters. 143, 65, 147 and 156 broods were used from the control, pH 5, pH 6 and pH 7 respectively.

Table S1. Summary of the life stage mortality of *O. binodis* progeny. Data presented as percent (%) of broods in total. Bold numbers indicate strong evidence of differences for mortality.

Table S2. Summary table of number of F1 progeny which emerged were measured and analysed for F1 statistics.

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