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Physicochemistry of Diet Influences Nutritional Indices and Performance of the Cotton Leafworm, *Spodoptera littoralis* (Boisd.)

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Abstract: Nine artificial diets were prepared by buffer solutions differing in their pH and molarities. They were fed by the fourth larval instar of *Spodoptera littoralis* for 4 days for testing the ability of alternation in physicochemical conditions of the diets to induce significant changes in nutritional indices, total carbohydrates and fitness of the larvae. pH had a significant reduction on weight gain, RCR (relative consumption rate) and total carbohydrates. On the other hand, the effect of different buffer molarities was significant on weight gain, RGR (Relative Growth Rate), RCR, ECI (efficiency of conversion of ingested food), ECD (efficiency of conversion of digested food) and total carbohydrates. The interaction between the both factors was high as observed in most parameters. AD (Approximate Digestibility) demonstrated that digestion was not adversely affected, which might indicates that midgut has a good buffering system. It was concluded that larvae probably to over come unsuitable physicochemical conditions of the introduced diets, increased their demand on energy through consuming digested or assimilated food like carbohydrates instead of building new tissues, which finally influencing larval performance parameters.

Key words: Physicochemistry, diet, *Spodoptera littoralis*, nutritional indices, performance, carbohydrates

INTRODUCTION

Phytophagous insects depend on plants as food which must fulfill their nutritional requirements for normal growth and development to occur. However, there are some aspects that might interfere with this rule. Nutrient deficiency and the change in food composition strongly influence performance parameters such as survival, growth and development in phytophagous insects (Lindroth *et al*., 1991; Mattson and Scriber, 1987; Lavy *et al*., 2001). Allelochemicals, water and nitrogen content and physical attributes of foliage are considered to be determinant factors greatly affecting the quality of plant as diet, which in turn suppress insect growth and survival (Felton *et al*., 1992; Appel, 1994). Since midgut pH has long been recognized as an important factor for the optimal activity of digestive enzymes, studies have been expanded to examine the physicochemical characters of diet or plant such as pH, redox potential and buffering capacity and their influencing on insects gut conditions (Schultz and Lechowiz, 1986; Appel and Maines, 1995; Johnson and Felton, 1996). But reports deal with effect of such characters on performance and fitness on phytophagous insects are few. Karowe and Martin (1993) found that the growth of fourth instar, *Manduca sexta* larvae on nutrient-rich artificial diets is significantly affected by the characteristics of the buffer system present in the diet. However, gut physicochemistry which may strongly impact digestion and nutrient assimilation in herbivorous insects varies among lepidopteran larvae (Johnson and Felton, 1996). The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) is a major pest of cotton in Egypt. The majority of literatures on the nutritional value of this pest's food deal with the effect of plant chemicals as gossypol (Meisner *et al*., 1978), or nutrient deficiency as ascorbic acid depriviation (Navon *et al*., 1985), or even starvation (Amin and El-Halafawy, 2001) on the physiology and normal growth of the larvae. To the best of our knowledge, no one has studied the effect of the physicochemical characters of the diet on

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this species. So hydrogen ion concentration and molarity of an artificial diet were chosen to test their ability to induce significant changes in nutritional indices and growth of the cotton leafworm larvae.

MATERIALS AND METHODS

The used strain of *S. littoralis* was a laboratory strain, obtained as newly hatched larvae from a culture of Central Agricultural Pesticides Laboratory, Dokki, Egypt.

Larvae were reared under constant conditions of 25±1°C and 70±5% RH at the laboratory of Pest Physiology Dept., Plant Protection Research Institute, Egypt. They were fed on an artificial diet prepared as described by Shorey and Hale (1965). Chemicals used throughout the study were of analytical quality and purchased from commercial suppliers.

Newly moulted 4th instar larvae were supplied with different artificial diets, prepared by using buffers had different pH values and different molar concentrations. Control diet prepared using distilled water. Citric acid-Na_2HPO_4 (McIlvaine) buffer solutions were chosen due to the presence of citrate in plant tissues, which largely determines their pH (Kurkdjian and Guern, 1989). In the present study, the buffer system consisted of solutions containing a mixture of weak acid (Citric acid) and salt of weak base (Na_2HPO_4).

Preliminary studies were done for choosing buffer solutions of acceptable values with respect to pH and buffer concentration (conc.) i.e., that makes artificial diets in the range as that of plants either from pH point of view or buffering capacity. The buffer capacity which is an indicator of solution effectiveness in minimizing the pH change that results from the addition of standard quantity of strong acid or strong base was used to predict the range of molar conc. used. Lechowicz (1983) found that pH of plant tissues from a number of different plants in spring 1980 ranged from 4.05-6.19 and buffer capacity 36.4-266.3/μmoles OH^-/g tissue. A quantitative measure of buffer capacity in this work was given by the number of μmoles of strong base (NaOH) required to change the diet pH to the average of lepidopteran midgut pH = 8.75 (Berenbaum, 1980). Buffer capacity was ranged, in the present study, from 158 to 246.2 μmoles NaOH/1 g fresh diet. By adding buffer solutions having 3 different pH values, each of which has 3 different buffer conc., we get 9 diets as shown in Table 1.

pH and titration were carried out at room temperature (25°C). One gram of freshly prepared diet was titrated against 0.02 N NaOH solution using a glass stirbar as a stirrer during the slow flowing of the base solution. pH measurements were carried out using universal indicator strips purchased from MERCK, Germany.

Thirty larvae in three replicates were used for each diet. Fresh diet was supplied during the time of experiment (4 successive days). The weight of the introduced diet was increased according to the increase of larval body weight (growth), but ensuring most of the diet consumed. Dead larvae were discarded. The fresh weight of survivors, diet and feaces in each rearing jar were recorded daily. Fresh diets were kept under the same conditions to estimate the natural loss of moisture, consequently the corrected weight of consumed food was calculated as described by Waldbaeur (1968). Standard formulas of Soo Hoo and Fraenkle (1966) and Waldbaeur (1968) were used for calculating Relative Consumption Rate (RCR), Relative Growth Rate (RGR), Approximate Digestibility (AD), efficiency of conversion of ingested food to body substances (ECI) and efficiency of conversion of digested food to body substances (ECD).

Total carbohydrates were estimated in acid extract of body homogenate by the phenol-sulphuric acid reaction of Dubois *et al.* (1956). Total carbohydrates were extracted from insect tissues and prepared for assay according to Crompton and Birt (1967). The colour produced was read by a spectrophotometer (Spectronic, 1201) at 490 nm and referred to glucose standard. Total carbohydrates were expressed as μg glucose/insect.

Results were statistically analysed by two way ANOVA completely randomized using Costat statistical software. Factors for the two-way analyses were pH and buffer concentration. When the ANOVA was significant ($p<0.05$), means were separated by the Duncan's multiple range test.

RESULTS AND DISCUSSION

Buffer solutions used were of pH 3,5 and 7 at 3 levels of morality; 0.1, 0.05 and 0.025 M. The addition of buffers during preparation of artificial diets, changed pH values and buffer capacities of these diets (Table 1). Diet 3 had the highest pH = 6, while diet 1 had the lowest one (pH = 4.11). They were prepared by buffer solutions of pH values equaled to 7 and 3, respectively (0.1 M). The lowest buffer capacity was that of Diet 3 (= 161 μmoles NaOH/1 g fresh diet). This diet had the highest pH value, so its buffer capacity or the number of μmoles NaOH needed to bring the diet to pH 8.75 was the lowest.

Table 1: pH and morality of buffer solutions and the corresponding pH values of artificial diets consumed by *S. littoralis* larvae

Diet	Morality (M)	pH of buffer solutions	The corresponding pH of the diet (mean±SD)	pH buffering capacity* (mean±SD)
1	0.1	3	4.11±0.060	246.2±2.00
2	0.1	5	5.03±0.100	210.0±0.26
3	0.1	7	6.00±0.155	161.0±3.80
4	0.05	3	4.25±0.072	230.0±4.40
5	0.05	5	5.12±0.100	202.00±4.00
6	0.05	7	5.72±0.100	173.00±1.80
7	0.025	3	4.44±0.050	217.00±2.40
8	0.025	5	5.22±0.058	193.00±3.00
9	0.025	7	5.51±0.095	179.60±4.00
Control	-	-	5.30±0.12	178.00±2.30

*Buffer capacity quantified as µmole of NaOH required to bring 1 g of fresh diet to the average of lepidopteran midgut pH = 8.75

Table 2: Two way ANOVA completely randomized indicating F-ratio and p-values and interaction of the effect of diets prepared by buffer solutions differing in their pH and buffer concentration (conc.) on some parameters of *S. littoralis* larvae

	pH		Buffer concentration		Interaction (pH X Conc.)	
Parameter	F-ratio	P	F-ratio	P	F-ratio	P
Weight gain	8.10	0.0031**	54.87	0.000***	16.18	0.000***
Survival	3.93	0.038*	1.11	0.35ns	0.74	0.571ns
RCR	8.79	0.0022**	9.64	0.0014**	8.50	0.0005***
RGR	2.50	0.1096ns	4.79	0.0214*	4.05	0.016*
AD	1.09	0.355ns	0.99	0.388ns	1.04	0.41ns
ECI	1.73	0.205ns	6.59	0.0071**	7.93	0.0007***
ECD	2.48	0.112ns	28.60	0.000***	6.80	0.0016**
Total carbohydrates	193	0.000***	177	0.000***	24.10	0.000***

*F is significant at p<0.05, **F is significant at p<0.01, ***F is significant at p<0.001

In contrary, Diet 1 had the lowest pH and highest buffer capacity = 246.2. Buffering capacity and pH were inversely correlated; well buffered plants were more acidic (Appel and Maines, 1995). In fact, pH and buffer capacity of a certain diet depend on pH and morality of the buffer solution. Control diet prepared by distilled water, but its pH = 5.3 and its buffer capacity = 178 referred only to its own components.

Data were statistically analysed by two way ANOVA test to detect the significance of using different buffer solutions on some parameters of *S. littoralis* larvae (Table 2). pH had a significant effect on weight gain, survival, RCR and total carbohydrates (p-values were 0.0031, 0.0038, 0.0022 and 0.000, respectively). On the other hand, the effect of different buffer molarities was significant on weight gain, RGR, RCR, ECI, ECD and total carbohydrates (p-values were 0.000, 0.0214, 0.0014, 0.0071, 0.000 and 0.000, respectively). The interaction between the two factors was high as observed in most parameters under study, except survival and AD (p = 0.571 and 0.411, respectively).

It was assumed that altering of diet physicochemistry by addition of buffer solutions to diets instead of water might affect midgut pH which has long been recognized as an important factor for the optimal activity of digestive enzymes or affect reactions associated with absorption and detoxification of food in the insect gut, which ultimately might affect herbivore fitness. Percent survival of *S. littoralis* larvae was non-significantly changed, while weight gain and RGR showed significant reduction as compared to control (Table 3). The highest reduction was among diets prepared by 0.1 M buffer solution. The weight gain was 35.19, 38.60, 30.85 and 66.27 mg/mg/day for diet 1, 2, 3 and control, respectively.

The depression of growth and weight gain can be explained by that the insect consumed less amount of food, since diet pH might affect palatability and thus affects consumption (Karowe and Martin, 1993). RCR

Table 3: Percent survival, weight gain and relative growth rate of *S. littoralis* larvae fed for 4 days on different artificial diets ($\bar{X}$ ±SD). Means with different letterers are different at p<0.05, ANOVA, Duncan's multiple range test

Diet	No. of survivors (%)	Weight gain (mg/larva)	RGR (mg/mg/day)
1	60.0±10.0^{ab}	35.19±2.24^{f}	0.24±0.005^{b}
2	46.6±5.7^{ab}	38.60±7.82^{ef}	0.23±0.025^{b}
3	43.3±5.8^{b}	30.85±4.00^{f}	0.23±0.045^{b}
4	63.3±15.0^{ab}	46.76±1.30^{de}	0.28±0.035^{ab}
5	47.0±15.0^{ab}	34.95±6.67^{f}	0.29±0.035^{ab}
6	53.3±20.0^{ab}	54.42±3.48^{cd}	0.33±0.01^{a}
7	66.0±6.0^{a}	59.55±2.00^{bc}	0.31±0.026^{a}
8	63.3±5.7^{ab}	56.90±3.00^{c}	0.24±0.03^{b}
9	46.0±15.0^{ab}	73.86±8.86^{a}	0.29±0.017^{ab}
Control	66.6±5.7^{a}	66.27±5.10^{ab}	0.31±0.05^{a}

RGR = Relative Growth Rate

Table 4: Dietary effects on nutritional indices of *S. littoralis* larvae after feeding for 4 successive days on different artificial diets ($\bar{X}$±SD)

Diet	RCR (mg/mg/day)	AD (%)	ECI (%)	ECD (%)
1	1.14±0.037^{bcd}	79.60±1.90^{abc}	24.88±2.87^{abc}	27.93±3.40^{cd}
2	1.26±0.11^{b}	71.70±3.65^{d}	19.00±3.60^{cd}	22.60±1.48^{de}
3	1.15±0.095^{bcd}	74.93±1.49^{bcd}	14.50±7.60^{d}	17.76±10.05^{e}
4	0.93±0.01^{d}	81.20±3.97^{ab}	25.66±0.28^{abc}	31.73±1.46^{c}
5	1.006±0.12^{cd}	69.30±7.77^{d}	29.86±6.18^{ab}	34.2±3.27^{c}
6	1.01±0.055^{cd}	72.80±2.30^{cd}	32.66±3.05^{a}	44.66±2.51^{a}
7	0.97±0.017^{cd}	72.96±2.05^{cd}	31.70±3.06^{ab}	43.43±4.72^{ab}
8	1.02±0.025^{bcd}	75.06±2.85^{bcd}	24.10±2.81^{bc}	32.06±4.20^{c}
9	1.54±0.280^{a}	83.10±4.54^{d}	25.56±2.97^{abc}	34.20±4.30^{c}
Control	1.55±0.245^{a}	73.86±3.88^{cd}	26.20±3.55^{abc}	35.53±6.71^{bc}

Within a column, means bearing different subscripts are significantly different (p<0.05). RCR = Relative Consumption Rate, AD = Approximate Digestibility, ECI = Efficiency of Conversion of ingested food to body substances, ECD = Efficiency of conversion of digested food to body substances

Table 5: Total carbohydrates of *S. littoralis* larvae fed for four days on nine different artificial diets ($\bar{X}$ ±SD)

	Diet									
	1	2	3	4	5	6	7	8	9	Control
Total carbohydrates (μg glucose/insect)	233±35.57^{cd}	87.6±9.23^{a}	90.0±3.33^{a}	578±19.00^{b}	124±25.20^{a}	215±22.70^{cd}	684±75.00^{a}	159±7.20^{de}	283±32.10^{c}	508±92.00^{b}

Means bearing different subscripts are significantly different at $p<0.05$, Duncan's multiple range test

(Table 4) was reduced by 40.0, 35.1, 34.9 and 37.5% for diets 4, 5, 6, 7 and 8, respectively as compared to control. Diets 1 and 3 resemble the most acidic and the most alkaline diets, respectively. Their consumption index was expected to be more reduced than that of the other diets (reduced only be 26.5% for diet 1 and 26.6% for diet 3). Larvae might consumed relatively low amount of food, but their body weight gain (Table 3) is very low and consumption index is related food eaten to the body weight, so RCR showed these values. Depression of weight gain and growth might also occur when the insect ability to digest food or assimilates it to body tissue was reduced and/or body substances like carbohydrates were exhausted.

AD measures the average digestion of food by the insect and termed approximate because it does not account for metabolic waste products in the feaces, including the peritrophic membrane and undigested executive (Waldbaeur, 1968). The results (Table 4) demonstrate that digestion not adversely affected. This might indicates that midgut which is considered the main site of digestion has a good buffering system i.e., not easily affected by the changes in the physicochemical parameters of the tested diets. Appel and Maines (1995) examined the effect of six different host plants on the midgut physicochemical conditions of Gypsy moth, *Lymantria dispar*. They found host plants differ in specific traits such as leaf pH, pH buffering capacity and redox potential and the pH of the midgut is independent of host plant.

ECD measures the ability of the insect to convert digested food to body tissues. The index decreases as the proportional of digested food metabolized for energy increased (Waldbaeur, 1968). The introduction of acidic food or high concentrations of buffer might necessitate a significant expenditure of energy, resulting in the diversion of assimilated food from growth and energy storage to energy metabolism (Karowe and Martin, 1993). Larvae fed diets 1,2 and 3 showed high reduction as compared to control (showed 21.4, 26.4 and 50% reduction as compared to control, respectively) (Table 4). The response to different buffer conc. was significant while pH values changes were nonsignificant (Table 2). This indicates that buffer conc. is determinant factor effecting ECD. ECI measures the overall ability of the insect to convert ingested food to body matter. Since this index is correlated to ECD (Waldbaeur, 1968), it showed pronounced reductions (Table 4), specially diet 3 (ECI was 14.5 and 26.2 for diet 3 and control, respectively).

Carbohydrates are one of the main metabolites and one of the major sources of energy. They were significantly affected by the changes or disturbances after feeding of *S. littoralis* larvae on buffered diets. Their reduction (s) was significant and high among diets and control diet and at different pH and buffer conc. values (Table 5 and 2). It is suggested that depression of carbohydrates means that this main metabolite was exhausted through the increased demand on larval energy budget to readjust midgut or haemolymph physicochemical conditions. Midgut epithelium has a powerful cation pump (Dow, 1984) and estimated to consume 10% of cellular ATP during ionic regulation (Dow, 1987).

It was clear that within the used ranges of pH and buffer conc., the morality of buffer solutions used in the present report was more responsible on the significant changes of the tested parameters than pH.

Karowe and Martin (1993) found that pH has a less pronounced effect on *M. sexta* larval growth parameters than do buffer conc. and buffering capacity. However, there were some differences like they found that AD affected by pH, while such effect was not observed on *S. littoralis* larvae. The differences might be species specific, or because they used different buffer conc. levels (0.05-0.25 M) and different pH (range of diet pH was 4.4-5.49). Carbohydrates provided as more good indicator for high energy demands, than total lipids tested by them. Also, the control diet of *M. sexta* was the least buffered diet and had the least buffering capacity, the case which was not observed during preparation of *S. littoralis* diet (Table 1).

Diet 9 represents the most closer diet, in its physicochemistry, to the control one. In most cases, it caused slight changes relative to control. Diets 1 and 3

were of relatively severe changes in their pH or buffer capacity and diet 2 of high buffer capacity value. They all caused high reductions in growth and nutritional indices. Buffer conc. used to prepare these diets was the highest (0.1 M) and Table 2 indicates that buffer conc. affected the tested parameters higher than pH do.

It can be said that larvae probably to overcome unsuitable physicochemical conditions of the introduced diets, increased their demand on energy through consuming digested or assimilated food like carbohydrates instead of building new tissues, which finally influencing larval performance. The present results point out to the need to take into account all factors viz. nutritional factors that might help in the control of *S. littoralis* in Egypt.

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