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Augmenting Laboratory Rearing of Stable Fly (Diptera: Muscidae) Larvae With Ammoniacal Salts

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Abstract

Stable flies are blood feeding parasites and serious pests of livestock. The immature stages develop in decaying materials which frequently have high ammonium content. We added various ammonium salts to our laboratory stable fly rearing medium and measured their effect on size and survival as well as the physical properties of the used media. The addition of ammonium hydroxide, ammonium phosphate and ammonium sulfate reduced larval survival. These compounds decreased pH and increased ammonium content of the used media. Ammonium bicarbonate had no effect on pH and marginally increased ammonium while increasing survival twofold. The optimal level of ammonium bicarbonate was 50 g (0.63 mol) per pan. Larval survival decreased when pH was outside the range of 8.5 to 9.0.

Key words: ammonium, bicarbonate, larval medium, development

Stable flies, *Stomoxys calcitrans* (L.), are serious pests of livestock worldwide. Their painful bites reduce weight gain and milk production in cattle (Bruce and Decker 1947, 1958; Campbell et al. 1977, 1987, 1993, 2001; Taylor et al. 2012). In addition, they are severe nuisances for humans, companion animals and wildlife (Hansens 1951, Newson 1977, Hogsette et al. 1987, Urban and Broce 1998). Immature stable flies develop in a wide variety of decomposing or fermenting vegetative materials, often mixed with animal wastes (Simmons and Dove 1941, 1942, Siverly and Schoof 1955, Hafez and Gamal-Eddin 1959, Sutherland 1978, Campbell and McNeal 1979, Hall et al. 1982, Meyer and Petersen 1983). The chemical and physical characteristics determining the quality of a substrate for stable fly larval development are poorly understood (Broce and Haas 1999, Talley et al. 2009, Wienhold and Taylor 2012, Friesen et al. 2016). A goal of our research has been to characterize the physical and chemical properties of stable fly substrates to delineate their defining properties.

Stable fly developmental substrates frequently have high levels of ammonia (Wienhold and Taylor 2012, Friesen et al. 2016) and stable fly larvae exhibit positive chemotaxis to ammonia (unpubl. data) even at very high concentrations. In addition, electric conductivity of the substrate, an indicator of the concentration of salts including ammonium, is positively correlated with the probability of the presence of stable fly larvae (Wienhold and Taylor 2012). These observations led us to speculate upon the possible effects of augmenting stable fly developmental media with ammonium salts. The objective of this study was to evaluate the effects of ammonium salts on size and survival of developing stable flies as well as physical properties of the substrates.

Materials and Methods

Stable Flies

Flies were from a laboratory colony reared using the standard operating procedures developed by the Agroecosystem Management Research Unit at the University of Nebraska-Lincoln. They were reared at 23 ± 2°C, 30–50% RH and a photoperiod of 12:12 (L:D) h. The larval media consisted of 500 g wheat bran, 200 g wood chips, and 115 g fish meal mixed with 1.6 liters of distilled water. Media (2,415 g) was placed in a 1.5 liter plastic pan and 1 ml of freshly collected stable fly eggs (≈8,000 eggs) was added to each pan. Eggs were covered lightly with media and pans were placed in pillow cases closed with rubber bands. After 14 d, pupae were harvested by removing aggregations from around the edges with a spoon. Remaining media was examined for isolated pupae which were removed as well. Pupae were cleaned by flotation in water and placed on paper towels to dry.

Biological Properties

After pupae dried, they were weighed (tWt) and a sample of 100 pupae was selected randomly and weighed (cWt). The number of pupae was calculated by (tWt/cWt) × 100. The 100 pupa samples were held for 10 d until adult fly emergence was complete and percent emergence and sex ratio were calculated.

Physical Properties of Substrate

A 16 g sample of used media from each pan was combined with 80 ml distilled water and agitated on a stirring plate for 1 h for determining pH, conductivity and ammonium concentration.

Table 1. Diet additives used in study

Compound	Formula	Molecular Weight	CAS	Source
Ammonium chloride	NH_4Cl	53.50	12125-02-9	Fisher Scientific[a], A649
Ammonium sulfate	$(NH_4)_2SO_4$	132.14	7783-20-2	Sigma-Aldrich[b], A5132
Ammonium bicarbonate	NH_4HCO_3	79.06	1066-33-7	JT Baker[c], 3003-05
Ammonium phosphate, dibasic	$H(NH_4)_2PO_4$	132.07	7783-28-0	Sigma-Aldrich[b], A5764
Ammonium hydroxide	NH_4OH	35.04	1336-21-6	JT Baker[c], 9721-05
Sodium bicarbonate	$NaHCO_3$	84.00	144-55-8	Fisher Scientific[a], S233
Potassium bicarbonate	$KHCO_3$	100.12	298-14-6	Fisher Scientific[a], P184

[a]Thermo Fisher Scientific Inc., Waltham, Massachusetts.
[b]Sigma-Aldrich, St. Louis, Missouri.
[c]JT Baker, Center Valley, Pennsylvania.

Conductivity and pH were measured with a pH conductivity meter (Oakton 510 series, Vernon Hills, Illinois). Ammonium levels were measured using an Oakton Ion 2100 series meter with a Cole Parmer (Vernon Hills, Illinois) ammonia ion-selective electrode. Moisture was calculated by weighing ≈50 g of media, drying at 100 °C for 48 h, and weighing the dried sample. Moisture of the sample was calculated relative to dry weight, Moist = $(W_{wet} - W_{dry})/W_{dry}$ (Verhulst et al. 2013).

Treatments

Salts were dissolved in the distilled water before adding dry diet components. Three experiments were conducted. In the first, five ammonium salts (ammonium bicarbonate, ammonium chloride, ammonium hydroxide, ammonium phosphate and ammonium sulfate) were evaluated at a concentration of 0.63 mol of ammonium per rearing pan. In the second experiment, five quantities (0, 12.5, 25, 50, and 100 g) of ammonium bicarbonate and sodium bicarbonate per rearing pan were compared. In the third experiment, three bicarbonate salts (ammonium bicarbonate, potassium bicarbonate and sodium bicarbonate) were evaluated at a concentration of 0.63 mol per rearing pan (Table 1).

Statistical Analysis

Data were evaluated with ANOVA and regression analyses (Proc Glimmix, SAS 9.3, SAS 2012). A negative binomial distribution with a log link function was used for number of pupae, a lognormal distribution for ammonium, a normal distribution for pupal weight, pH, and conductivity and a binomial distribution with a logit link function was used for sex ratio and emergence. All experiments were replicated three times.

Results

Ammonium Salts

The first experiment evaluated the effects of several ammonium salts at a dosage of 0.63 mol of ammonium per rearing pan. No stable fly larvae completed development to pupariation with ammonium hydroxide and very few completed development with ammonium phosphate (6.0 ± 3.5) and ammonium sulfate (20.0 ± 17.6; $F = 24.36$, df = 5,12, $P < 0.05$; Fig. 1). We collected twofold more pupae from media supplemented with ammonium bicarbonate relative to control media ($t = 2.87$, df = 6, $P = 0.05$). Pupal weight ($F = 1.08$, df = 4,8, $P = 0.43$), percent adult emergence (42 ± 6; $F = 0.75$, df = 4,8, $P = 0.58$) and sex ratio (0.42 ± 0.06; $F = 0.74$, df = 4,7, $P = 0.59$) did not vary among treatments.

Ammonium chloride, ammonium phosphate and ammonium sulfate reduced the pH of the used media relative to that of the control ($t = 7.83$, 4.42, and 5.58, respectively, df = 12, $P < 0.05$; Fig. 1) whereas ammonium bicarbonate had no effect on pH ($t = 1.53$, df = 12, $P = 0.44$). Ammonium chloride, ammonium hydroxide, ammonium phosphate, and ammonium sulfate increased the ammonium content of the media ($t = 6.31$, 3.85, 6.79, and 7.10, respectively, df = 12, $P < 0.05$) whereas ammonium bicarbonate produced a marginally significant increase ($t = 2.64$, df = 12, $P = 0.08$). Moisture content did not vary among treatments ($\bar{x} = 2.6 \pm 0.07$; $F = 0.11$, df = 5,12, $P = 0.11$).

Ammonium Bicarbonate and Sodium Bicarbonate

Effects of four different quantities of ammonium bicarbonate and sodium bicarbonate were compared. Both compound and quantity had significant effects upon the number of pupae per pan ($F = 6.59$, df = 1,20, $P < 0.05$, $F = 3.96$, df = 4,20, $P < 0.05$, respectively). The interaction term was marginally significant ($F = 2.62$, df = 4,20, $P = 0.07$). Over twofold more pupae were produced in pans with ammonium bicarbonate (2,528 ± 513) relative to pans with sodium bicarbonate (1,112 ± 360). Regression analysis indicated that the relationship between number of pupae and amount of ammonium bicarbonate was curvilinear although the level of significance for both terms was marginal ($F = 3.16$, df = 1,12, $P = 0.10$ for quantity and $F = 3.54$, df = 1,12, $P = 0.08$ for quantity squared). The regression equation $e^{7.2+0.04q-0.0004q^2}$ has an optimum of 4,277 pupae at 47 g of ammonium bicarbonate per pan. In our 50 g per pan treatment, we collected 4,589 ± 521 pupae compared with 1,708 ± 1,051 pupae per pan for the control treatment. The amount of ammonium bicarbonate per pan had no effect on pupal weight ($F = 1.11$, df = 4,10, $P = 0.40$). Significant effects of the amount of ammonium bicarbonate were observed on pupal emergence ($F = 15.04$, df = 4,9, $P < 0.05$) and sex ratio ($F = 3.65$, df = 4,9, $P = 0.05$). Pupal emergence was higher for the 25 and 50 g treatments, 79 and 81%, than the controls, 64% ($t = 4.05$ and 4.43, respectively, df = 9, $P < 0.05$). Sex ratio was probably an anomaly because the percentage of the adults that were female differed from the control value only for the lowest treatment level, 12.5 g, and a single replicate with very low survival accounted for most of that difference (Fig. 2).

Media pH **varied** relative to compound added, quantity and their interaction ($F = 216.7$, df = 1,19, $P < 0.05$, $F = 90.4$, df = 4,19, $P < 0.05$, $F = 57.2$, df = 4,19, $P < 0.05$, respectively). Change in pH relative to quantity was marginally significant for ammonium bicarbonate ($F = 3.43$, df = 4,9, $P = 0.06$) as a result of a higher pH with the highest dose, 8.96, compared with 8.72–8.76 for the 0–50 g treatments. pH increased curvilinearly relative to the quantity of sodium bicarbonate ($F = 164.9$ and 61.4, df = 1,11, $P < 0.05$ for quantity and quantity squared, respectively). The regression

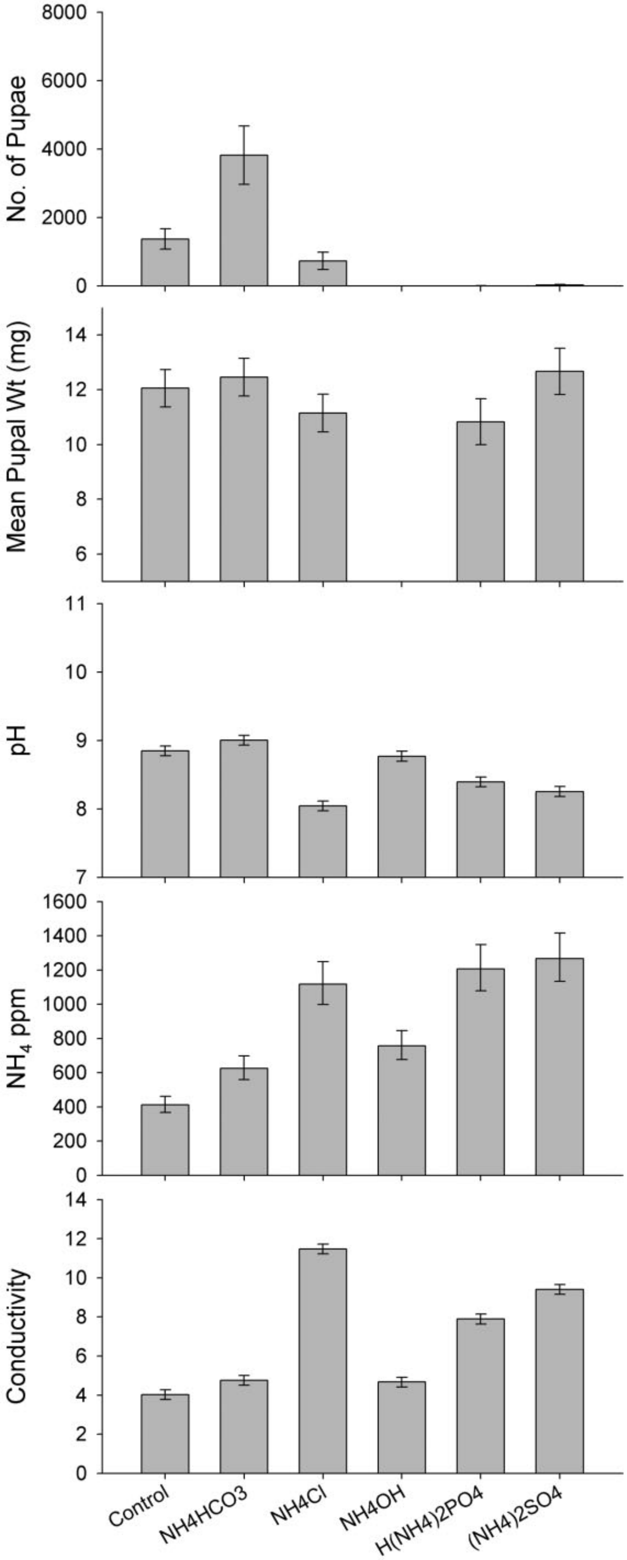

Fig. 1. Comparison of effects of ammonium salts on development of stable fly larvae and physical properties of used substrate.

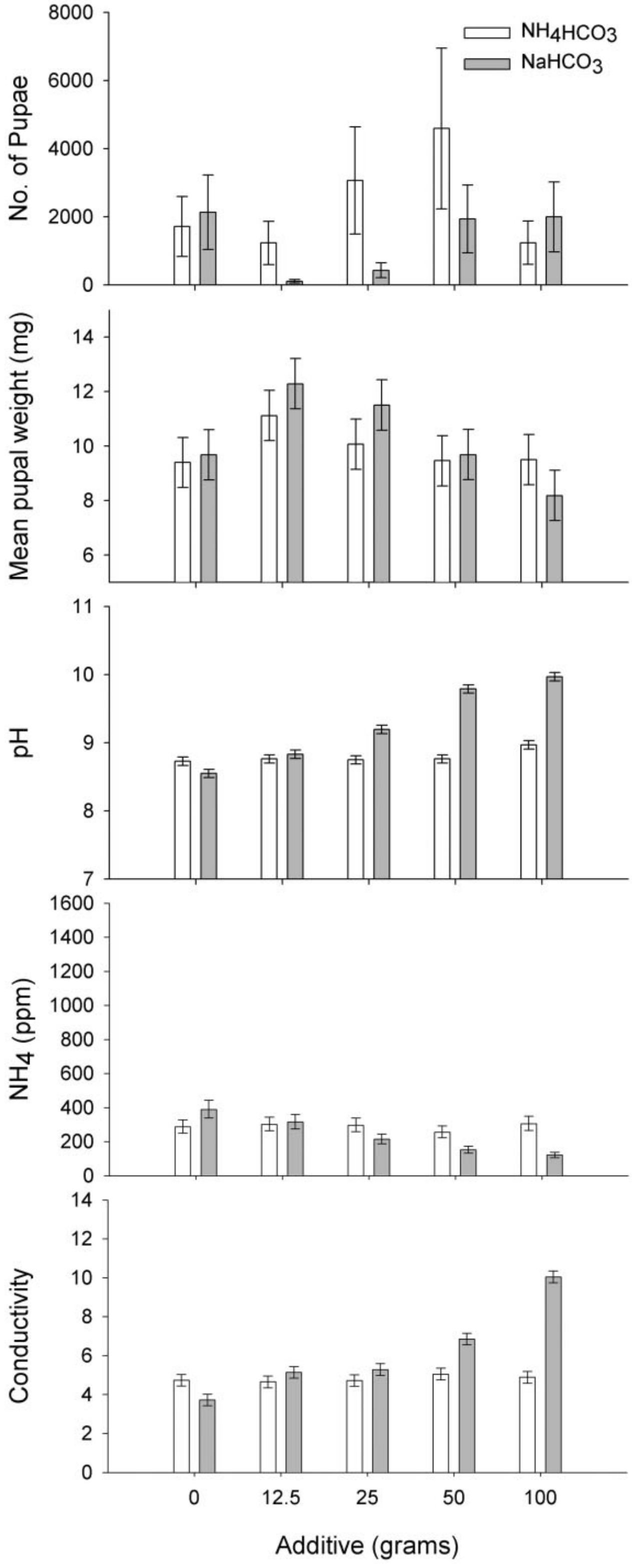

Fig. 2. Comparison of effects of 0–100 g of ammonium bicarbonate and sodium bicarbonate on stable fly development and physical properties of used substrate.

equation was $pH = 8.54 + 0.035q - 0.0002q^2$. Ammonium content of the used media varied relative to compound, quantity and their interaction ($F = 7.5$, df = 1,19, $P < 0.05$, $F = 6.5$, df = 4,19, $P < 0.05$, $F = 5.3$, df = 4,19, $P < 0.05$, respectively). Overall, ammonium levels were higher when ammonium bicarbonate was added (287 ± 27 ppm) than with sodium bicarbonate (197 ± 20 ppm) ($F = 16.92$, df = 1,19, $P < 0.05$). For ammonium bicarbonate, the ammonium content did not vary from the control nor with the quantity added ($F = 0.35$, df = 4,9, $P = 0.84$). With sodium bicarbonate, the ammonium content in the used media decreased curvilinearly relative to the quantity added ($F = 111.95$ and 51.91, df = 1,11, $P < 0.05$ for quantity and quantity squared, respectively). The regression equation was ammonium ppm = $e^{5.8+0.03q-0.0002q^2}$. Conductivity was highly correlated with pH ($r = 0.87$) and the pattern observed relative to compound, quantity, and their interactions were very similar. Moisture levels in the used diet did not vary relative to the compound added ($F = 1.48$, df = 1,19, $P = 0.24$) but did vary relative to the quantity of compound added ($F = 6.98$, df = 4,19, $P < 0.05$). The interaction term was insignificant as well ($F = 0.46$, df = 4,19, $P = 0.77$). Moisture decreased curvilinearly ($t = -4.33$ and 3.54, df = 26, $P < 0.05$ for quantity and quantity squared, respectively). The regression equation was $2.60\text{-}0.018q + 0.0001q^2$.

Bicarbonates

To evaluate whether the beneficial effects of ammonium bicarbonate were in response to the ammonium or bicarbonate moiety, we compared two additional bicarbonate salts, potassium bicarbonate and sodium bicarbonate, with ammonium bicarbonate. The bicarbonate salts had a significant effect upon the number of pupae produced ($F = 12.52$; df = 3,20, $P = 0.5$). Pans with ammonium bicarbonate and sodium bicarbonate produced 2× and 1.5× more pupae than did the control pans (Fig. 3; $t = 6.11$ and 3.51, respectively, df = 20, $P < 0.05$). Pupal weight did not vary among compounds ($F = 0.14$, df = 3,20, $P = 0.94$). The pattern of variation in pupal emergence was similar to that observed for number of pupae. Emergence varied among treatments ($F = 11.52$, df = 3,20, $P < 0.05$) with ammonium bicarbonate (85 ± 4%) being higher than the control (80 ± 5%; $t = 2.94$, df = 20, $P < 0.05$) and sodium bicarbonate being lower (75 ± 6%; $t = -2.70$, df = 20, $P < 0.05$). Emergence of pupae developing with potassium bicarbonate did not differ from that of the control (84 ± 4%; $t = 1.65$, df = 20, $P = 0.27$). Sex ratio was 50 ± 0.9% female and did not vary among treatments ($F = 0.60$, df = 3,20, $P = 0.62$).

Media pH **varied** among treatments ($F = 50.6$, df = 3,20, $P < 0.05$); media with potassium bicarbonate and sodium bicarbonate were more basic than the control media ($t = 9.83$ and 9.81, respectively, df = 20, $P < 0.05$) whereas the pH of media with ammonium bicarbonate did not differ from the control (Fig. 3; $t = 1.38$, df = 20, $P = 0.41$). Ammonium in the used diets varied by treatment ($F = 32.57$, df = 3,20, $P < 0.05$) being lower than the control in media with potassium bicarbonate and sodium bicarbonate ($t = -6.56$ and -6.76, respectively, df = 20, $P < 0.05$) and similar to the control for media with ammonium bicarbonate ($t = 0.52$, df = 20, $P = 0.92$). Conductivity was highly correlated with pH ($r = 0.93$) and followed the same pattern of variation. Used media with sodium bicarbonate had a lower moisture level (2.9 ± 0.12) than did the control media (3.2 ± 0.12; $t = -2.67$, df = 20, $P < 0.05$). Ammonium bicarbonate and potassium bicarbonate had no effect upon the moisture level of the media ($t = -1.55$ and -1.39, df = 20, $P = 0.32$ and 0.41, respectively).

Pooled Analysis

As with many studies dealing with insect rearing and diets, variation, especially in biological parameters, was high and number of replicates for individual studies was relatively low. This situation resulted in weak statistical support for some of the experiments. In order to address this concern for the primary comparison of the study, we repeated the analysis using pooled data from the three experiments for the control and 0.63 M (50 g) ammonium bicarbonate treatments. The result was 18 replicates with ammonium bicarbonate and 21 without. Two times more pupae were recovered from pans with ammonium bicarbonate (4,440 ± 199) compared with control pans (1,959 ± 486; $F = 30.04$, df = 1,37,

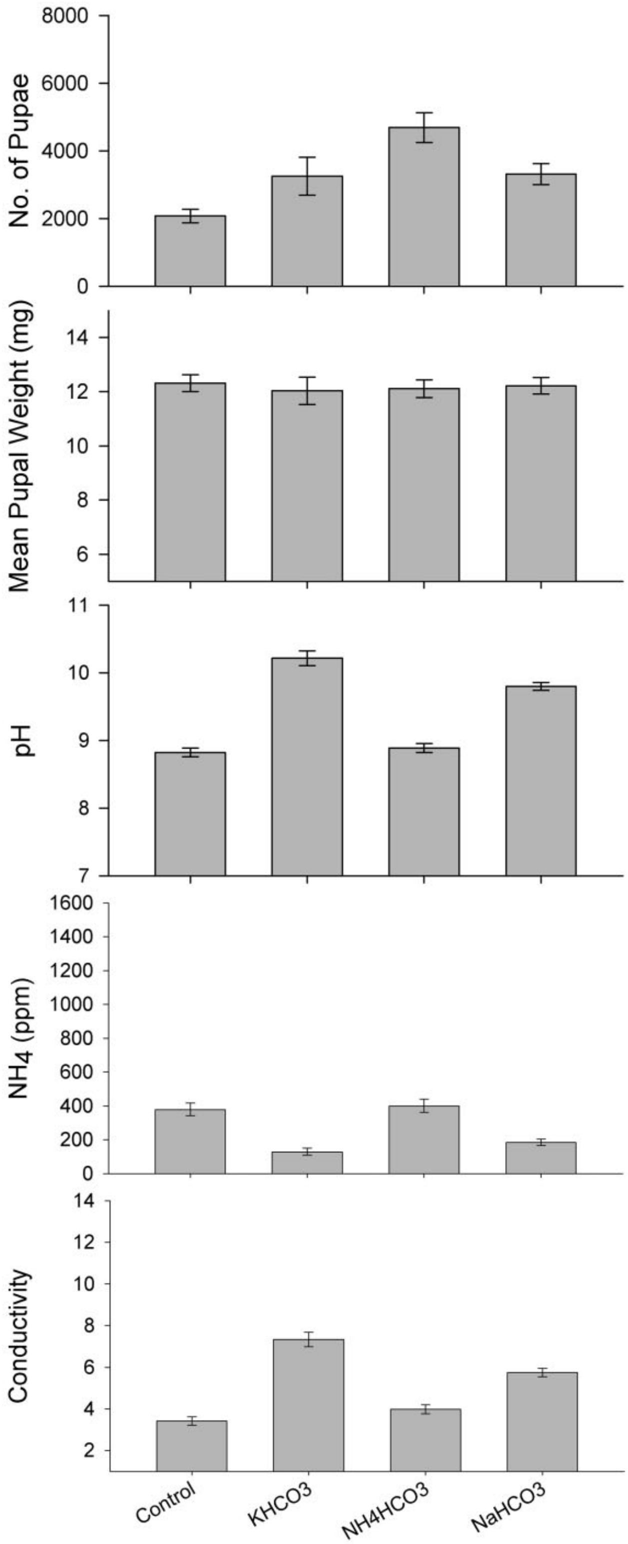

Fig. 3. Comparison of effects of potassium bicarbonate, ammonium bicarbonate and sodium bicarbonate on stable fly development and physical properties of used substrate.

Table 2. Correlation matrix for biological and physical properties

	Weight	Emergence	Sex ratio	pH	NH_4	Conductivity	Moisture
No. Pupae	−0.19	0.49*	0.37*	0.31*	−0.34*	−0.28*	0.24*
Weight		0.09	−0.33*	−0.13	0.09	−0.31*	0.45*
Emergence			0.48*	0.06	−0.24*	−0.33*	0.37*
Sex Ratio				0.10	−0.19	−0.20	0.17
pH					−0.68*	0.11	−0.05
NH_4						0.39*	−0.03
Conductivity							−0.48*

*$P < 0.05$.

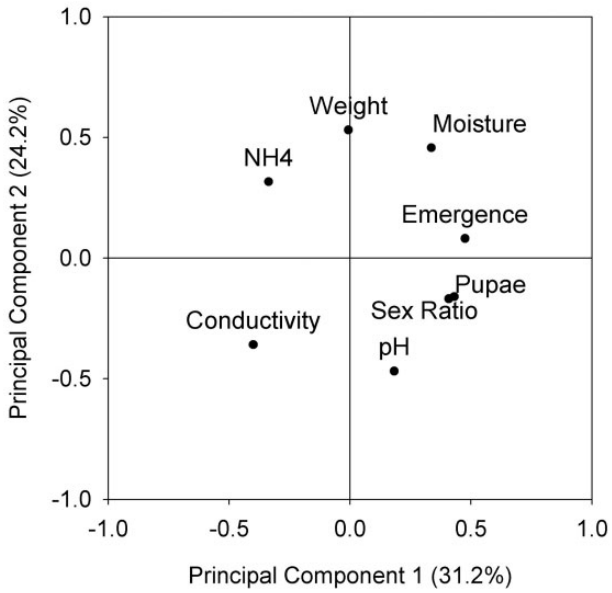

Fig. 4. Principal Component analysis of pooled dataset.

$P < 0.05$). Pupae reared on the two media were the same size (11.9 ± 1.2 mg; $F = 0.07$, df = 1,37, $P = 0.80$). Emergence was higher for pupae reared with ammonium bicarbonate (76 ± 1%) compared with the control (69 ± 1%; $F = 24.22$, df = 1,37, $P < 0.05$) and sex ratio (52% female) did not differ among treatments ($F = 2.14$, df = 1,37, $P = 0.15$). pH of media amended with ammonium bicarbonate was slightly higher (8.95 ± 0.04) compared with control media (8.78 ± 0.03; $F = 10.63$, df = 1,37, $P < 0.05$). Ammonium concentration tended to be higher in media with ammonium bicarbonate (426 ± 32 ppm) compared with control media (353 ± 29 ppm) but the difference was not significant ($F = 2.81$, df = 1,37, $P = 0.10$). Conductivity of the treated media (4.33 ± 0.17 U) was higher than that of control media (3.71 ± 0.16; $F = 6.80$, df = 1,37, $P < 0.05$).

Evaluation of biological and physical parameters in the complete pooled dataset (all compounds included) revealed that three biological parameters, number of pupae, emergence and sex ratio were positively correlated (Table 2; Fig. 4). Those biological parameters were positively correlated with moisture and pH and negatively correlated with ammonium and conductivity. Pupal weight was positively correlated with moisture.

Discussion

The addition of ammonium bicarbonate to our standard stable fly larval diet more than doubled the number of pupae produced per pan with no negative affect on pupal weight. The optimal dose of ammonium bicarbonate was 50 g per pan with 2,415 g of media or 2% on a weight basis. In addition to improving rearing efficiency, the increased number of pupae harvested represents improved larval survival and hence decreased selection and bottleneck during the colonization process. Therefore, laboratory colonies of stable flies reared with ammonium bicarbonate amended diet should be more representative of the natural population from which they were derived.

Detailed **discussion** of the properties of each of the salts used is beyond the scope of this study. However, none of the moieties are directly toxic. Therefore, the observed effects of the salts on the biological parameters are most likely the result of changes in the physical properties of the media affecting either the immature stable flies themselves or the microcosm upon which they depend for development. Compounds which reduced the pH of the media to below 8.5 or increased it to above 9.0 had negative effects larval survival. Both the ammonium and bicarbonate moieties appeared to have a positive effect upon larval survival when the pH was maintained within the acceptable range.

Interestingly, the **ammonium** concentration and conductivity of the used diet were negatively correlated with the number of pupae produced. Given that both of these properties are positively correlated with the presence of immature stable flies in natural habitats (Wienhold and Taylor 2012) and the experimental diets all began with similar ammonium concentrations, this was probably the result of increased nitrification of the ammonium by the microcosm in those media more conducive to stable fly larval survival and development.

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References Cited

Broce, A. B., and M. S. Haas. 1999. Relation of cattle manure age to colonization by stable fly and house fly (Diptera: Muscidae). J. Kansas Entomol. Soc. 72: 60–72.

Bruce, W. N., and G. C. Decker. 1947. Fly control and milk flow. J. Econ. Entomol. 40: 530–536.

Bruce, W. N., and G. C. Decker. 1958. The relationship of stable fly abundance to milk production in dairy cattle. J. Econ. Entomol. 51: 269–274.

Campbell, J. B., R. G. White, J. E. Wright, R. Crookshank, and D. C. Clanton. 1977. Effects of stable flies on weight gains and feed efficiency of calves on growing or finishing rations. J. Econ. Entomol. 70: 592–594.

Campbell, J. B., and C. D. McNeal. 1979. A guide to integrated pest management at feedlots and dairies. Univ. Nebr. Coop. Ext. Serv., EC 80-1536. Lincoln, NE. 21. pp.

Campbell, J. B., I. L. Berry, D. J. Boxler, R. L. Davis, and D. C. Clanton. 1987. Effects of stable flies (Diptera: Muscidae) on weight gain and feed efficiency of feedlot cattle. J. Econ. Entomol. 80: 117–119.

Campbell, J. B., M. A. Catangui, G. D. Thomas, D. J. Boxler, and R. Davis. 1993. Effects of stable flies (Diptera: Muscidae) and heat stress on weight gain and feed conversion of feeder cattle. J. Agric. Entomol. 10: 155–161.

Campbell, J. B., S. R. Skoda, D. R. Berkebile, D. J. Boxler, G. D. Thomas, D. C. Adams, and R. Davis. 2001. Effects of stable flies (Diptera: Muscidae) on weight gains of grazing yearling cattle. J. Econ. Entomol. 94: 780–783.

Verhulst, N., R. Cox, and B. Govaerts. 2013. Soil water content: A practical guide for comparing crop management practices. CIMMYT. Mexico, DF (Mexico). 4. pp.

Friesen, K., D. R. Berkebile, B. J. Wienhold, L. Durso, J. Zhu, and D. B. Taylor. 2016. Environmental parameters associated with stable fly (Diptera: Muscidae) development at hay feeding sites. Environ. Entomol. 45: 570–576.

Hafez, M., and F. M. Gamal-Eddin. 1959. Ecological studies on *Stomoxys calcitrans* L. and *sitiens* Rond. in Egypt, with suggestions on their control (Diptera: Muscidae). Bull. Soc. Entomol. Egypte. 43: 245–283.

Hall, R. D., G. D. Thomas, and C. E. Morgan. 1982. Stable flies, *Stomoxys calcitrans* (L.), breeding in large round hay bales: initial associations (Diptera: Muscidae). J. Kans. Entomol. 55: 617–620.

Hansens, E. J. 1951. The stable fly and its effect on seashore recreational areas in New Jersey. J. Econ. Entomol. 44: 482–487.

Hogsette, J. A., J. P. Ruff, and C. J. Jones. 1987. Stable fly biology and control in northwest Florida. J. Agric. Entomol. 4: 1–11.

Meyer, J. A., and J. J. Petersen. 1983. characterization and seasonal distribution of breeding sites of stable flies and house flies (Diptera: Muscidae) on eastern Nebraska feedlots and dairies. J. Econ. Entomol. 76: 103–108.

Newson, H. D. 1977. Arthropod problems in recreational areas. Ann. Rev. Entomol. 22: 333–353.

SAS. 2012. SAS 9.3 help and documentation. SAS Institute, Cary, NC.

Simmons, S. W., and W. E. Dove. 1941. Breeding places of the stable fly or "dog fly" *Stomoxys calcitrans* (L.) in northwestern Florida. J. Econ. Entomol. 34: 457–462.

Simmons, S. W., and W. E. Dove. 1942. Waste celery as a breeding medium for the stable fly or "dog fly," with suggestions for control. J. Econ. Entomol. 35: 709–715.

Siverly, R. E., and H. F. Schoof. 1955. Utilization of various production media by muscoid flies in a metropolitan area. I. Adaptability of different flies for infestation of prevalent media. Ann. Entomol. Soc. Am. 48: 258–262.

Sutherland, B. 1978. The suitability of various types of dung and vegetable matter as larval breeding media for *Stomoxys calcitrans* L. (Diptera: Muscidae). Onderstepoort J. Vet. Res. 45: 241–243.

Talley, J., A. Broce, and L. Zurek. 2009. Characterization of Stable Fly (Diptera: Muscidae) Larval Developmental Habitat at Round Hay Bale Feeding Sites. J. Med. Entomol. 46: 1310–1319.

Taylor, D. B., R. D. Moon, and D. R. Mark. 2012. Economic impact of stable flies (Diptera: Muscidae) on dairy and beef cattle production. J. Med. Entomol. 49: 198–209.

Urban, J. E., and A. B. Broce. 1998. Flies and their bacterial loads in greyhound kennels in Kansas. Curr. Microbiol. 36: 164–170.

Wienhold, B. J., and D. B. Taylor. 2012. Substrate properties of stable fly (Diptera: Muscidae) developmental sites associated with round bale hay feeding sites in eastern Nebraska. Environ. Entomol. 41: 213–221.