Comparative Study on the Resistance of Three Commercial Strains and Balady (Local) Breed of Chickens to Infection With *Salmonella gallinarum*

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ABSTRACT : Three commercial strains bred in the US (Hubbard), Holland (Lohman), and Canada (Shaver) and Balady breed of chickens were orally infected with 10^6 cfu of *Salmonella gallinarum* per chick. Chicks were compared for weight gain, feed intake, feed conversion ratio, mortality rate and contamination of the liver, spleen and intestine with *Salmonella gallinarum* on the day of slaughter. The Balady chicks had significantly the lowest mortality rate ($p<0.01$) and weight gain ($p<0.001$) and poorest feed conversion ratio ($p<0.001$). *Salmonella gallinarum* could be shed from the liver, spleen and intestine with alternative rates in all strains. Lohman was the most resistance over the other two commercial strains, while Hubbard had the highest susceptibility. The Balady chicks were the most resistance. However, they may serve as a possible reservoir of *Salmonella gallinarum* and it may play a role of spreading the infection to the commercial farms in Jordan. (*Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 1 : 96-100*)

Key Words : Disease Resistance, Broiler, Mortality, Growth Performance, *Salmonella gallinarum*

INTRODUCTION

A significant rise in number of outbreaks of *Salmonella gallinarum* was reported in Jordan (Alshawabkeh and Yamani, 1996, 1998; MA, 1999), as in other countries (Nicholoas et al., 1990; Pomeroy and Nagaraja, 1991; Barrow, 1992). This would cause a serious economic problems to poultry farms at different production stages.

The most effective means of control of Salmonellosis in chicken is a combination of stringent management procedures and eradication (Snoeyenbos, 1991). Therapeutic treatment of salmonelosis has limitation and cannot be recognized as an effective means to clean up *Salmonella gallinarum* contaminated flocks because carriers may remain (Smith and Tucker, 1975; Alshawabkeh, 1987). Competitive exclusion (Normi and Rantala, 1973; Nisbet et al., 1997), vaccination (Smith and Tucker, 1975; Alshawabkeh, 1987) and feed additives enhance resistance to Salmonella colonization in broiler chicks (McHan et al., 1991; Nisbet et al., 1994; Alshawabkeh, 1997), with varying outcomes.

Selection for disease resistance in poultry strains can be an effective strategy for prevention and control of several important infectious diseases (Smith, 1956; Bumstead and Barrow, 1993; Lindell et al., 1994). It has been suggested that some strains of chickens are more genetically susceptible or resistant to infection with Salmonella than others (Smith, 1956; Benjamin et al., 1991; Protais et al., 1996). Moreover, *Salmonella gallinarum* should be given special consideration, because of the coexistence of Balady (Local) breed of chicken near commercial poultry farms in Jordan. It is reared cheaply in free range system in small number of 5-50 chickens. It is self-breeding and better adapted to the local environmental and management conditions. Although this breed are characterized by low productivity, farmers keep it for domestic consumption and selling the excess for extra family income. However, it might be a possible reservoir and potential means of spreading Salmonella infection either vertically or horizontally in chicken farms in Jordan. The purpose of this study was to compare several outbred lines of various genetic origins or innate to resistance of infection by *Salmonella gallinarum*.

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MATERIALS AND METHODS

Experimental subject

Three hundred eighty one-day old chicks were obtained from three commercial strains bred in the US (Hubbard), Holland (Lohman), and Canada (Shaver) and from the Balady breed of chicken. Parents of Hubbard, Lohman and Shaver were imported from the US, Holland and Canada, respectively. Upon arrival, five chicks from each strain were sacrificed, samples were taken from internal organs and cultured. They were found to be negative for Salmonella. Birds from each strain were randomly divided into three equal groups (thirty chicks each). The twelve groups were randomly allocated in a floor reared pens in a house located in an Isolation Unit on the research farm of the Faculty of Agriculture at University of Jordan. Chicks were provided with unmedicated feed (NRC, 1984) and water *ad libitum*. Before use, the feed and water were cultured for the presence of Salmonella using a standard culture method (Andrew et al., 1978), and they were proven to be Salmonella free.

Inoculation with *Salmonella gallinarum*

Salmonella gallinarum isolates were obtained from the Animal Health Institute, Ministry of Agriculture, Amman, Jordan, and selected for resistant to nalidixic acid at 20 μg/ml and novobiocin at 25 μg/ml to inhibit the growth of other bacteria. Challenge inocula were prepared from selenite F broth cultures incubated at 42℃ for 24 hours. The cultures were serially diluted to 10^6 cfu/0.25 ml in sterile distilled water. The viable cell concentration of the challenge inoculation was confirmed by colony counts on brilliant green agar (BGA) plates. Upon arrival, each chick was inoculated *per os* in the crop using a syringe.

Experimental protocol

The body weight of 10 chicks from each group was measured on day one and weekly after challenge inoculation. The average body weight, feed intake and mortality recorded weekly. Clinical signs and mortality were recorded daily and necropsy was carried out on all dead and cultured for Salmonella. Biweekly after challenge inoculation, 4 chicks from each group were killed by cervical dislocation and portions of each chick liver, spleen and intestine were transferred aseptically into tetrathionate broth (TTB) (Oxoid) tubes to give an approximate 1: 10 ratio and were incubated at 42℃ for 24 hours. TTB cultures were streaked on to BGA plates. Plates were incubated at 37℃ for 24 hours and resulting Salmonella colonies were evaluated for number of cfu according to the method of Smith and Tucker (1975). Typical Salmonella colonies were confirmed by biochemical tests on triple sugar iron agar and lysine iron agar and serological tests with somatic 0 antiserum, poly 0-1 and group D factors 1, 9, 12.

Statistical analysis

Measures used for evaluating differences among different strains of chicks were both weekly and final, body weights, weight gains, feed intake, feed conversion ratios and mortality rates. Analysis of variance was performed on the data to test the differences due to strain of chicks as main plot and week as subplots for weekly measures. The final measures were analyzed for the differences among strains of chicks only. Significant differences were further separated using protected LSD test. To test for an association between positive bacteriological results and chick strains, the Chi-square test was utilized. All analyses were performed using the Statistical Analysis System (SAS, 1988).

RESULTS

Growth performance

Body weights at weekly intervals and overall weight gain at six weeks of age are presented in table 1. Significant differences ($p<0.001$) in weekly body weights were observed among the four chick strains, with a significant ($p<0.001$) interaction between chick strain and week. The growth pattern for the three commercial strains was curve linear, while a linear slow growth pattern was observed for the Balady breed (Balady). Strain Hubbard had always the heaviest body weight and Strain Balady had the lightest weight, while Lohman and Shaver were intermediate. At week six, the overall weight gain had a similar result (table 1).

Feed intake and feed conversion results are presented in table 1. Similar feed intake was observed for four chick strains for the first three weeks of the experiment, however strain Shaver consumed more feed toward the end of experiment. The interaction effect of chick strain and week on feed intake and feed conversion ratio was significant ($p<0.001$). The overall feed intake was significantly higher ($p<0.05$) for Shaver than that of Hubbard and Balady and similar to Lohman. Although, Balady chicks had similar feed intake to Hubbard and Lohman, feed conversion ratio was significantly ($p<0.001$) lower for the Balady breed than that for the commercial chicks for the whole experimental period.

Mortality rate

Mortality was not initiated in all groups until day three postinoculation. Most deaths occurred from day five to eight postinoculation and was significantly ($p<0.01$) lowest in the Balady chicks (3.3%) (table 1). Strain Hubbard showed higher, but not significant- ly, mortality rate than that of the other commercial strains.

Reisolation of *Salmonella gallinarum* from internal organs of dead and sacrificed chicks.

The incidence of *Salmonella gallinarum* among

Table 1. Performance and mortality at week 6 for the four strains infected with *Salmonella gallinarum*

Strain[1]	Feed intake (g/chick)	Feed conversion ratio	Weight gain (g)	Mortality rate
Probability>F	*	***	***	**
Hubbard	2,417^{b}	1.39^{c}	1,740^{a}	26.7^{a}
Lohman	2,634^{ab}	1.72^{cb}	1,534^{b}	16.5^{a}
Shaver	3,039^{a}	1.95^{b}	1,569^{b}	22.2^{a}
Balady	2,233^{b}	7.02^{a}	319^{c}	3.3^{b}
LSD	459	0.45	141	11.5

^{a,b,c} Values in the same column with different letters are different at $p<0.05$.

*** $p<0.001$; ** $p<0.01$; * $p<0.05$.

[1] n=90 birds per strain kept in three groups.

liver, spleen and intestine of dead chicks are illustrated in table 2. The mean incidence of *Salmonella gallinarum* of the infected Balady chicks was lower than that of the commercial strains. All internal organs (liver, spleen and intestine) of the infected chicks were positive for *Salmonella gallinarum* except Balady chicks. The isolation rates of Salmonella from internal organs of dead chicks during the first three weeks postinoculation were highest in Lohman, while Hubbard and Shaver did not differ. In week six postinoculation, the incidence of *Salmonella gallinarum* was subside in all strains except in strain Shaver. The Chi-square value for overall isolation rates of *Salmonella gallinarum* among strains of chicks in the whole experiment was significant ($p<0.05$) from liver isolates only.

The incidence of *Salmonella gallinarum* among liver spleen and intestine of sacrificed chicks are shown in table 3. The organism could be shed from the liver, spleen and intestine with alternative rates. The internal organs contamination was more frequent and sever in strain Lohman and Balady. However, Balady had a dramatic reduction in organs contamination in the later weeks more than the other strains. The Chi-square values for incidence of *Salmonella gallinarum* among the sacrificed chicks was significant ($p<0.05$) from liver and ($p<0.1$) intestine isolates in week two only.

DISCUSSION

Large genetic variation for resistance to *Salmonella gallinarum* has been confirmed here by comparing several outbred lines. This is in consistence with findings of many researchers whose results suggested that immunity and susceptibility to diseases of chicken may be genetically related (Bumstead and Barrow, 1993; Protais et al., 1996; Poulsen et al., 1998; Pinard-Van der Laan et al., 1998; Okoye and Aba-Adulugba, 1998). Mortality rate of different strains of chicks disclosed susceptibility differences to *Salmonella gallinarum* infection. Because some strains of chicks were resistant and others were susceptible to *Salmonella gallinarum*, it seems unlikely that susceptibility is a result of fixation of a single deleterious recessive gene. Rather, it is more likely that susceptibility is a polymorphism present in general chicken population. A previous study have indicated that resistance to Salmonella is a result of dominant autosomal genetic effect (Bumstead and Barrow, 1988). More recent study however, demonstrated that resistance to Salmonella is inherited as a complex trait, since linking to genes such as Nramp1 and TNC explained only 33% of the differential resistance to Salmonella infection (Hu et al., 1997).

Results of this study revealed that differences among the four strains of chickens were significant in weight gain, feed intake, feed conversion and mortality rate (table 1). Based on mortality differences among chicken strains, the heavy Hubbard was the most susceptible commercial strain to *Salmonella gallinarum* infection in this experiment. The stress of high growth rate and conversion ratio may had an inverse effect on its resistance to Salmonella. However, the internal organs contamination of Hubbard for both dead and sacrificed chicks was among the lowest groups (table 2 and 3). Albers and Verheijen (1992) suggested that some lines are able to withstand more than others the consequences of disease and would survive. Hubbard had the highest mortality rate, but at the same time had the lowest weight gain depression. These results are agreeable with those of Albers and Verheijen (1992) and Pinard-Van der Laan et al. (1998) who found that lines differences in mortality does not always reflect differences in weight gain. Lohman and Shaver had similar productivity. However, Lohman had the lowest observed mortality. This may suggest that Lohman had the highest resistance over the other two commercial strains, in spite of having the highest internal organs contamination for both dead and sacrificed chicks in almost all weeks.

Balady chicks in this study, had the lowest mortality rate compared to the three commercial strains, with the lowest weight gain and poorest feed

Table 2. Weekly frequency of *Salmonella gallinarum* positive organ samples for the dead chicks

	Strain											
	Liver				Spleen				Intestine			
Week	Hubbard	Lohman	Shaver	Balady	Hubbard	Lohman	Shaver	Balady	Hubbard	Lohman	Shaver	Balady
1	3/5	2/2	2/3	0/1	3/5	1/2	2/3	1/1	2/5	2/2	2/3	0/1
2	7/9	2/2	1/2	-	7/9	2/2	1/2	-	6/9	2/2	0/2	-
3	1/3	2/3	0/3	-	0/3	0/3	1/3	-	1/3	2/3	1/3	-
4	0/2	3/3	1/3	-	2/2	1/3	1/3	-	1/2	1/3	2/3	-
5	1/1	1/1	-	-	0/1	1/1	-	-	0/1	1/1	-	-
6	-	-	2/3	-	-	-	1/3	-	-	-	3/3	-
Overall	12/20	10/11	6/14	0/1	12/20	5/11	6/14	1/1	10/20	8/11	8/14	0/1

Table 3. Frequency of *Salmonella gallinarum* positive organ samples for the sacrificed chick at 2-week intervals

	Week								
	Liver			Spleen			Intestine		
Strain[1]	2	4	6	2	4	6	2	4	6
Hubbard	7/12	5/12	10/12	9/12	6/12	6/12	8/12	6/12	9/12
Lohman	12/12	6/12	8/12	12/12	8/12	9/11	12/12	8/12	8/11
Shaver	10/12	8/12	8/12	10/12	7/12	9/12	11/12	7/12	10/12
Balady	11/12	4/12	6/11	11/12	7/12	6/10	11/12	6/12	6/10
Chi-square	8.40*	2.92	2.23	3.81	0.69	3.21	6.86†	0.93	1.54

[1] n=12 birds per strain were sacrificed at 2-weeks intervals in three groups.
* $p<0.05$; † $p<0.1$; others were not significant at $p>0.25$.

conversion ratio. This poorest conversion ratio was because they were ruffling the feed around feeders before consuming it more than the other chicks. Therefore; the actual feed intake of Balady chicks is much less than that found (table 1). However, it could not be measured more accurately. Also, they were much more active than chicks of the other strains. These facts made feed conversion ratio for the Balady breed, over the whole 6 weeks period, to be 7.02, while the superior conversion ratio was 1.39, for Hubbard strain. On the other hand, it was the most resistant breed in this study. Its organs contamination was among the highest for the sacrificed chicks after two weeks of inoculation with *Salmonella gallinarum*. In the later weeks however, dramatic drop in percentage of organs contamination was observed. Balady breed has been observed to survive better with lower productivity than exotic breeds under semi-extensive or free range system, because it is more adapted to the harsh environmental conditions, such as severe temperature, poor nutrition and bad housing system, common to the semiarid areas. Contradictory to our results, local chicken of Nigeria was higher susceptible to infectious bursal disease than commercial broiler and pullets (Okoye and Aba-Adulugba, 1998). However, differences in genotypes and mechanisms of resistance to different diseases may be claimed for this contradictions (Lindell et al., 1994; Thorp and Luiting, 2000). Results of this experiment suggested that Balady chicks may serve as a possible reservoir of *Salmonella gallinarum* and then as a potential means of spreading infection to commercial chicken farms in Jordan.

The mechanism of genetic differences in resistance or susceptibility of different strains to *Salmonella gallinarum* is not known yet. Further studies on the molecular level are needed to determine the involvement of certain genes with such phenomena.

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