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Antimicrobial activity of some cephalosporins with special reference to their effects on body weight and immune response to Newcastle disease vaccine in fayoumy chicks

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The susceptibility of the most common bacterial pathogens, namely *E. coli*, *P. mirabilis* and *Ps. aeroginosa* which were isolated from egg incubators and yolk sacs of randomly selected one day old Fayoumy chicks to three selected cephalosporins (cephradine, ceftiofur and cefquinome) were studied. The minimum inhibitory concentration (MIC), the minimum bactericidal concentration (MBC) of the tested drugs and the effect of these antibiotics on the body weight gain, mortality and immune response against Newcastle disease (ND) vaccine of the same bread of chicks were also estimated. The tested organisms were sensitive to ceftiofur and cefquinome whereas E.coli and ps. aeroginosa were found to be resistant to cephradine. The results showed that mortalities were higher in control and cephradine treated groups, while it was lower in the ceftiofur and cefquinome treated groups. On the other hand, the lowest mean body weight was recorded in control group (155.7±6.55 gm) followed by ceftiofur treated group (162.5±2.06 gm) and the highest mean body weight was recorded in cefquinome treated group (183.5±1.66 gm, $p < 0.01$) at 30 days of age. The study revealed that the tested antibiotics not exert any immune suppressive effect against (ND) vaccine.

Colibacillosis refers to localized or systematic infection caused entirely or partially by *Escherichia coli (E.coli*), including colisepticaemia, coligranuloma, air sac disease, cellulites, swollen head syndrome, peritonitis, salpingitis, osteomylitis, synovitis, omphalitis and yolk sac infection (Barnes and lozano, 1994).

Proteus mirabilis (P. mirabilis) was isolated from dead-in-shell chicken embryos (Orajaka and Mohan, 1985). The organism can penetrate egg shell and survive within the egg (Al-Aboudi *et al.,* 1988). On the other hand, *Proteus mirabilis* infection mainly causes mortality in young chicks up to 4 weeks of age with suppurative osteomylitis (Venkanagouda *et al.,* 1996).

Pseudomonas organism can cause localized or systemic diseases in young and growing poultry, invade fertile eggs causing death of embryos and newly hatched chicks. *Pseudomonas aeroginosa (P. aeroginosa*) can be highly virulent causing 100 % mortality in experimentally inoculated chickens Lin *et al.,* (1993), and from 0 to 90% in broiler chicks (Walker *et al.,* 2002).

Cephradine is 1st generation cephalosporin that can be administered orally and used successfully in treating infection of the respiratory tract and soft tissue. The gram-negative aerobic enteric bacilli (*E.Coli, P. mirabilis, Salmonella spp., and Shigella spp.)* were reported to be highly susceptible to the first generation cephalosporins, (Quintilani *et al.,* 1982).

Ceftiofure is a broad spectrum 3rd generation cephalosporrin approved for veterinary use to treat a variety of Gram-negative, Gram–positive bacterial pathogens and anaerobic pathogens including *Pasterilla* spp., *Streptococcus* spp., *Staphylococcus* spp., *salmonella* spp. and *Escherichia coli* (Brown *et al.,* 1991; Salmon *et al.,* 1996; Tragesser *et al.,* 2006). Ceftiofur was recomended for the control of *P.multocida* infection in balady chickens and for control of terminal bacterial infection in one day old broiler chickens (El-Naeneey and lotfy, 2000).

Cefquinome is the 4th generation cephalosporin developed for use in veterinary medicine. It had in vitro and in vivo efficacy against a wide range of gram-negative and gram-positive bacterial pathogens, (Limbert *et al.,* 1991; Rohdich *et al.,* 2009).

The present study was carried out to.

Determine the susceptibility of *E.coli*, *P. mirabilis* and *Ps. aeroginosa* isolated from egg incubators and yolk sac of randomly selected day

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old Fayoumy chicks to three selected cephalosporins (cephradine, ceftiofur and cefquinome).

Determine the effect of these antibiotics on chicks' performance.

Studied the effect of the selected antibiotics on chicks' immune response to (ND) vaccine.

Materials and methods

Drugs.

Cephradine (velocef)®. It was supplied as pure powder for injection from Bristol-Myers Squibb Company, Cairo, Egypt.

Ceftiofur sodium (Excenel)®. It was supplied as seterile powder (1 gram vail) for injection from Pharmacia & upjohn Company, Cairo, Egypt.

Cefquinome sulphate (Cobactan)®. It was obtained as suspention 2.5% for injection from Intervet International Company Cairo, Egypt.

Samples. A total of 10 samples in the form of cracked shells, dust and fluffs were collected from hatcheries, as well as eighty day old Fayoumy chicks were randomly selected for isolation of bacterial pathogens from their yolk sacs. Fourty day old chicks were randomly selected for collection of blood sampls for application of Heamagglutination Inhibition test for (ND) maternal antibodies .

Media for bacterial isolation and identification. Bacterial fluid and solid media were used for isolation of diferent bacterial pathogens, as each swab was inoculated into tetrathionate broth, tryptone soya broth and MacConkey broth. All fluid media were incubated at 37 ◌̊C for 18 hours. A loopfull from each fluid medium was seeded onto the surface of the following solid plating media; salmonella-shigella agar, Tryptone soya agar and MacConkey-bile salt-lactose agar media, followed by aerobic incubation at 37 ◌̊C for up to 72 hours. The obtained colonies were identified morphologically and smears were taken stained with Gram's staining technique and identified biochemically according to Collee *et al.,* (1996).

Experimental chicks and grouping. A total number of 1400 one day-old Fayoumy chicks were divided into 4 equal groups; each of 350 chicks. All chicks were floor reared in El-Azab project for poultry production at Fayoumy Governorate. Feeding and lighting programmes were applied according to the catalogue of the project.

In vitro antimicrobial sensetivity test. The bacterial isolates from the egg incubators and egg yolk were examined for sensitivity against three cephalosporines (cephradine, ceftiofur and cefquinome) .

Cephradine, ceftiofur and cefquinome sensitivites were determined using the disc and agar diffussion method as described by Collee *et al.,* (1996) as follow, A portion of a single bacterial colony was selected and inoculated into 4 ml Muller Hinton broth and incubated at 37 ◌̊C for 18 hours. After incubation the concentration of the M.O was equal to 1×10^8 cfu/ml. The culture was flooded onto the surface of well-dried Muller Hinton agar plates. The plates were tipped and excess of fluid was removed with a pipette after being sure that entire surface was covered with inoculum. The bacterial culture was allowed to settle for 15 minutes at 37 ◌̊C. The antimicrobial discs of cephradine and ceftiofur (30 ug/disc) and cefquinome (30 ug/well) were asceptically overlaied. The plates were allowed to stand 30 minutes at room temperature, after application of the discs. Then after overnight incubation at 37 ◌̊C, the plates were examined for inhibition zones. The interpretation of the results were carried out according to Collee *et al.,* (1996).

Minimum inhibitory and bactericidal concentrations [(MIC) and (MBC)]. According to Collee *et al.,* (1996) the drugs were dissolved in sterile distilled water to obtain a concentrations of 10 mg ml^{-1},1 mg ml^{-1}, 0.1 mg ml^{-1} as stock solutions.

From the 10 mg ml^{-1} solution 256 ul, 128 ul, 64 ul, 32 ul were taken and added to tubes containing 20 ml Muller Hinton broth to obtain the following dilutions of antibiotics (128, 64, 32 and 16 µg ml^{-1}, respectivelly.

From the 1mg ml^{-1} solution 160 ul, 80 ul and 40 ul were taken and added to tubes of Muller Hinton broth to obtain the following dilutions of (8, 4, 2 µg ml^{-1}).

From the 0.1 mg ml^{-1} tube 200 ul, 100 ul, 50 ul and 25 ul were added to Muller Hinton broth tubes to obtain dilutions of (1, 0.5, 0.25 and 0.125 µg ml^{-1}).

Each tube was inoculated with 100 ul of 1×10^8 cfu/ml (prepared by using MecFarland opacity tube) suspention of each of the microorganisms isolated from the egg incubators and chicks.

All tubes were incubated at 37 ◌̊C for 24 hours.

At the end of incubation period the tubes were examined visually for turbidity .

The tubes which has no visible growth indicate the MIC points.

To determine the MBC, a loopfull from the tubes which showing no visible growth were subcultured onto Muller Hinton agar and incubated at 37 ºC for 24-48 hours. After incubation of the subculture plates they were examined for growth. The tube containing the lowest concentration of antibiotic that fails to yeiled growth on the subculture plate was regarded as containing MBC of antibiotic for the tested strain.

Vaccination programme. Chicks groups were received the following viral vaccines.

At 1 day old. Marek's virus vaccine s/c in the neck 0.2 ml/chick.

At 2 day old. Infectious bronchitis living attenuated vaccine in drinking water.

At 6 days of age. ND (Hitchner B1) living attenuated vaccine in drinking water.

At 9 days of age. Infectious bursal disease vaccine in drinking water which repeated at 19 days of age.

At 18 days of age. ND (Lasota) vaccine in drinking water which was repeated at 28 days of age.

Experiment NO 1 (The effect of subcutanous injection of the tested drugs on the chicks performance). In this experiment the effect of subcutaneous injection of three doses (at one, 16 and 26 days of age) of cephradine, ceftiofur and cefquinome was studied on the performance (general health condition, body weight, feed intake, food conversion rate and mortality rate) of 1400 one day old chicks about 27 gm body weight. The chicks were divided into 4 groups each of 350 chicks. The 1st group was injected with cephradine at adose of 50 mg kg^{-1} b.wt (Oishi *et al.,* 1976), the 2nd group injected ceftiofur (10 mg/kgb.wt.), Aziza *et al.,* (1998), whereas the 3rd group injected cefquinome (2 mg kg^{-1} b.wt.) Block (1996) and the 4th group was was kept as a non treatrd control. The chicks of each group was weighed at 1, 7, 14, 21, 30 days of age.

Relative growth rate and feed conversion ratio were calculated according to Crampton and Lioyd (1959) as the following:

$$\text{Relative growth rate} = \frac{(w2-w1)}{(w1+w2)\div 2} \times 100$$

Where:

w1= body weight at the begining of the period.

w2 = body weight at the end of the period.

The feed conversion rate was measured by dividing the amount of food consumed, in a certain period, by the gain in weight at the same period, expressed in the same weight units.

Experiment NO 2 (The effect of the tested drugs on chicks immune response against ND vaccine). Serum samples were collected at the first day by scarifying 40 chicks, then at the 10th, 19th and 29th day of age by scarifying 15 chicks from each group and collection of their blood for serum. Collected sera were subjected to for heamagglutination inhibition test (HI) by the conventional microtitre method according to Calnek (1979).

Statistical analysis. The mean and standerd deviatons were calcluted as described by Snedecor (1969), the F- test was carried out by using INSTAT program.

Results and Discussion

Bacterial isolates from examined hatchery samples, 6 samples were found to harbour bacterial pathogens from 10 samples, (2 *Ecoli*, 3 *ps. aeroginosa* and 1 *p. mirabilis* isolate) and 22 bacterial pathogens were isolated from the yolk sac of day old chicks, and identified as (9 isolates *Ecoli*, 8 isolates *ps. aeroginosa*, 3 isolates *p. mirabilis and 2* isolates were spore forming aerobic gram- positive bacilli).

These findings nearly coincide with the results obtained by Choudhury *et al.,* (1993) who isolated *E.coli* from 57 (67.04 %) of infected yolk sacs on 58 farms. Barnes and Lozano (1994) revealed that egg transmission of pathogenic *E.coli* is common and can be responsible for high chick mortality. Radwan and Hassan (2004) experimentally induced 68 % embryonic death using *p.mirabilis* through egg shell.

E. coli and *ps. aeroginosa* were found to be resistant and *p .mirabilis* was found to be sensitive to cephradine. These finding resemble those obtained by Lacey *et al.,* (1983); Gakuya *et al.,* (2001).

The tested organisms was sensitive to Ceftiofur, this is similar to the result obtained by (El –Naenaey and lotfy 2002) who recorded that in chicks 39 out of 40 tested strains of *E.coli* were sensitive to ceftiofur, for *Ps. aeruginosa* the activity percentage of 80 % was recorded to ceftiofur as 4 strains were sensitive from the tested 5 isolates, all tested isolates of *proteus* species from chicks were completely sensitive. Huang *et al.,* (2009) reported that *E.coli, salmonella* and *p. multocida* isolates form poultry were sensitive to ceftiofur. On the other hand our study not agrees with the result obtained by (Deshpande *et al.,* 2000) who found

that the isolates of *E.coli* which produce extended spectrum β-lactamase (ESBL) were resistant to ceftiofur. This may be due to the acquired resistance of E.coli. Also (Walker *et al.,* 2002) recorded that antibiotic sensitivity tests showed that *p. aeruginosa* isolates obtained from hatcheries and broiler chicks were resistant to ceftiofur.

The result of our investigations proved that *E. coli, P. mirabilis and ps. aeroginosa* were sensitive to cefquinome. This finding agreed with that reported by Sheldon *et al.,* (2004) and Rohdich *et al.,* (2009).

MIC and MBC of cephradine for the tested *E. coli, P. mirabilis* and *Ps. aeroginosa* were 32, 128 and >128 µg ml^{-1} and 64, 128, >128µg ml^{-1}., respectively (Table 1).

The MIC and MBC of ceftiofur for *E. coli, P. mirabilis and ps. aeroginosa* investigated in this study were 0.5, 16, >128 µg m^{-1} and 0.5, 16 and >128 µg ml^{-1} respectively. These results similar to that recorded by (Deshpande *et al.,* 2000), the MIC of ceftiofur and cefquinome for *E.coli* were (0.06-2 and ≤ (0.03 -1 µg ml^{-1}), respectively but resistant strains of *E.coli* exert MIC >32 µg ml^{-1} for both drugs. Meyer *et al.,* (2008) recorded that the MIC for *E.coli* in foals was ≤ (0.5-1 µg/ml).

This study revealed that MBCs for ceftiofur is similar to its MICs against most tested strains strongly suggest that ceftiofur exerts bactericidal effect. This result confirmed the findings of Franklin (1992) and Klein *et al.,* (1996); they reported that ceftiofur sodium exerts bactericidal effect on tested isolates at concentration equal to or at most one doubling dilution above MIC.

The MIC and MBC of cefquinome for the tested bacteria *E. coli, P. mirabilis and Ps. aeroginosa* investigated in this study were 0.5, 1, 16 µg $ml^{-1}$and 1, 16 and >128 µg ml^{-1}, respectively. This finding similar to that obtained by Limbert *et al.,* (1991) who recorded that the MIC for *E. Coli* (<0.006-0.781 µg ml^{-1}), *P. mirabilis* (0.024-0.39 µg ml^{-1}*), ps. aeroginosa* (0.391-50 µg ml^{-1}). Also the MIC for *E Coli* and *P. mirabilis* was less than or equal to 0.5 µg ml^{-1}, while MIC for *ps. aeroginosa* 8 ug ml^{-1} and MBC for most species except Enterobacter were within a dilution of MIC, Chin *et al.,* (1992); Murphy *et al.,* (1994). Cefquinome had MIC value for *E.coli* <0.06 µg/ml in cattle by Sheldon *et al.,* (2004) and Thomas *et al.,* (2006).

Performance of different groups of chicks under study. As shown in Tables (2 and 3), mortalities were higher in control (53 chicks) and cephradine treated group (33) and lower in the other two groups (21 and 20) in ceftiofur and cefquinome treated groups, respectively). Regarding total feed intake, the control non-treated group showed the highest quantity of feed intake (632.78 gm/chick in 30 days) followed by cefquinome treated group (593.21 gm/chick). On the other hand, the lowest mean body weight was recorded in control group (155.7 gm ± 6.55) followed by ceftiofur treated group (162.5gm±2.06) and the highest mean body weight was recorded in cefquinome treated group (183.5 gm ± 1.66), ($p< 0.01$) in 30 days. Mean weight gain was highest in cefquinome treated group (156.5) and lowest in control group (128.7).The excellent feed conversion rate was obtained in the third cefquinome treated group (3.79) followed by ceftiofur treated group (4.09) mean while it was (4.18) and (4.92) in cephradine and control groups, respectively. Relative growth rate was highest (148.69) in cefquinome treated group and lowest (140.88) in control treated group (Table 3).

Egg-born diseases are transmitted from the infected dam to newly hatched offspring by means of fertile eggs. Some disease agents are carried inside the shell as a result of shedding into the egg piror to the addition of shell membrane. Others are carried out on the shell or penetrate from the shell surface through pors after the egg is laied (Radwan and Hassan, 2004). The ability of ceftiofur and cefquinome to reduce mortality rate were detected in this study and the efficacy of both drug were also evident by improved mean body weight gain, feed intake and feed conversion. This previous data is supported by Alxander, (1985) who reported that after the lapse of the acute phase of the infection the drugs improve weight gain in consequence of an increased feed intake and increased absorption of nutrients.

The used chicks had ND maternal antibodies titers of $\log_2$ 6. Serum samples were collected from all groups at 10, 19 and 29 days of age and the mean antibody titer of each group was showed in Table (4).

It was clear that ceftiofur treated group recorded non significant decrease antibody titer against ND vaccine, mean while cefquinome treated group recorded non significant increase antibody titer.

Conclusion. From the previous study we could concluded that:

Cephradine could be used for treatment of potential infections with *Proteus mirabilis*.

Table (1): Minimum inhibitory and bactericidal concentrations {(MIC) and (MBC)} of the tested drugs.

Tested M.Os	Cephradine		Ceftiofur		Cefquinome	
	MIC ($\mu g\ ml^{-1}$)	MBC ($\mu g\ ml^{-1}$)	MIC ($\mu g\ ml^{-1}$)	MBC ($\mu g\ ml^{-1}$)	MIC ($\mu g\ ml^{-1}$)	MBC ($\mu g\ ml^{-1}$)
E. coli (1 isolates)	32	64	0.5	0.5	0.25	1
P. mirabilis (1 isolates)	128	128	16	16	1	16
Ps.aeroginosa (1 isolates)	>128	>128	>128	>128	16	>128

Table (2): Effect of cephradine, ceftiofur and cefquinome on mortalities and performance in chicks.

Group (350 chicks/group)	Mortality per number (350 chicks)	Feed intake (gm) per chick	Mean body weight (gm) per chick n =80 chicks	Mean weight gain (gm) per week	Feed conversion rate (week)	Relative growth rate
7 days of age						
1st group (Cephradine)	8 chicks	87.7	57.3±0.47	30.25	2.89	71.8
2nd group (Ceftiofur)	9	87.98	54.75±1.49	27	3.25	65.06
3rd group (Cefquinome)	6	86.48	58.5±1.5	31.5	2.74	73.68
4th group (Control group)	27	91.33	57.5±0.645	30.3	3.014	71.7
14 days of age						
1st group (Cephradine)	13	149.5	94±2.81	36.8	4.06	48.6
2nd group (Ceftiofur)	6	158.9	92.4±1.43	36.4	4.4	49.06
3rd group (Cefquinome)	10	156.86	100.6±1.79	42.1	3.72	52.9
4th group (Control group)	13	148.4	92.75±0.36	35.25	4.2	46.93
21 days of age						
1st group (Cephradine)	2	179.1	130.4±1.46*	36.2	4.95	32.3
2nd group (Ceftiofur)	1	160.4	132.1±2.27**	39.7	4.04	35.3
3rd group (Cefquinome)	2	166.1	135.2±0.71***	42.1	3.94	34.6
4th group (Control group)	3	206.7	123.6±1.03	31.6	6.54	24.73
30 days of age						
1st group (Cephradine)	10	152.06	163±3.93	32.6	4.66	22.3
2nd group (Ceftiofur)	5	146.96	162.5±2.06	30.4	4.83	20.6
3rd group (Cefquinome)	2	183.77	183.5±1.66**	40.8	4.51	25
4th group (Control group)	10	186.35	155.7±6.55	32.1	5.8	22.1

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Table (3): Comparison between different groups of chicks demonstrating important items of performance.

Performance	Group			
	1st group (Cephradine)	2nd group (Ceftiofur)	3rd group (Cefquinome)	4th group (control non treated group)
Total mortalities	33	21	20	53
Total average Feed intake (gm)/chick in 30 days	568.36	554.24	593.21	632.78
Mean body weight (gm) at 30 days of age	163±3.93	162.5±2.06	183.5±1.66**	155.7±6.55
Mean body gain in 30 days	136	135.5	156.5	128.7
Food conversion rate at 30 days of age	4.18	4.09	3.79	4.92
Relative growth rate at 30 days of age	143.16	143	148.69	128.7

** $P < 0.01$

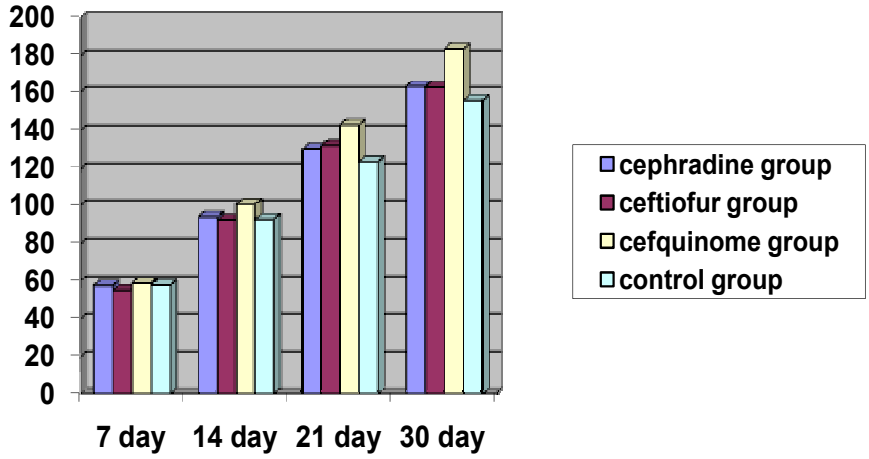

Fig. (1): Mean body weight in the different chicks groups.

Table (4): Effect of cephradine, ceftiofur and cefquinome on ND Mean HI log_2 of antibody titers.

Age per days	Group			
	Cephradine group	Ceftiofur group	Cefquinome group	Control non treated group
One day age	6	6	6	6
At 10 days of age	11	11	9.85±0.40	10±0.45
At 19 days of age	9.57±0.68	6.714±0.61	9±0.44	8.16±0.6
At 29 days of age	8.33±0.45	5.7 ±0.39	9.1±0.655	7.33±0.65

Non significant variation ($p > 0.05$).

Cefquinome and ceftiofur could be used for treatment of potential infections with *Esherchia coli, Proteus mirabilis* and *pseudomonas aeroginosa* microorganisms.

Mortalities were lower in chicks treated by ceftiofur and cefquinome while the highest mean body weight was recorded in cefquinome treated chicks.

Cephradine, ceftiofur and cefquinome did not exert any immunosuppressive effect against Newcastle disease vaccine thus they could be used safely before vaccination without adverse effect on the immune response of chickens**.**

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النشاط المضاد للبكتيريا لبعض مركبات السيفالوسبرون مع الإشارة الخاصة لتأثيرها على وزن ومناعة الكتاكيت الفيومى المحصنة ضد مرض النيوكاسل

استهدفت هذه الدراسة معرفة حساسية ميكروب البروتيس ميرابيلس والقولون الاشيريكى والزائفة الزنجارية المعزولة من الحضانات وكتاكيت فيومى عمر يوم لثلاثة من المضادات الحيوية من مجموعة السيفالوسبورن وهى السيفرادين والسفتيفيور والسيفكينوم وتم حساب تركيزاتها المثبطة و القاتلة لهذة البكتريا وتم ايضا دراسة تاثير هذة المضادات الحيوية على معدل نمو ونفوق الكتاكيت و دراسة تأثير هذة المضادات الحيوية على الجهاز المناعي للكتاكيت المحصنة بمصل النيوكاسل. وأتضح من الاختبار المعملى أن هذة الميكروبات كانت حساسة للسفتيفيور والسيفكينوم ولكن ميكروب القولون الاشيريكى والزائفة الزنجارية أكثر مقاومة لعقار السيفرادين. ووجد أن اقل معدل نمو كان فى الكتاكيت التى لاتاخذ مضادات حيوية(١٥٥,٧±٦,٥٥جم) تليها مجموعة الكتاكيت المحقونة بالسفتيفيور(١٦٢,٥±٢,٠٦جم) وكان أعلى معدل نمو فى المجموعة المحقونة بالسيفكينوم (١٨٣,٥ ± ١,٦٦جم) عند عمر ٣٠ يوم. ووجد ان اعلى نسبة نفوق كانت فى المجموعة التى لاتاخذ مضادات حيوية تليها مجموعة الكتاكيت المحقونة بالسيفرادين تليها المجموعة المحقونة بالسفتيفيور ثم المجموعة المحقونة بالسيفكينوم . ومن هذة الدراسة تبين ان هذة المضادات الحيوية ليس لها تاثير مثبط للجهاز المناعى فى الكتاكيت المحصنة ضد مرض النيوكاسل.