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Gut Endosymbiont Disruption through Antibiotics Influences the Metabolic Homeostasis in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) Larvae

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NOG and RA conceptualized and designed the work. Author TDW performed the work, analyzed the data and drafted the manuscript. Authors NOG, RA, VB and SJ corrected the manuscript and helped in analysis. All authors read and approved the final manuscript.

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ABSTRACT

Fall armyworm as a polyphagous voracious feeder reported causing yield losses in most agriculturally important crops. As this insect had developed resistance against most of the insecticides, there is a need for an alternate approach to management. Gut endosymbiotic bacteria play a significant role in host feeding, digestion, and defense response throughout the life stages of insects. In the present study, we have isolated and identified the gut endosymbiotic bacteria of fall armyworm and the larvae were treated with antibiotics. The results showed that the maximum bacterial population was observed in the fourth instar of field-caught larvae and the least population was observed in the fourth instar artificially reared population. Based on the biochemical results the

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isolated gut endosymbiotic bacteria mainly comprised of *Bacillus* sp, *Enterococcus* sp., and *Enterobacter* sp. Based on the susceptibility of gut bacteria to different antibiotics, 6 antibiotic treatments with one insecticide treatment were administered to an artificial diet reared with third instar larvae and their dietary indices were evaluated. Among the antibiotic treatment, there was a reduction in the dietary indices in the larvae treated with Ciprofloxacin CIP^{5} (45.33%) and Cefotaxime CTX^{30} (41.73%) and an increase in dietary indices in the larvae treated with Nalidixin NA^{30} (31.58%), Doxycycline DO^{30} (8.82%), Vancomycin VA^{30} (22.05). Elimination of gut bacteria with a suitable antibiotic will affect the insect's feeding and dietary indices subsequently decreasing the relative growth rate and insect's physiology. Hence, gut bacteria-based green control measures might be used as an alternative approach for insecticides for the effective management of fall armyworm.

Keywords: Fall armyworm; insect gut; endosymbiont; antibiotics.

1. INTRODUCTION

Insect gut act as a medium for microbial colonization as they provide preferential conditions for microbial metabolism mechanisms. In Scarab beetle larvae, microbial fermentation products like formate, acetate, and lactate were produced abundantly in the midgut [1]. Meanwhile, the insect endosymbiont can help to produce nutrients that do not exist in the ingested food. The obligate endosymbiont of *Wigglesworthia glossinidia* expressed genes that resulted in the synthesis of nutrients and transport [2]. Symbiotic microbes can be endosymbionts (inside the host) or ectosymbionts (outside the host). It is reported that most of the insects are involved in symbiosis [3]. The mutualism between herbivorous insects and symbiotic microbes could secrete cellulolytic enzymes causing hydrolysis which helps in the biomass deconstruction and digestion function [4-7].

Gut microorganisms can control herbivore-induced defensive responses and improve insect adaptability [8,9], This mostly affects insect survival and will give vital information for pest control. The metabolic process may be impacted by gut microbial dysbiosis. Through controlling gene expression, changes in the diversity and composition of the microbial community in the insect's stomach can have an impact on crucial physiological activities of the host [10]. An increased mortality rate is brought on by the dysbiosis of the gut microbial population by antibiotic exposure in Honeybees (*Apis mellifera*), primarily as a result of increased susceptibility to pathogens [11,12].

Antifungal and antibacterial compounds can be toxic to insects which ultimately affects bioassay results even at a low concentration that is mainly due to detrimental effects on the growth and development of insects [13,14]. Thus, the use of antibiotics may cause deviation in the gut microbial symbionts and highly influences insect fitness and survival [15].

Fall armyworm (FAW) (*Spodoptera frugiperda*) is a regular and serious pest that disperses mainly during the summer months. Though it is a polyphagous insect, prefers mainly maize [16]. Besides there were natural enemies identified viz., larval parasitoids, *Coccygidium melleum*, *Eriborus* sp., *Exorista sorbillans* and predators, *Harmonia octomaculata*, *Coccinella transversalis* [17], chemical insecticides were widely used for their management [16]. However, insects developed resistance to almost all insecticidal groups [18,19]. Hence treating fall armyworms with antibiotics may affect the gut bacterial community and causes detrimental effects on the insect's physiology. Being an important economic pest FAW has been extensively used for various studies under laboratory conditions where they were mass-reared on an artificial diet. Hence, in this study we have first identified variation in bacterial communities in the gut of field caught and artificially reared FAW populations then, we have treated larvae with different antibiotics injected into their diet to evaluate their fitness and survival by enumerating their quantitative food use efficiency by disrupting gut microflora.

2. MATERIALS AND METHODS

2.1 Insect Collection

The fall armyworm, *Spodoptera frugiperda* (J.E.Smith), used in this experiment was collected from both the infested field and laboratory-reared populations. The larval collection was carried out from the maize field of Tamil Nadu Agricultural University, Coimbatore

(11.0123° N, 76.9355° E) and from Dharapuram (10.7343° N, 77.51861° E), Tiruppur district during November 2021 to January 2022. While the laboratory reared populations were obtained from the Department of Biotechnology, Tamil Nadu Agricultural University, Coimbatore, India. In the laboratory, the larvae were reared on the artificial diet (CIMMYT diet). The diet ingredients (in grams or ml per 3 liters of diet) were maize leaf powder (75.6), common bean powder (265.2), brewer's yeast (68.1), ascorbic acid (7.5), sorbic acid (3.9), methyl-p-hydroxybenzoate (6.0), vitamin E capsules (6.3), sucrose (105.9), agar (37.8), formaldehyde 40% (6.0) [20]. The temperature during insect rearing was maintained at 25±1 ° C under a 16:8 hr light/dark photoperiod and relative humidity (RH) of 75±5%.

2.2 Enumeration of Gut Bacterial Isolates

The fourth and fifth instar larvae cause considerable damage to the host plants hence both the instar larvae were selected for isolation of gut endosymbionts to know about their significance in their life stages. Twenty-five individuals from both the larval instars of the field caught population and artificially reared population was taken for isolation and enumeration of gut bacterial isolates. The larvae were surface sterilized with 70% ethanol for 3 mins followed by 3- 5 times wash with sterile distilled water. Larvae were dissected using a sterile dissection knife and forceps. The gut samples were collected in 0.1 mol phosphate buffer (pH-7.0) containing in sterilized pestle and mortar. All the dissection procedures were carried out in a sterile environment in the laminar airflow chamber. The gut samples were homogenized and serially diluted.100µl from fourth and fifth dilutions were spread on the plates containing eleven different isolation media viz., Eosin methylene blue (EMB) agar, Corn Meal agar, Czapex dox agar, Endo agar, Luria Bertani agar, Mac Conkey agar, Yeast Extract peptone dextrose (YPD) agar, Reasoner's 2A (R2A) agar, Nutrient agar, Tryptose soy agar, and De Man, Rogosa and Sharpe (MRS) agar and were incubated for 72 hrs and colonies were observed for every 24 hrs. The bacterial colonies obtained on different media plates were enumerated based on their color, size, and morphology. Colonies with similar morphology were considered as single morphotypes and were maintained as pure cultures through streak plates. And the bacterial population was calculated in the unit of log CFU ml^{-1}.

2.3 Biochemical Characterization

Biochemical tests were done to identify the various enzymes and products produced by the organisms based on their enzymes and end products, the bacteria associated with FAW were tentatively identified.

2.3.1 Gram staining

The test bacterial cultures were smeared on a clean glass slide and allowed to dry. The dried smears were heat fixed with a flame for 2 minutes and crystal violet dye was added to the smears and allowed to dry for 30 seconds and the slides were washed with distilled water. Then, iodine solution was poured onto the glass slides, after washing of iodine solution 95 percent ethanol was added to the glass slides. Again, the glass slides were washed with distilled water and finally, safranin (counter stain) was added and allowed to dry for 30 secs. The slides were washed with distilled water, blot dry with absorbent paper, and air dried. The slides were observed under a light microscope, the visibility of the blue color around the cells was considered gram-positive and the pink color indicates gram-negative bacteria [21].

2.3.2 Starch hydrolysis test

Ten microliters of the test bacterial isolates (1× 10^8 cfu/ml) were spotted on starch agar medium plates and incubated for 48 hrs at 28 ± 2°C. Then the plates were flooded with Lugol's iodine solution for one minute. The clear zone surrounding the spots indicates the hydrolysis of starch.

2.3.3 Gelatin hydrolysis test

A hundred microliters of the test bacterial cultures (10^8cfu/ml, 24 hrs) were inoculated in 10 ml gelatin broth and incubated for 48 h at 28 ± 2C. After incubation, the isolates were placed in the refrigerator at 4°C for 30 mins. The culture tube that remained liquefied state indicates gelatin hydrolysis by gelatinase enzyme [22].

2.3.4 Indole production test

A hundred microliters of the test bacterial cultures were inoculated in 10 ml tryptone broth and incubated for 48 h at room temperature. Then one ml of Kovac's reagent was added. Cultures producing a red layer indicate indole

positive that the organism can utilize tryptophan and convert it into indole [23].

2.3.5 Methyl red test

A hundred microliters of the test bacterial cultures were inoculated in 100 mL MR-VP broth and incubated for 48 h. After incubation, a methyl red indicator was added. The formation of red color in the broth indicated that the sugars were fermented by the organism and led to acid production which decreases the pH of the medium [24].

2.3.6 Voges –Proskauer test

A hundred microliters of the test bacterial cultures were inoculated in 100 mL MR-VP broth and incubated for 48 h. After incubation Barritt's reagent was added (10 drops of solution A and 10 drops of solution B, Solution A- Naphthol 6 g dissolved in 100 mL of 95 percent ethanol; Solution B-Potassium hydroxide 16 g was dissolved in 100 mL of distilled water). The deep rose color developed within 15 mins indicates positively that shows the presence of acetoin in the liquid medium [24].

2.3.7 Hydrogen sulfide production test

The test bacterial cultures were inoculated in nutrient broth and a strip of filter paper impregnated with lead acetate was held in place by the cotton plug. After incubation, the blackening of paper indicated hydrogen sulfide production [25].

2.3.8 Catalase test

The test bacterial cultures were grown in nutrient agar plates. After incubation, drops of hydrogen peroxide were added to the grown cultures. The effervescence of oxygen indicates the presence of catalase enzyme and is aerobic [23].

2.4 Antibiotic Susceptibility Test for Gut Bacterial Isolates of FAW

To test the most effective antibiotic for insect treatment, -twenty-one gut bacterial isolates were selected and subjected to antibiotic susceptibility tests by seeded plate technique using sixteen different antibiotics (Polymyxin-B PB^{300}, Vancomycin VA^{30}, Cefotaxime CTX^{30}, Doxycycline DO^{30}, Ciprofloxacin CIP^{5}, Colistin CL^{10}, Ampicillin AMP^{10}, Nalidixin NA^{30}, Bacitracin B^{10U}, Tetracycline TE^{30}, Carbenicillin CB^{100}, Kanamycin K^{30}, Chloramphenicol C^{30}, Streptomycin S^{10}, Rifampicin RIF^{5}, Erythromycin E^{15}) (M/s. HiMedia Laboratories, Mumbai, India) as described by Bauer et al . [26]. Gut bacterial isolates were inoculated in tryptose soy broth and incubated for 24 h at 28± 2°C. Then 10 mL of inoculated culture were added in 100 ml TSA medium at its bearable temperature and the plates were allowed for 5 mins for solidification. Using sterile forceps, the antibiotic disc was then placed on the agar's surface and incubated for 24 h at 28±2°C. To interpret the antibiotic sensitivity of the isolates, the diameter of the inhibition zone produced around the disc was measured and compared with the diameter of the inhibition zone as detailed by the Clinical Laboratory Standards Institute (CLSI).

2.5 Effects of Antibiotics on Quantitative Food Use Efficiency

To know how the bacterial endosymbionts influence the growth parameters, feeding efficiency and food utilization of FAW, an insect bioassay was performed. Antibiotic treatments were T_1-Vancomycin (30 µg/ml), T_2-Cefotaxime (30 µg/ml), T_3-Doxycycline (30 µg/ml), T_4-Ciproflaxacine (5µg/ml), T_5- Nalidixic acid (30 µg/ml), T_6- Insecticide [Spinetoram (1.25 ppm)], T_7- Control. The bioassay was done as per the protocol developed by Insecticide Resistance Action Committee susceptibility test 016 (IRAC 2009) *i.e.,* antibiotics were injected into the artificial diet and fed to the 4th instar larvae. Each treatment contains 15 larvae and the experiment was conducted in a completely randomized block design with 3 replications. The bioassay was conducted for 3 days and observations were made every 24 h. The consumption rate (CI), Relative growth rate (RGR), Approximate digestibility (AD), the efficiency of the conversion of ingested food (ECI) and the efficiency of the conversion of digested food (ECD) were gravimetrically calculated by using the formulae, CI=E/TA, RGR=P/TA, AD=100(E-F)/E, ECI=(P/E)100, EDI=100 (P/(E-F)) where A = the mean dry weight of the larvae during the experimental period (T), E = the dry weight of the food eaten, F = the dry weight of the faeces produced, and P = the dry weight gain of the larvae.

2.6 Statistical Analysis

Data were analyzed by performing an analysis of variance (ANOVA), and the means were compared using generalized linear models

(GLMs) with Tukey's HSD test. All the analyses were performed using IBM SPSS Statistics 22 (Spss 2013).

3. RESULTS

3.1 Enumeration of Gut Bacterial Isolates

The maximum number of the bacterial population (log 8.07 CFU mL of gut suspension) was recorded from the fourth instar larvae of the field caught population from NA medium, while the least bacterial population (log 4.7 CFU mL of gut suspension) was recorded from fourth instar larvae of artificial diet reared population from endo agar medium (Table 1). There were no bacterial colonies observed in CMA agar plates containing gut suspensions from fourth and fifth instar artificially reared larval populations. Bacterial colonies were observed in the MRS medium only after 48 h of incubation. Among the isolates from the artificially reared larval gut samples, maximum bacterial populations were observed in the TSA medium (log 8.06 CFU mL^{-1}) and the least population was observed in the Endo agar medium (log 5.7 CFU mL^{-1}). Among the isolates from field caught FAW larval gut samples, the maximum population was observed in NA medium (log 8.07 CFU mL^{-1}) and the least bacterial population was observed in Mac Conkey agar medium (log 6 CFU mL^{-1}). While analyzing the instar-wise bacterial population, the growth of colonies in all the eleven isolation media was observed in the plates with gut suspensions of the fourth instar of the field caught FAW population. And the least bacterial population was observed in the fifth instar artificially reared FAW population as bacterial colonies were observed only on 7 isolation mediums (NA, LB, MRS, Mac Conkey, YPD, R2A, TSA). There was a significant difference in bacterial population in both fourth and fifth instars and field caught and artificially reared FAW populations ($F = 4.542$, $df=21$, $P < 0.05$).

3.2 Biochemical Characterization

For conducting biochemical tests twenty-one bacterial isolates with different morphology were selected. The results were depicted in Table 2. The results showed that among 21 isolates, seven isolates showed gram negative and fourteen showed gram positive. All the isolates were catalase positive except 5T6, 5T6, 5T9, 5N2, 5N5, and 5FCL2. Eight isolates could hydrolyse starch. Thirteen isolates showed positive results for gelatin hydrolysis. All the isolates were positive for Vogues –Proskauer test. And all the isolates showed a negative result for the methyl red test except 5CZ9 and 5N2 indicating that they are acid producers. All the isolates showed negative results for both the hydrogen sulfide test and the Indole production test.

3.3 Antibiotic Susceptibility Test

Twenty-one bacterial isolates were tested for their antibiotic susceptibility with sixteen antibiotic discs using disc assay. Among these, most of the bacterial isolates were resistant to Polymyxin-B PB^{300}, Ampicillin AMP^{10}, Colistin CL^{10} and Rifampicin RIF^{5}. Five bacterial isolates were susceptible to ChloramphenicolC^{30}. Eighteen bacterial isolates were susceptible to Doxycycline DO^{30}, eight isolates were susceptible to Vancomycin VA^{30}, and nine were susceptible to Cefotaxime CTX^{30}, ten for NalidixinNA^{30} and 12 were susceptible to Ciprofloxacin CIP^{5}. Therefore, Vancomycin VA^{30}, Doxycycline DO^{30}, Cefotaxime CTX^{30}, NalidixinNA^{30}, and Ciprofloxacin CIP^{5} were selected based on their highest susceptibility to bacterial isolates and maximum zone of inhibition and these five antibiotics were used for insect bioassay studies to test the effect of antibiotics in endosymbionts infectivity and their role in insect's nutrition and food use efficiency (Table 3).

3.4 Effects of Antibiotics on Quantitative Food Use Efficiency

Based on the results, all the five parameters [Consumption rate (CI), Relative growth rate (RGR), Approximate digestibility (AD), efficiency of the conversion of ingested food (ECI), efficiency of the conversion of digested food (ECD)] were significantly low in larvae treated with insecticide [Spinetoram (1.25ppm)] whereas nearly similar values as that of control were observed in those larvae treated with Nalidixin NA^{30} (Fig. 1). A significant reduction in the nutritional index next to insecticide was observed in larvae treated with Ciproflaxacine CIP^{5} and Cefotaxime CTX^{30} except for approximate digestibility. Approximate digestibility was higher in those larvae treated with antibiotics *viz.,* Doxycycline DO^{30}, Cefotaxime CTX^{30}, and Nalidixin NA^{30} than in untreated larvae (Control). The relative growth rate was higher in those larvae treated with Vancomycin VA^{30}, Doxycycline DO^{30}, and Cefotaxime CTX^{30} than in untreated larvae (Control). The highest relative

growth rate (0.83 mg/day) was observed in the larvae treated with vancomycin and similarly, its efficiency of conversion of ingested food (32%) was also higher than that of control (30%) larvae and Vancomycin has the maximum efficiency of conversion of digested food (21%) next to the control larvae. Nalidixic acid has the maximum approximate digestibility value (45%) and similarly the maximum efficiency of conversion of ingested food (40%) but their relative growth rate was low (0.51 mg/day) compared to that of control (Untreated) larvae. The antibiotic that influences more in consumption index more than that of control (3.79) was ciprofloxacin (4.8) but had low values of digestibility (13%) next to the insecticide [spinetoram] (1.3%).

Quantitative food use efficiency values were calculated based on the formulae given by Nathan *et al*., (2005). Values in each column are the mean of 3 replications of ± standard error value (SE). Means in the column followed by different letters are significantly different ($F = 4.159$, $P < 0.05$, Tukey's HSD test).

4. DISCUSSION

Our present finding demonstrated that the maximum number of bacterial colonies were observed in the fourth instar field caught population. The maximum bacterial population in the gut of the field caught population might be due to the availability of more nutrients from the natural host plants while the availability of nutrients might be lower in the artificially reared population. Dongbiao et al. [27] suggested that the high abundance of Firmicutes in the gut of *Spodoptera frugiperda* larvae is due to the better absorption of different nutrients. Studies showed that *Enterococcaceae* and *Lactobacilli* were stable across the different growth stages of *S*. *littoralis* and *H*. *armigera* [28]. A study indicated the persistence of the core community of bacteria in the gut throughout the life stages irrespective of diet and other factors [29]. There was no or nearly very low population observed in corn meal agar medium which might be due to the low abundance of the fungal population in the gut. As fungi are more frequent in the guts of insects that feed on wood and debris, and those organisms play a role in digestion [30]. On interpreting the results of 21 gut isolates, 4FCM1, 5MC2, 5CZ3, 4EM2, 4MC2, 4L1, 4N5, 5N2 and 5T5 seems to show similar results with *Bacillus* sp. in biochemical tests as they are Gram-positive organisms with catalase, gelatin hydrolysis, starch hydrolysis, Voges-Proskauer positive and methyl red, hydrogen sulfide and indole production negative. Similar results with a high density of *Bacillus* sp. were obtained from the gut of the nymphal stage of rugose spiralling whitefly (*Aleurodicus rugioperculatus*) [31]. And the isolates 4FCR1, 4N11, 4N6, 4MC5, and 5FCT2 also showed results positive for *Enterobacter* sp. as they are gram negative organisms with catalase, Voges-Proskauer positive and gelatin hydrolysis, starch hydrolysis methyl red, hydrogen sulfide and indole production negative. Notably, members of *Enterobacteriaceae* were detected in the gut of both wild and mass reared fruit fly species. This family was more predominant in wild *Zeogodacus cucurbitae* adults as compared to matured larvae and newly emerged larvae [32].Then the isolates 5FCL2,5T9,5N5,5T6 showed positive results for *Enterococcus* sp. by being Gram positive but catalase negative representing facultative anaerobes. Similarly, the cultured gut bacterium,

Table 1. Enumeration of gut bacteria associated with fall armyworm (*Spodoptera frugiperda*) using different growth media

Media	Laboratory reared		Field caught	
	4th instar	5th instar	4th instar	5th instar
NA	7.57±0.06^{a}	7.97±0.06^{a}	8.07±0.02^{a}	6.88±0.22^{cd}
LB	6.88±0.31^{ab}	6.4±2.85^{b}	6.55±0.2^{ef}	6.7±0.31^{d}
Mac Conkey	7±0.24^{ab}	7.05±0.04^{a}	6±2.66^{f}	7.26±0.18^{bc}
MRS	7.92±0.04^{a}	7.68±0.05^{a}	7.92±0.02^{c}	7.67±0.05^{ab}
EMB	ND	ND	7.15±0.13^{ef}	7.71±0.06^{ab}
CMA	ND	ND	6.85±0.29^{ef}	ND
CZ	7.12±0.11^{a}	ND	7.6±0.03^{d}	6.78±0.24^{d}
Endo	5.7±2.5^{bc}	ND	6.75±0.22^{ef}	7.96±0.02^{ab}
YPD	6±2.66^{bc}	6.85±0.13^{a}	6.55±0.2^{ef}	7.21±0.03^{cd}
R2A	6.91±0.12^{ab}	7.63±0.04^{a}	8.01±0.03^{b}	7.69±0.07^{ab}
TSA	7.95±0.02^{a}	8.06±0.02^{a}	7.68±0.07^{d}	7.93±0.07^{ab}

The first column in the table represents isolation media. Values in each column are mean of three replications of ± standard error (SE). Means in column with the same letter are not significantly different at 0.05 levels (Tukey's HSD test)
ND-Not Detected

Table 2. Biochemical characterization of FAW gut associated bacteria

S. No.	Isolates	Gram's reaction	Catalase	Starch hydrolysis	Gelatin hydrolysis	Methyl red test	Voges-Proskauer test	Hydrogen sulfide production	Indole production	Species identified
1	4FCM1	+	+	+	+	-	+	-	-	*Bacillus* sp.
2	4FCR1	-	+	-	-	-	+	-	-	*Enterobacter* sp.
3	4FCY1	-	+	-	-	-	+	-	-	*Klebsiella* sp.
4	5MC2	+	+	+	+	-	+	-	-	*Bacillus* sp.
5	5CZ3	+	+	+	+	-	+	-	-	*Bacillus* sp.
6	5T6	+	-	-	+	-	+	-	-	*Enterococcus* sp.
7	5CZ9	+	+	-	-	+	+	-	-	*Kocuria* sp.
8	5T5	+	+	+	+	-	+	-	-	*Bacillus* sp.
9	5N5	+	-	-	+	-	+	-	-	*Enterococcus* sp.
10	5N3	+	+	+	+	-	+	-	-	*Bacillus* sp.
11	5T9	+	-	-	+	-	+	-	-	*Enterococcus* sp.
12	5N2	-	-	-	-	+	+	-	-	*Pantoea* sp.
13	5FCT2	-	+	-	-	-	+	-	-	*Enterobacter* sp.
14	5FCL2	+	-	-	+	-	+	-	-	*Enterococcus* sp.
15	4N11	-	+	-	-	-	+	-	-	*Enterobacter* sp.
16	4MC5	-	+	-	-	-	+	-	-	*Enterobacter* sp.
17	4N5	+	+	+	+	-	+	-	-	*Bacillus* sp.
18	4N6	-	+	-	-	-	+	-	-	*Enterobacter* sp.
19	4L1	+	+	+	+	-	+	-	-	*Bacillus* sp.
20	4MC2	+	+	+	+	-	+	-	-	*Bacillus* sp.
21	4EM2	+	+	+	+	-	+	-	-	*Bacillus* sp.

The serial number 1-21 in the table represents the bacterial isolates, where 1-3 represents isolates from the fourth instar field caught FAW population,4-12 represents isolates from the fifth instar artificially reared FAW population, 13 & 14 represents isolates from fifth instar field caught FAW population, 15-21 represents isolates from the fourth instar artificially reared FAW population

+; Positive result; -; Negative result

Table 3. Antibiotic susceptibility test for FAW gut associated bacterial isolates

Isolates	Ampicillin (10 mcg)	Streptomycin (10 mcg)	Kanamycin (30 mcg)	Colistin (10 mcg)	Polymyxin 300 U	Tetracyclin (30 mcg)	Carbenicillin (100 mcg)	Rifambicin (5 mcg)	Erythromycin (15 mcg)	Chlorophenicol (30 mcg)	Bacitracin (10 U)	Doxycycline hydrochloride (30 mcg)	Vancomycin (30 mcg)	Cefotaxime (30 mcg)	Nalidixic acid (30 mcg)	Ciproflaxacine (5 mcg)
4FCM1	R	R	R	R	R	R	R	I	I	I	R	S	S	S	R	I
4FCR1	R	R	I	R	R	R	R	R	I	S	R	S	R	I	S	S
4FCY1	R	R	R	R	R	R	R	R	R	R	R	I	R	I	I	S
5MC2	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	I
5CZ3	S	S	S	R	R	S	S	S	S	S	R	S	R	S	S	S
5T6	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
5CZ9	S	R	R	R	R	R	R	R	I	S	R	S	R	R	R	I
5T5	S	R	I	R	R	I	S	R	S	S	R	S	R	I	R	S
5N5	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
5N3	R	I	R	R	R	R	R	R	R	R	R	S	R	R	I	R
5T9	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
5N2	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
5FCT2	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	I
5FCL2	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R
4N11	R	R	R	R	R	R	R	R	R	R	R	S	S	R	S	S
4MC5	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R
4N5	R	R	R	R	R	R	R	R	R	R	R	S	R	R	S	I
4N6	R	R	I	R	R	R	R	R	R	R	R	S	S	R	I	S
4L1	R	R	R	R	R	R	R	S	I	I	R	S	R	S	S	S
4MC2	R	S	S	R	R	S	R	R	S	S	R	S	R	S	S	S
4EM2	R	R	R	S	R	R	R	R	R	R	R	S	S	S	S	S

The resistance and susceptibility to different antibiotics of FAW gut bacterial isolates were analyzed based on the diameter of the inhibition zone published by the Clinical Laboratory Standards Institute (CLSI) where R-Resistant, I-Intermediate, S- Susceptible

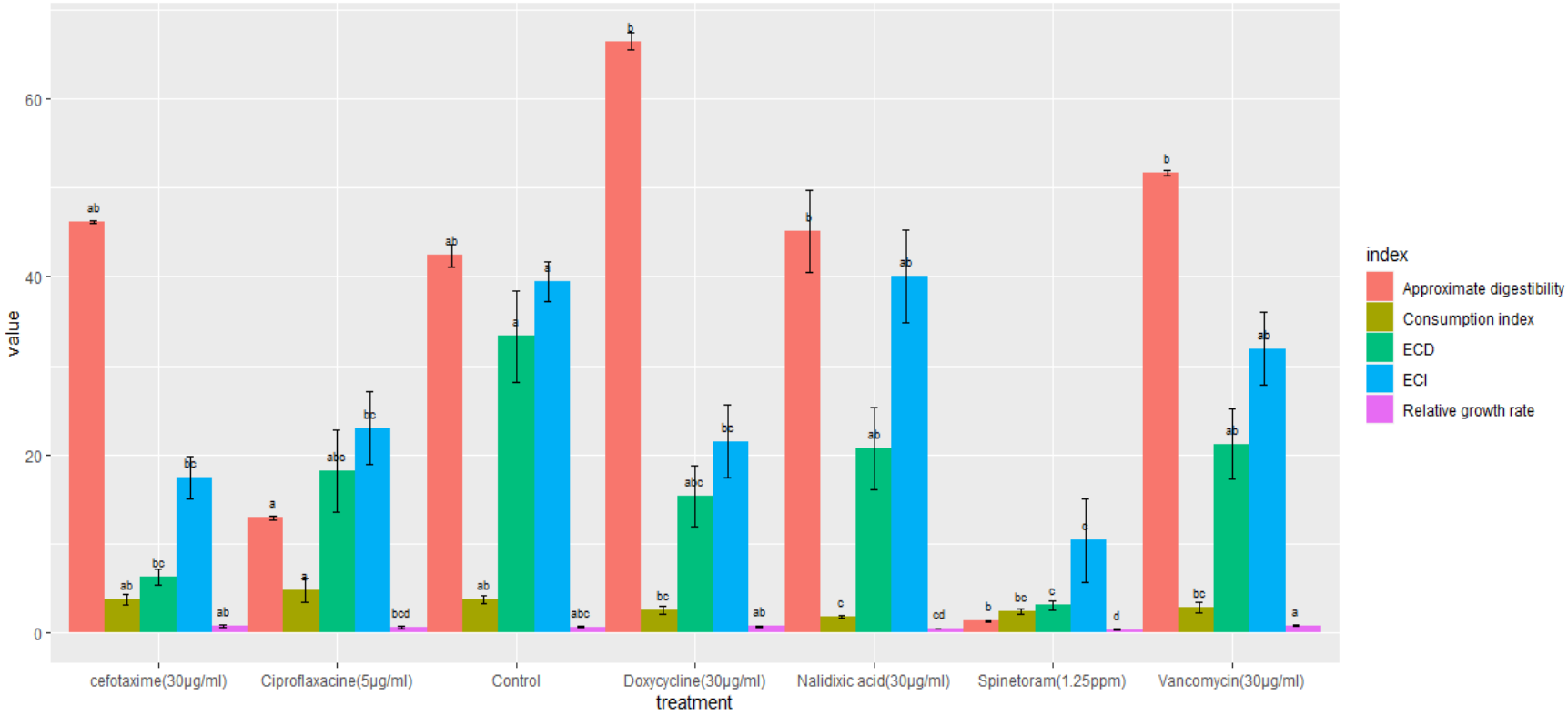

Fig. 1. Quantitative food use efficiency of FAW larvae after treatment with susceptible antibiotics

Enterococcus mundtii, represented the most existing taxon isolated from *Spodoptera littoralis* [33]. Our results of antibiotic treatment against the larvae exhibited the lowest dietary indices to those larvae treated with insecticides and almost the next lowest quantitative dietary indices were observed in those larvae treated with ciprofloxacin CIP^5. Ciprofloxacin is known to inhibit gram negative *Enterobacteriaceae* [34] and we also have isolates positive for *Enterobacte*r sp. from biochemical observations which clearly defines most of the important larval metabolisms at the late instars were highly influenced by the Gram negative bacteria. Many studies suggested the universal presence of the *Enterobacteriaceae* family in the Mediterranean fruit fly, *Ceratitis capitata* and *Enterobacteriaceae* bacteria were found to influence the biological traits of fruit fly by shortening immature development stages, increasing fecundity, prolonging survival rate, and improving male mating competitiveness and female mating receptivity [35]. Subsequently, in our results, the relative growth rate was higher in the larvae treated with Vancomycin VA^{30}, Doxycycline DO^{30}, and Cefotaxime CTX^{30} than in the control larvae. Studies with *Spodoptera frugiperda* by treating the larval diet with streptomycin sulphate showed similar results as the relative growth rate of larvae treated with diet was significantly increased by 2.81 to 3.52-fold over control (Untreated larvae) [36]. *B. methylotrophicus* and *B*. *amyloliquefaciens* isolated from *S. litura* larvae have been known to produce digestive enzymes [37,38]. A higher abundance of these bacteria on the guts of larvae treated with antibiotics in comparison with untreated larvae was reported [36]. Thus, it can be assumed that microbes with digestive enzymes might have helped the insect to utilize the nutrients of the diet, and hence there was an increase in values for relative growth rate, approximate digestibility and efficiency of conversion of ingested food over control. Similar findings with increased dietary utilization were reported by Indiragandhi et al. [39] while treating *Plutella xylostella* larvae with chitinase-producing strain and concluded that the increase in relative growth rate and efficiency of conversion of ingested food was due to colonization of chitinase producing strains. Similarly, the chitinase-producing bacteria attach to the peritrophic membrane of the insect gut and positively influence food digestion eventually maintaining membrane integrity and thickness [40]. In our study, we have isolates positive for *Bacillus* sp.as many of the *Bacillus* spp. were reported [41], to produce chitinase enzyme and our results were more relevant as concluded by Indiragandhi et al. [39].Interestingly, few bacterial species *viz., Enterococcus casseliflavus* with low abundance in antibiotic free diet were significantly increased after antibiotic treatment and their enrichment might be due to their strong resistance to antibiotics [42]. Hence in our study, the difference in dietary index on treatment with different antibiotics, the significant increase in RGR, CI, and ECI over control might be due to an increase in the colonization of either chitinase producing organisms or might be due to higher resistance of digestive enzymes producing organisms against the antibiotic. And the decrease in the dietary index by FAW larvae may be due to the susceptibility of several gram negative bacteria which plays a major physiological role in the larval stages.

5. CONCLUSION

Fall armyworm is a serious and invasive pest that can be widely controlled by insecticides. Continuous use of insecticides causes the development of resistance upon generations and negatively affects natural enemies. In our present study, by disrupting the gut endosymbionts of FAW through antibiotics, both significant reduction and increase in dietary indices were observed. Hence, utilizing suitable antibiotics that cause a reduction in gut microflora which has a significant role in food digestion and physiology, can be efficiently used as an alternative approach for sustainable pest management. The application of antibiotics for the management of FAW and their persistence in the environment under pot and field studies is yet to be studied.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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