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Field study on control of chronic respiratory disease in vertically infected broiler chicks

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Our field studies had been carried out after in vitro antibiogram of *E. coli* to compare the effect of pulmotil (macroloide), enerofloxacin (fluoroquinolones) and doxycycline (tetracycline) in controlling mycoplasma and *E. coli* as a cause of CRD in broilers. The drugs were used in single or in combination. Two doses at the 3rd and 23rd day of age on performance of commercial broiler Ross derived from mycoplasma SPA-test positive breeders and *E. coli* positive isolation at the 1st day of age. The prevalence of marked air sac gross lesions in non treated control group indicated the development of CRD and severity of lesions increased with age. The used drugs played a role in controlling infection as treated groups showed milder lesions while more sever lesions were in doxycycline treated group. Protection against mortality was less in the treated pins than untreated ones. Cumulative culls % was low (1.1) in pen treated with enrofloxacin, (1.5) in pulmotil + enrofloxacin, (1.6) in doxycycline, and (1.7) in pulmotil + enrofloxacin; while pulmotil and control were the same (2.2%). Losses expressed as total mortality and culls % were the lowest in pulmotil + enrofloxacin and enrofloxacin (3.2 and 3.6), other treated pins showed the same values (4.2), while the highest was in non treated ones (5.8%). Average Body wt. in pulmotil + enrofloxacin, pulmotil, and enrofloxacin treated pens were higher (1934, 1924 and 1819 gm) than doxycycline (1802 gm), Pulmotil + Doxycycline (1705 gm) and non treated control (1708 gm). CFCR in pulmotil or enrofloxacin and in combination medicated pens were higher than other treatments and non medicated pen. Average day/ week/ gain in control non treated was equal to that of pulmotil or enrofloxacin (65g), slight lower value was in their combination (63g) followed by 58 g in doxycycline. The lowest ADG /w/g value was in pulmotil + doxycycline (52 g). Calculated EEF of treated and non medicated pens were higher than > 280. The medicated pens with either pulmotil or/ enrofloxacin and there compilation were superior (333, 313 and 330; respectively) and close to the farm stander (346). This study pointed out that *E. coli*, and Mycoplasma with life ND vaccine reduced broiler performance and the used drugs were of values in control such infections. The in vitro antibiotics sensitivity testing of *E. coli* is important to obtain good results and drug combinations must be carefully performed.

Poultry had developed to be industry to fulfill world to animal protein through fast growing broiler chickens. Many bacterial pathogens had been incriminated as a cause of losses in broilers including Colibacillosis (Azzam, 1983; Ibrahim and Sheha, 1985, and Srivasan et al, 2003).

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Respiratory colibacillosis is a relatively common disease that causes growth retardation in broilers, either as primary infection between 0 and 3 weeks of age or as secondary infection from around 3 weeks of age (Dho-Moulin and Fairbrother, 1999). The disease itself causes stress (Metveit, 1984) and the growth retardation results in reduced profit. Additionally, Infection of chickens with Mycoplasma gallisepticum *(MG)* and Mycoplasma synoviae *(MS)* increase susceptibility to pathogenic and potential

pathogenic organisms including *E. coli* (Gross, 1990 and Nakamura 1994).

As broiler poultry industries developed, almost the chickens are grown in high crowded with low air conditioned houses. In such situations many of the flocks which infected by MG, become predisposed or their disease condition have been aggravated. After predisposing the air sacs to Mycoplasma or other virus infections, colibacillosis developed and the chronic respiratory disease CRD complex occurs *(*Fotina-Tatiana, 2004). CRD in broiler chickens characterized by respiratory signs, low conversion, decreased growth, quality and downgrading of carcasses at slaughter because of airsacculitis and low final product (Neuman *et al.,* 1986; Kleven, 1998; Saif *et al.,* 2003). In the other hand the increased medication costs makes this infection as one of the costliest disease problems confronting poultry production (Ley and Avakian, 1992).

Many drugs had been reported to be effective in control of infected chickens with MG and /or *E. coli* by improving performance and reducing losses. These drugs included: enerofloxacin (Fotina -Tatiana, 2004; Amer and Abd El-Gahny, 2006), pulmotil (Hinz and Rottmann, 1990, Amer *et al.,* 2009) and doxycycline (George *et al.,*1977 ; Pakpinyo and Sasipreeyajan, 2007)

Antibiogram is recommended for detection of suitable drug for control of these organisms (Saif *et al.,* 2003; Zhao *et al.,* 2005; Miles *et al.,* 2006) as they acquired drug resistance by long and hazard use (Jordan and Horrocks, 1996; Stipkovits 2000; Saif *et al.,* 2003; Dai *et al.,* 2008). Therefore our field studies had been carried out as recommended by results of in vitro antibiogram. of isolated E. coli to compare effect of three drugs from different categories including Pulmotil (Macroloide) , Enerofloxacin (Fluoroquinolones) and Doxycycline (Tetracycline) in controlling of the possible effect of mycoplasma and E. coli as a couse of CRD in broilers. The drugs were used singly or in combination in two doses 1st at the 3rd day of life and the 2nd at 23 day on performance of commercial broiler Ross breed derived from infected breeders as showing MG and MS serological positive and E.coli isolation at the 1st day of age.

Material and Methods

Chicks. A total of 22740, 1 day-old Ross chicks hatched from commercial breeders proved to be infected with MG and MS by Amer, et al. (2009) these chicks were checked for the presence of pathogenic bacteria by examination of 20 sacrificed chicks and 15 died during transportation. Also individual serum was collected from the sacrificed chicks.

Ration. The chicks were feed on prepared ration according to the Ross broiler management manual and National Research Council (NRC, 1984). Ration without feed additives was given to the chicks ad libitum.

Bacteriological examination. Aseptically collected livers, heart blood and contents of non absorbed yolk sac of 10 sacrificed and 15 died chicks during transportation were directly cultured on Mac Conkey agar plates. After 24 hours incubation at 37 ^{0}C all plates were examined for bacterial growth. The suspected E. coli colonies were examined for colonial morphology, stain character and subjected to biochemical identification according to (Cruickshank *et al.,* 1975; **Quinn** *et al.*, 2002).

Serum plate agglutination (SPA) test. Individual collected serum from sacrificed 20, 1-day old chicks was subjected to SPA-test was as 0.02 ml of was mixed with 0.02 ml of stained antigen of MG or MS, clumping indicates positive result (Ewing *et al.,* 1996; Kleven, 1998). Stained M.G and MS antigens for SPA test were obtained from "Intervet International BV Boxneer, Holland". The tested sera were positive to MG. and MS in rates of 35% (7/20) and 40 % (8/20); respectively.

Antibiogram. The obtained *E. Coli* isolates were tested for their in vetro sensitivity for the following chemotherapeutic discs including neomycin 30 µg, doxycycline 30 µg, ampicillin 10 µg , colistin 10 µg, erythromycin 15 µg and enerofloxacin 10 µg using disc diffusion methods (Cruickshank *et al.,* 1975) and the results were interoperated according to Bio-Merieux (1980). The tested *E. coli* isolates were sensitive to enerofloxacin and doxycycline, resistant to neomycin, erythromycin and colistin, while intermediate sensitivity was recorded to ampicillin.

Drugs and Medications. The following drugs tilmilcosin (Pulmotil AC®), enerofloxacin (bytril®) and doxycycline were used in drinking water in two doses the 1st at 3 days and the second at the 23 day of age singly or in combination. Each drug or combination was used in and repeated in the same

Table (1): Age of chicks, Dose of drug and route of administration.

Active principle	Dose at 3 days old (mg)	Dose at 22 days (mg/kgm)	Conc. in D.W/200L
Tilmilcosin	2/bird	15	60ml
Doxycycline	20/kgm	20	83 gm.
Enrofloxacin	10/kgm	10	200 ml
Tilmilcosin +	2/bird	15	60 ml
Doxycycline	20/kgm	20	83 gm.
Tilmilcosin +	2 /bird	15	60 ml
Enrofloxacin	10/kgm	10	200 ml

Table 2: Broiler vaccination program.

Age/days	Vaccine	Route
5	ND (Hitchner B1)+IB	DW
6	IBD Gumboro L	DW
12	IBD 228E	DW
18	ND La Sota	DW

broiler pin as shown in table (1). The 1st dose was given before vaccination; while the 2nd was after. Vaccination. All chicken pins were vaccinated against Newcastle disease (ND); Infectious bronchitis (IB) and Infectious bursal disease (IBD) via drinking water as in table (2). The used vaccines were produced by Intervet International BV Boxneer, Holland**.**

Broiler performance parameter. In this field study broiler performance parameters including average cumulative mortality rate (CMR), cumulative culls, total mortality and culls percentage, livability, body weight/gm., cumulative feed intake/gm (CFI/gm), cumulative feed conversion rate (CFCR) and European efficacy factor (EEF) were used and calculated as shown in table2 fig 1-8 (Sainsbury, 1984). Good efficiency is considered to have an EEF > 280, while low efficiency has an EEF < 220. The obtained results were compared with farm standard obtained from the non infected Ross breed. Died chickens in all pins were subjected to post-mortem examination to compare the air sac gross lesions (Nakamura *et al.,* 1992).

Experimental design. The remaining chicks were divided into 6 groups and stoked in pins in floor density to be 12 chickens /m^2 at 35 days of age (table 3). The chicks were reared in closed automatic controlled pins and fed on ration prepared in the farm. At the 3ed day of age the chicks were received the 1st dose for 3 days. The same drug was repeated at 23 day of age for another 3 days. All pins were daily observed with recording of daily mortality and feed intake as well as weekly body weighty gain. At the 35 day of age all chickens were sold with recording of average body weight and number of culls. The performance parameters were calculated to evaluate effect of used drugs as compared with non treated pin 6 and farm standard as shown in (Table 3, Fig. 1-8).

Results and Discussion

The used chicks in this field study were proved to be derived from MG and MS infected breeders by the results of SPA-test ,where tested sera were positive to MG. and MS in rates of 35% (7/20) and 40 % (8/20); respectively. Both mycoplasmas are vertically transmitted from dam hen to their progeny (Saif *et al*, 2003). In the other hand bacteriological examination of dead chicks proved the isolation and biochemical identification of E. coli from internal organs. The result indicated that the used chicks had Colibacillosis infection. At PM examination the prevalence of marked air sac gross lesions in non treated control group indicated the development of CRD, the lesions increased in severity with age. The treated groups showed milder lesions varied from normal to slight turbidity without marked difference between medicated groups as recorded (Jordan and Horrocks, 1996; Stipkovits *et al*., 2004) where the prevalence of gross lesions of the air sac was similar in all the medicated groups and was less than that for the infected unmedicated group. The result indicated that the used drugs played a role in

Table (3): Effect of drugs on performance of Mycoplasma and *E. coli* positive broiler chicks.

Treatment	Pin No	No housed chicks	Cum Mor. Rate	Cum Culls %	Cum. Mor. culls%	Liv.1	Av. Body wt. (g)	Cum Feed intake /g	CFCR[2]	ADG /w/g[3]	CADG /g[4]	EEF ****
Pulmotil	1	3900	2.0	2.2	4.2	95.8	1924	3044	1.58	65	54.9	333
Pulmotil + Doxycycline	2	3560	2.7	1.5	4.2	95.8	1705	2830	1.66	52	48.7	281
Pulmotil + Enrofloxacin	3	3970	1.5	1.7	3.2	96.8	1934	3133	1.62	63	55.2	330
Doxycycline	4	3910	2.7	1.6	4.3	95.6	1802	2975	1.65	58	51.5	298
Enrofloxacin	5	3824	2.5	1.1	3.6	96.4	1819	2883	1.60	65	52.0	313
Control non treated	6	3540	3.6	2.2	5.8	94.2	1708	2815	1.64	65	48.8	280
Farm stander						97	1948	3040	1.56	78	55.6	346

1; Liv. : Livability, 2;CFCR: Cumulative Feed Conversion Rate. 3;ADG :Average day/week gain/g:
4;CADG: Cumulative average body weight gain/day/g. 5;EEF: European Efficacy Factor.

controlling infection (Saggiorato *et al.*, 2000; Saif *et al.*, 2003) and limitation of gross lesions (Kempf *et al*., 1997; Charleston *et al*., 1998; Guarini *et al.*, 1999; Jordan and Horrocks, 1996; Saggiorato, *et al*., 2000). The severe lesions of CRD in non medicated group may be also due to the extra complication of used live Newcastle vaccines as stated by (Saif *et al*., 2003; Fotina-Tatiana, 2004). Chickens of pen 2 and 4 those received combination of pulmotil + doxycycline and doxycycline alone showed more severe lesions. These results disagree with those of George *et al*. (1977) who found that doxycycline was effective in E. coli infection in young chicks, while Dai *et al*. (2008) who found that 75% of tested *E. coli* was resistant to doxycycline. This results indicates that pulmotil and enrofloxacin either each alone or in combination were more effective than pulmotil + doxycycline and doxycycline in reducing gross lesions.

Chicken treated with pulmotil + enroflxacin showed the lowest CMR (1.5%), followed pulmotil (2.0%), enroflxacin (2.5%), in pulmotil + doxycycline and doxycycline (2.7%), while the non treated control showed the highest CMR (3.6%) (Fig. 1). These results agree with Jordan and Horrocks (1996) where mortality was significantly less in the infected treated groups than infected untreated group; while, Protection against mortality was best in enrofloxacin than tiamulin (Jordan, *et al*., 1989). Cumulative culls% were lowest in pens treated with enrofloxacin (1.1%), intermediate rates were in (1.5, 1.6, 1.7) in pulmotil + doxycycline, doxycycline, pulmotil + enrofloxacin; respectively; while pulmotil and control were the same (2.2%) (Fig. 2). In the other hand the losses expressed as total mortality and culls % were the lowest in pulmotil + enrofloxacin and enrofloxacin (3.2 and 3.6). The other treated pins showed nearly the same values (4.2), while the non treated pen 6 gives the highest results 5.8% (Fig. 3). This result indicated that CRD complicated with respiratory vaccinal virus cousing increased mortality and under weights (Saif *et al*., 2003). Livability in pens given Enrofloxacin alone or in combination was higher (96.4- 96.8%) than other treated pens (95.6-95.6%), while non treated showed the lowest values (94.2%). The recorded results (Fig. 4) are showing the effect of used drugs in keeping of treated birds in apparent good condition by

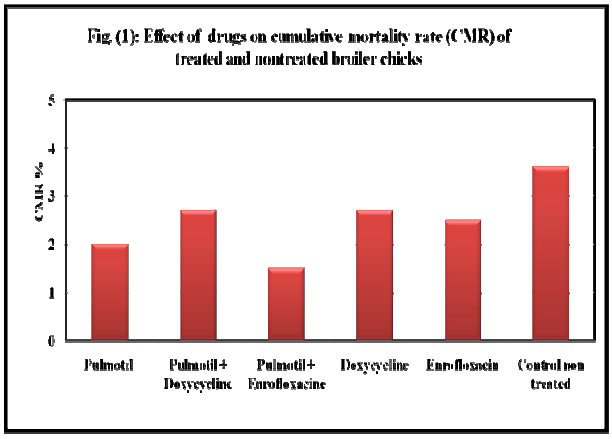
Fig. (1): Effect of drugs on cumulative mortality rate (CMR) of treated and nontreated broiler chicks
CMR %
0
1
2
3
4
5
Pulmotil
Pulmotil + Doxycycline
Pulmotil + Enrofloxacine
Doxycycline
Enrofloxacin
Control non treated

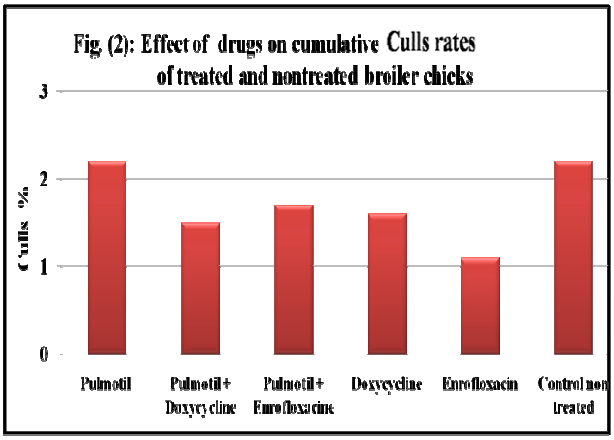
Fig. (2): Effect of drugs on cumulative Culls rates of treated and nontreated broiler chicks
Culls %
0
1
2
3
Pulmotil
Pulmotil + Doxycycline
Pulmotil + Enrofloxacine
Doxycycline
Enrofloxacin
Control non treated

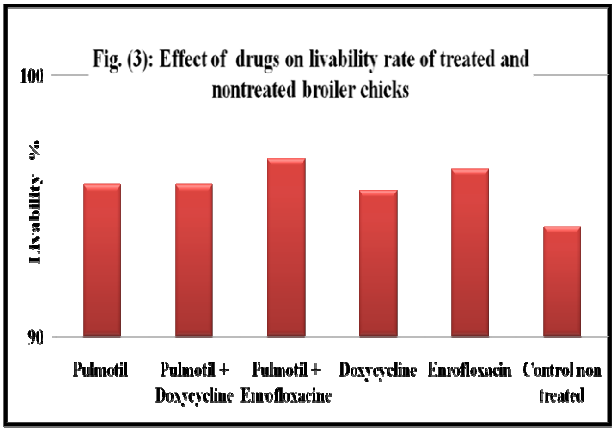
Fig. (3): Effect of drugs on livability rate of treated and nontreated broiler chicks
Livability %
90
100
Pulmotil
Pulmotil + Doxycycline
Pulmotil + Enrofloxacine
Doxycycline
Enrofloxacin
Control non treated

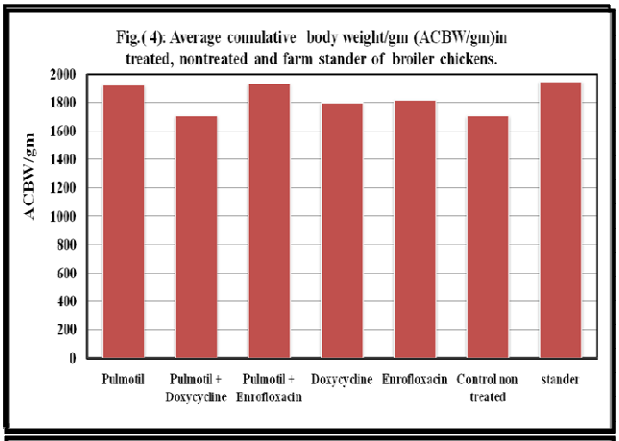
Fig.(4): Average cumulative body weight/gm (ACBW/gm)in treated, nontreated and farm stander of broiler chickens.
ACBW/gm
0
200
400
600
800
1000
1200
1400
1600
1800
2000
Pulmotil
Pulmotil + Doxycycline
Pulmotil + Enrofloxacin
Doxycycline
Enrofloxacin
Control non treated
stander

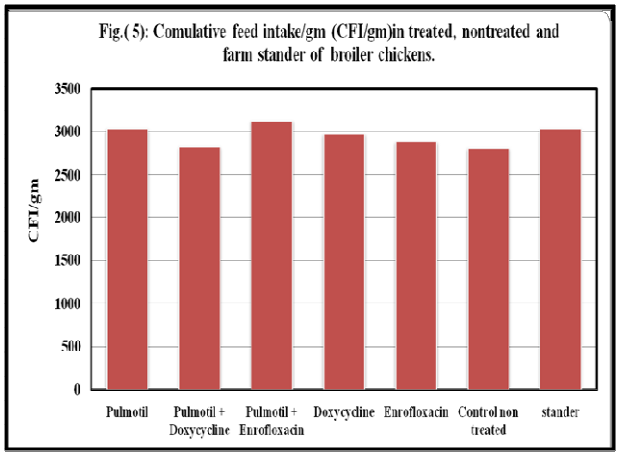
Fig.(5): Comulative feed intake/gm (CFI/gm)in treated, nontreated and farm stander of broiler chickens.
CFI/gm
0
500
1000
1500
2000
2500
3000
3500
Pulmotil
Pulmotil + Doxycycline
Pulmotil + Enrofloxacin
Doxycycline
Enrofloxacin
Control non treated
stander

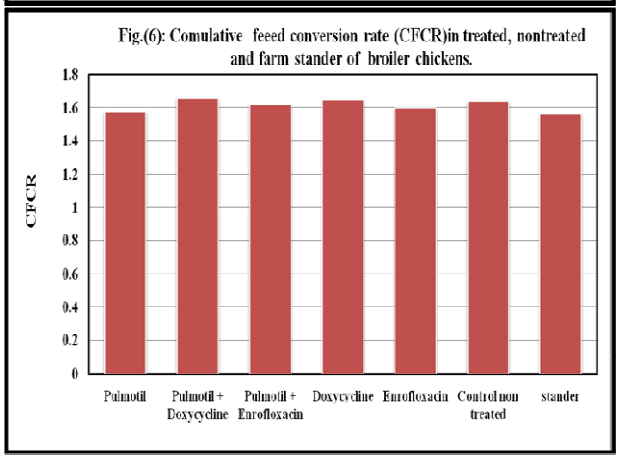
Fig.(6): Comulative feeed conversion rate (CFCR)in treated, nontreated and farm stander of broiler chickens.
CFCR
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
Pulmotil
Pulmotil + Doxycycline
Pulmotil + Enrofloxacin
Doxycycline
Enrofloxacin
Control non treated
stander

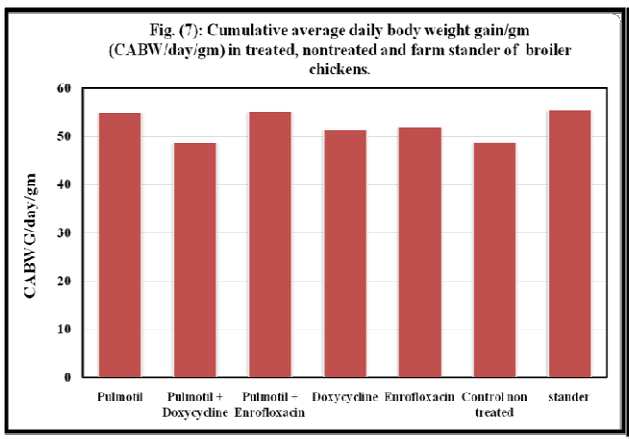
Fig. (7): Cumulative average daily body weight gain/gm (CABW/day/gm) in treated, nontreated and farm stander of broiler chickens.
CABWG/day/gm
0
10
20
30
40
50
60
Pulmotil
Pulmotil + Doxycycline
Pulmotil + Enrofloxacin
Doxycycline
Enrofloxacin
Control non treated
stander

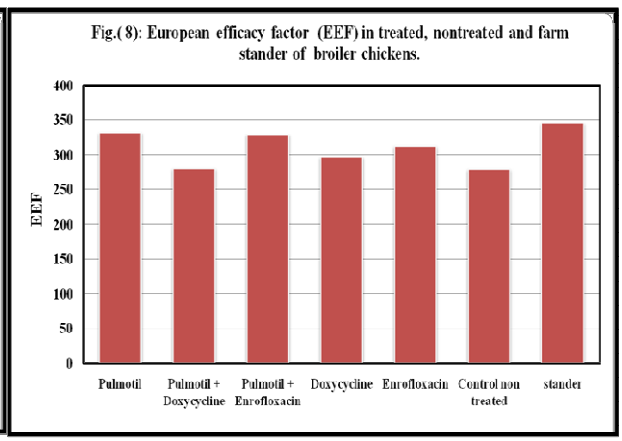
Fig.(8): European efficacy factor (EEF) in treated, nontreated and farm stander of broiler chickens.
EEF
0
50
100
150
200
250
300
350
400
Pulmotil
Pulmotil + Doxycycline
Pulmotil + Enrofloxacin
Doxycycline
Enrofloxacin
Control non treated
stander

limitation of infection especially under the vaccinal stress.

Average body wt. (ABW) in pens received pulmotil + enrofloxacin, pulmotil and enrofloxacin (1934, 1924 and 1819 gm; respectively) were higher than doxycycline (1802 gm), pulmotil + doxycycline (1705 gm) and non treated control (1708 gm). ABW in treated pens were higher than non treated but all were lower than farm stander. pulmotil + doxycycline treated pen (2) showed ABW nearly similar to non treated pen (Fig. 4). CFI/gm. in control pen (2815 gm) and pulmotil + doxycycline (2830 gm) were lower than those of treated ones. CFI in pen 1 treated with pulmotil (3044 gm) was close too the farm stander (3040 gm); while pulmotil + enrofloxacin was higher (3133 gm). Other treatments showed lower feed intake (Fig. 5).

CFCR in pulmotil or enrofloxacin and in combination medicated pens is generally higher than other treatments and non medicated pen (Table 3, Fig. 6). All pens showed values lower than farm stander. While, Jordan, *et al*., (1989) reported that enrofloxacin was best for prevention of growth depression than tiamulin. Average day/ week/ BW gain in control non treated was equal to that of pulmotil and enrofloxacin each alone (65g), while the two drug combination showed slight lower values (63g) followed by 58 g in doxycycline and the lowest value were in pulmotil + doxycycline (52 g) . All of recorded values were lower than stander (78g).

CADG/g in pulmotil (54.9g) and pulmotil+ enrofloxacin (55.2g) were the higher (fig 8) and close to the stander (55.6g), doxycycline was lower (51.5g), while both pulmotil + doxycycline (48.7g) was low than all and control non treated (48.8g).

Calculated EEF of treated and non medicated pens are higher than > 280 (Fig 8) can be evaluated as good. The medicated pens with either pulmotil or enrofloxacin and both in compilation were the higher (333, 313 and 330; respectively) and close to the stander (346). Similar results were investigated by (Amer *et al.* 2009). These results is supported the use of such drugs in improvement of infected broilers (Jordan *et al*., 1999; Scolari and Guarini, 1999; Saif *et al*, 2003) and the reputation of its use must be controlled by testing of organism sensitivity as Gautier-Bouchardon *et al*. (2002) concluded that resistance of MG and MS to enrofloxacin, tylosin, tiamulin and oxtetracycline in vivo might relatively frequently occurred. The combination between enrofloxacin and pulmotil was better indicating their synergistic action or to their good tissue distribution and affinity to lung tissue. In the other hand doxycycline that show lower effect can be attributed to its hepatointestinal circulation. Bad results of doxycycline and pulmotil combination may can be attributed to their antagonistic action especially on *E.coli*. Their for, combination of drugs must be controlled by tissue distribution, mode of action and residual time in relation to pathogenesis of organism. The results of this study proved that the both pulmotil or enrofloxacin and their combination were the more effective in control of both mycoplasma and *E. coli* in broilers under field condition, while administration of doxycycline alone or with pulmotil in such condition was not effective. It was also noticed that there was some sort of antagonistic effect when doxycycline and pulmotil were given in combination.

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دراسة حقلية على مقاومة المرض التنفسى المزمن فى دجاج بدارى التسمين المصابة راسيا

أجريت هذه الدراسة الحقلية بعد تحديد حساسية الميكروب القولوني للمضادات الحيوية لمقارنة كفاءة البالموتيل ، الإنروفلوكساسين و الدوكسيسيكلين فى مقاومة عدوى المكوبلازما والميكروب القولوني كمسببات للمرض التنفسي المزمن فى دجاج التسمين واستعملت العقارات فى جرعتين على عمر 3 و 23 يوما من العمر كل على حده أو في خلطات مزدوجة لدراسة التأثير على معدلات دجاج التسمين التجاري من سلالة "الروس" المنتجة من أمهات ايجابية الاختبار المصلى التجمعي للميكوبلازما و ايجابية العزل للميكروب القولوني في اليوم الأول من العمر، وأظهرت المجموعة غير المعالجة آفات مرضية هي الأكثر وضوحاً للمرض في الأكياس الهوائية أما المجموعات المعالجة فتراوحت تلك الآفات بين العتامه والمظهر الطبيعي بينما أظهرت المجموعة المعالجة بالدوكسيسيكلين آفات أكثر وضوحاً بين المجموعات المعالجة مما أثبت قدرة الأدوية المستخدمة على مقاومة المرض، وكانت الوفيات في المجموعات المعالجة اقل من المجموعة الضابطة غير المعالجة وكانت أقلها في المجموعات المعالجة بالإنروفلوكساسين و البالموتيل والخليط منهما عنه في الدوكسيسيكلين كما كانت نسب التقزمات 1.1 و 1.5 و 1.6 ثم 1.7 في الانرو ، البالموتيل + الانرو ، الدوكسى ثم البالموتيل + الدوكسى على التوالى بينما كانت 2.2 في كل من البالموتيل والمجموعة الضابطة. وكانت معدلات النفوق والتقزم هي الأقل في البالموتيل + الانرو ثم الانرو (3.2 و 3.6) وكانت في المجموعات المعالجة الأخرى 4.2 وفي المجموعة غير المعالجة كانت الأعلى (5.8) ، وأعطت المجموعات المعالجة بالبالموتيل + الانرو و كلٍ منهما علي حده أعلى أوزان ومعدلات تحويل غذائي على التوالي يليهما مجموعات :الدوكسى ثم البالموتيل + الدوكسى والمجموعة الضابطة، وأعطى معامل الكفاءة الاوروبى المحسوب للمعاملات (اعلى من 280) دلالة على جودة الأداء، بينما كان أعلاها عند العلاج بالبالموتيل أو الإنرو (313-333) وكانت الأقرب لمعدل المزرعة القياسى وأوضحت نتائج الدراسة أن الإصابة بالميكروب القولوني و الميكوبلازما واستخدام لقاح النيوكاسل الحي يؤدى إلي انخفاض معدلات إنتاج التسمين و أن الأدوية المستخدمة كان لها الأثر في الحد من أثار العدوى، كما أن إجراء اختبار حساسية الميكروب للمضادات البكتيرية هام للحصول على نتائج جيدة وان الخلط بينها يجب أن يتم بعناية.