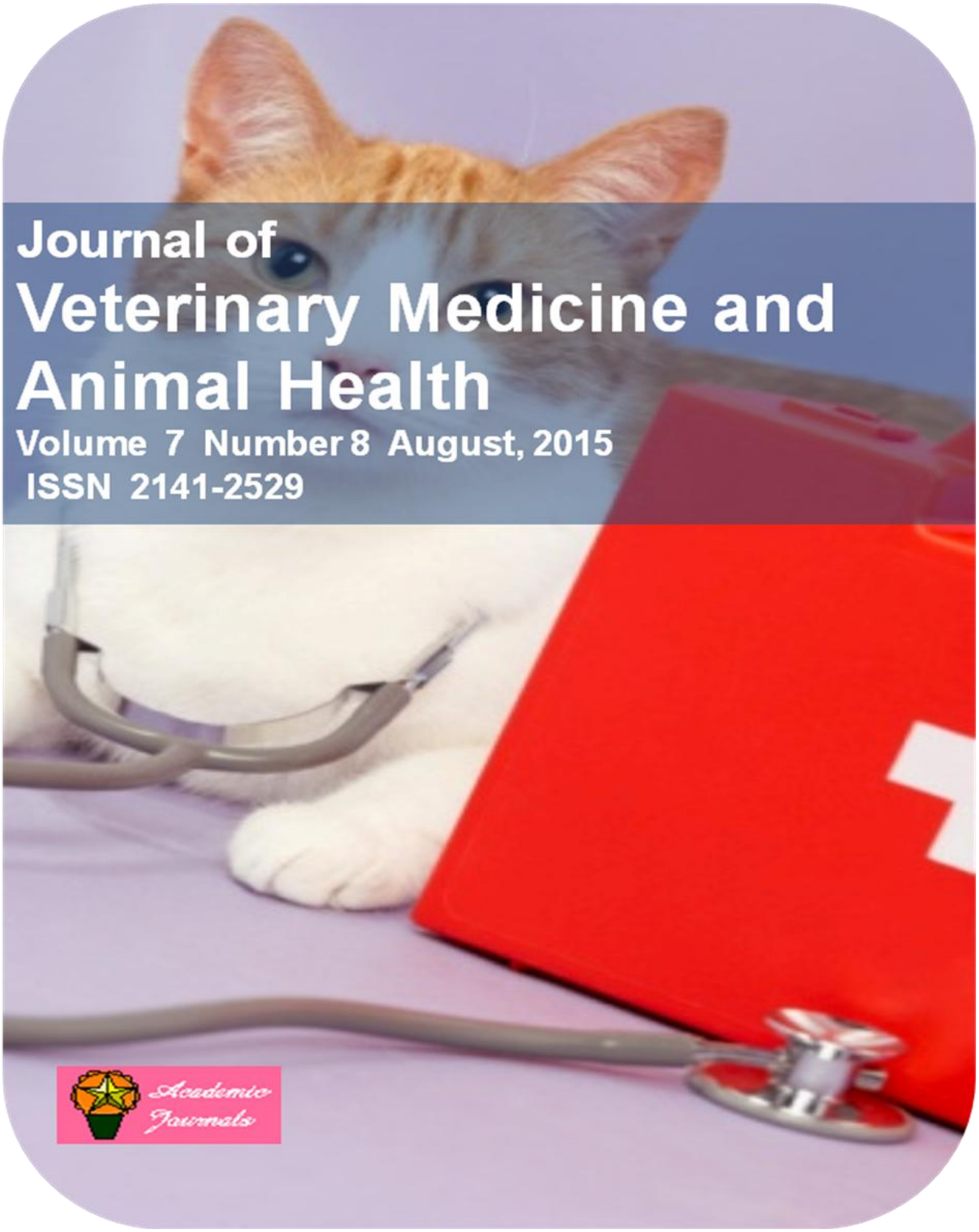

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Full Length Research Paper

The effects of different growth promoters on performance and carcass characteristics of broiler chickens

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This research was conducted to study the efficacy of different growth promoter's on the productive performance and carcass yield of broiler chickens. 840 male ROSS and 308 hybrid chickens were used according to completely randomize the design in six treatments and one control (Five growth promoters and control). Thus, there were six groups of chickens: group 1; control diet (without any promoter's), group 2; control diet + antibiotic, group 3; control diet + probiotic, group 4; control diet + prebiotic, group 5; control diet + phytobiotic and group 6; control diet + symbiotic. The productive indicators evaluated were: feed intake, weight gain, feed conversion ratio (FCR). The carcass yield and the main portions (breast, thigh and abdominal fat) was also determined. In all current studies, there wasn't any significant difference between treatments in body weight gain (P > 0.05) but all of them had beneficial effect compared to control. Lowest feed conversion ratio was observed in probiotic group and caused more efficient feed intake. Treatments vs. control increased carcass yield significantly but the difference between treatments was not significant. Breast and thigh was not affected by treatments and there wasn't any significant difference between treatments and control group. Lowest abdominal fat were seen in antibiotic group. According to our results, probiotic and symbiotic appeared to be superior compared to other growth promoters.

Key words: Growth promoters, performance, carcass, broiler.

INTRODUCTION

Nutrition is the most expensive factor in poultry production; therefore to reduce the cost of raising, one should improve the feed efficiency. The use of food additives as growth promoters in poultry nutrition is one way to accomplish this goal. Growth promoters used to stimulate growth, protect the health of poultry and to

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maintain the maximum potential are added to the poultry diets (Adams, 1999). Antibiotics are the chemical products obtained from certain strains of micro-organisms at low concentrations that can inhibit the growth of other micro-organisms, and may even cause their death. In the past, the use of antibiotics in food, as treatment and either at a lower level of care (as growth promoters) was widespread (Visek et al., 1987 and Shane, 2005), but the use of antibiotics in livestock and poultry may increase bacterial resistance. So in recent years, the use of antibiotics as additives in animal feed has been banned or restricted and the use of other additives as alternative compounds considered by livestock industry, especially in poultry industry has been. Probiotics are live microbial compound that stimulates the growth of beneficial micro-organisms and have very positive impact on the health of the host animal. Therefore, these materials are totally against antibiotics (Modir et al., 2001). The beneficial effects of probiotic supplementation can improve growth and has positive effects on livestock and poultry, increase food consumption, improve nutrient digestion and absorption, increase egg production, health improvement and reducing pathogenic enzymes secretion (Cole et al., 1987). Prebiotics are complex carbohydrates that with entering the digestive system, established suitable setting for beneficial micro-organisms of the digestive tract; therefore have a positive effect on the health of the host (Cummings and Macfarlane, 2002). Prebiotic mechanism stabilize the intestinal flora by stimulating the growth of beneficial bacteria, preventing the growth of undesirable bacteria, reducing serum cholesterol and improving immune responses (Gue et al., 2004). Synobiotics are the combination of prebiotics and probiotics and have properties of these two together; they have the effect such as reduced pH, preventing *Salmonella* infection, positive effect on performance and microbial population of the gastrointestinal tract, daily weight gain and also increase the final weight (Etuk et al., 2007). Liong et al., (2006) reported that the using of Synobiotics can cause concentration of organic acids, reduce cholesterol level and change the population of beneficial poultry intestinal bacteria. Pelica et al., (2004) also reported that improvement of poultry performance and strengthened immune system can be attributed to Synobiotics. Awad et al., (2008) showed that Synobiotics can cause better glucose absorption in poultry and have affect on stomach and intestines extent. Cho et al., (2006) reported that phytobiotics can increase the ration's protein and dry matter digestibility. Sirvydis et al., (2003) also reported that food containing phytobiotics have important influences on the development of physiological processes. This material increases the metabolism of proteins, fats and carbohydrates and improves growth rate of broiler chickens, also average daily gain, final body weight and feed conversion ratio improves. This experiment was designed to compare the effects of growth promoting additives such as; Avilamycin, Gallipro, immunoval, Digestarom and mixed Gallipro and immunoval on performance and carcass characteristics of male Ross 308 broilers.

MATERIALS AND METHODS

The trial was conducted at one of the Amol's Zarbal farm in the summer of 2013. In this study, 840 Ross and 308 male broilers were used. The project included 6 treatments (control, antibiotic, probiotic, prebiotic, phytobiotic and Symbiotic) and 4 replicates for each treatment. Thus, there were 24 experimental units; each had 35 chicks. A basal diet based on nutritional requirements for the Ross 308 commercial strain include starter (1 to 10 days), grower (11 to 28 days) and finisher diet (29 to 42 days) were adjusted using UFFDA software. Composition of the basal diet is reported in Table 1. Avilamycin as antibiotic was added at a rate of 100 g per ton of basal diet. Probiotic that was used in this study as feed additives with Gallipro. Its commercial name derived from the Bacillus subtilis brand (DSMZ 17299) according to the manufacturer's recommended level of 200 ppm was added to the diet. Prebiotic that was used in this study with immunoval. Its commercial name formed from Beta-glucan and manan oligosaccharides was added to basal diets at a rate of 2 kg/ton and afterwards was added at a rate of 1 kg/ton in the first week of the rearing period. Phytobiotic used in this experiment with Digestarom brand as herbal preparation to the level of 150 ppm was added to the basal diet. As symbiotic treatment, immunoval and Gallipro both in listed values were added to the basal diet. During the experiment, the chickens were given water and feed ad libitum. Temperature and humidity were adjusted accordingly to raising chickens Ross 308 standard. Light intensity was equal in halls. Antibiotics consumption was discontinued one week before slaughter. Feed intake, body weight gain and feed conversion ratio were measured weekly and the weight of each experimental unit's fatality was recorded daily. At the end of experiment, 42 days from each experimental unit, 2 chicks weighing close to average weight of the experimental unit (pen) were selected and after slaughter, carcass traits (carcass weight, breast weight, thigh and abdominal fat) were measured. Statistical models for the project were:

$$Yijk = \mu + Ti + eijk$$

That Yijk was each of the observations (performance), μ was the total mean, Ti was the effect of each treatment (probiotic, prebiotic, antibiotics, phytobiotic and Symbyotic) and eijk was the residual effect or error. Data were analyzed by using SAS statistical software (Version. 9.1) and GLM procedure. Average comparison was performed by using Duncan's multiple range tests in the statistical level of 5%.

RESULTS AND DISCUSSION

The results of performance parameters (feed intake,

Table 1. Composition of experimental diets in different rearing periods (%).

Diet composition	1 to 10 days	11 to 28 days	28 to 42 days
Corn	55.2	62.37	66.56
Soy bean meal (44%)	38.1	27.16	23.44
DCP	2	1.90	1.65
Slaughter by-products powder	2	5.00	4.00
Fatty acid	0.31	0.78	1.11
DL-Methionine	0.04	0.26	0.21
L-Lysine	0.03	0.21	0.18
L-Threonine	0.01	0.05	0.04
Mineral vitamin Premix	0.50	0.50	0.50
Salt	1	0.9	1.2
Sodium Bicarbonate	0.20	0.27	0.24
Formaycin gold	0.01	0.1	0.1
Oyster powder	0.40	0.45	0.72
Salinomycin	-	0.05	0.05
Zeolite	0.2	-	-
Chemical composition of calculated nutrient (%)			
Metaboisable energy (kcal/kg)	2890	3000	3050
Crude Protein	21.30	19.20	17.51
Calcium	1.01	0.86	0.81
Available phosphorus	0.48	0.40	0.35
Sodium	0.16	0.18	0.18
Arginine	1.41	1.23	1.10
Lysine	1.38	1.15	1.05
Methionine	0.70	0.55	0.48
Methionine+cysteine	1.03	0.88	0.78
Threonine	0.91	0.78	0.70
Vitamin premix			
Vitamin A: 7200 mg		Vitamin D_3: 1600 mg	
Vitamin E: 14400 mg		Vitamin B_1: 700 mg	
Vitamin B_2: 2640 mg		Vitamin B_3: 3920 mg	
Vitamin B_5: 11880 mg		Vitamin B_6: 1176 mg	
Vitamin B_9: 400 mg		Vitamin B_{12}: 6 mg	
Vitamin H_2: 40mg		Vitamin K_3: 800 mg	
Anti-oxidant: 400 mg		Choline chloride: 100000 mg	
Carrier(wheat bran): 1000 gm			

weight gain and feed conversion) for each of the starter, grower, finisher and overall periods are listed in Table 2. Symbiotic Group (immunoval + Gallipro) have more intake in each period and in the entire period of rearing ($P < 0.05$). Probiotic group (Gallipro) on days 11 to 28 had the lowest intake, although no significant differences were observed in feed intake between control treatment and the other treatments (except Symbiotic) ($P > 0.05$). In the final period, the probiotic treated group had a lower feed intake than in the other group ($P < 0.05$). The results showed that probiotic treatment has the lowest feed intake between days 1 to 42 in comparison with other treatments. In 1 to 10 days, antibiotic treatment, probiotic and Symbiotic had better weight gain than other treatments have. In the growth period, prebiotic group showed less weight gain than other treatments ($P < 0.05$). But in the final period, phytobiotic (digestarom) and control groups showed less weight gain ($P < 0.05$). Early

Table 2. Growth-stimulating effect on feed intake, body weight gain and feed conversion in broilers.

Source of variation (days)	Treatment	Antibiotic (avilamicin)	Probiotic (Gallipro)	Prebiotic (immunoval)	Phytobiotic (digestarom)	Symbiotic (Gallipro+ immunoval)	SEM
Feed intake (g)							
1 to 10	277.37^{c}	283.68^{b}	277.008^{c}	280.66^{bc}	277.20^{c}	287.19^{a}	0.95
11 to 28	1332.66^{b}	1330.91^{b}	1292.25^{c}	1322.35^{b}	1335.16^{b}	1375.90^{a}	6.25
29 to 42	2177.32^{ab}	2199.589^{a}	2149.97^{b}	2231.74^{a}	2216.74^{a}	2179.04^{a}	14.98
1 to 42	3787.36^{b}	3814.17^{ab}	3719.47^{c}	3834.75^{a}	3829.10^{a}	3860.13^{a}	16.49
Weight gain (g)							
1 to 10	177.67^{c}	201.47^{a}	199.48^{a}	194.64^{ab}	187.35^{b}	197.81^{a}	1.24
11 to 28	629.94^{b}	592.77^{bc}	637.01^{ab}	568.82^{c}	672.90^{a}	679.12^{a}	9.66
29 to 42	1.91.64^{b}	1147.07^{a}	1145.74^{a}	1154.79^{a}	1.96.74^{b}	1.70.40^{a}	13.51
1 to 42	1899.25^{b}	1941.31^{a}	1982.23^{a}	1918.25^{a}	1956.99^{ab}	2047.33^{a}	20.18
Feed convertion ratio							
1 to 10	1.56^{a}	1.4^{ab}	1.38^{c}	1.44^{b}	1.48^{ab}	1.45^{b}	0.015
11 to 28	2.11^{b}	2.24^{a}	2.02^{c}	2.32^{a}	1.98^{c}	2.02^{c}	0.029
29 to 42	1.99^{a}	1.91^{bc}	1.87^{c}	1.93^{b}	2.02^{a}	1.87^{c}	0.027
1 to 42	1.99^{a}	1.96^{b}	1.87^{c}	1.99^{a}	1.95^{b}	1.88^{c}	0.018

Means that have been shown in a row with dissimilar letters indicate statistically significant differences ($p < 0.05$).

in the study, the probiotic and control groups showed the lowest and highest Feed Conversion Ratio (1.38 and 1.56, respectively). Thus, probiotic treatment in this period has shown the best performance ($P < 0.05$). In the growth period, antibiotic and prebiotic group had the highest FCR, but in this course, no significant difference in feed conversion ratio in Probiotics, Photobiotic and Symbiotic treatments was observed ($P > 0.05$). In the final period, control and phytobiotic treated groups showed the highest FCR while the probiotic and symbiotic groups showed lowest FCR in this study. In the entire period, the control and prebiotic group had the highest FCR ($P < 0.05$). However, there was no significant difference between these two treatment groups ($P > 0.05$). In other words, in the whole course, probiotic and Symbiotic treatment groups had lowest FCR (1.87 and 1.88, respectively) and the best performance among the other treatment groups were. The main results of carcass traits examined in this study are shown in Table 3. Carcass yields were not affected by any of the treatments, so that all the treatments significantly had better carcass production performance versus control treatment ($P < 0.05$). Although among the control and antibiotic treatment, there was no significant difference in terms of carcass production ($P > 0.05$). Breast and thigh weight as a percentage of carcass weight was not affected by any of treatments ($P > 0.05$). Thus, no significant difference was observed between treatments and control groups in these traits. On the other hand, data from this trial showed that treatment with antibiotic, probiotics, and symbiotic have less effect on abdominal fat while the highest percentage of abdominal fat was observed in phytobiotic and control groups. Growth stimulants as feed additives are added to poultry diet to enhance growth rate and the economic meat production (Bunyan et al., 1997). Studies have shown that the use of growth stimulants have a positive impact on the growth of broiler chickens (Milligan et al., 1995 and Denli et al., 2003). Yang et al., (2009) reported that adding antibiotics in broiler chicken diets improves body weight gain, feed intake and feed conversion ratio. Bedford, (2000) found that the antibiotics as growth promoters are in direct contact with intestinal microflora, because these compounds had no effect on the Sterile Animals. Intestinal microflora by interaction with nutrient digestion may cause a significant effect on the host animal nutrition, health and performance of their growth (Barrow et al., 1992). When pathogens are attached to the intestinal mucosa, intestinal functions are strongly influenced (Droleskey et al., 1994) and the immune system is threatened (Neish et al., 2002). Chickens that were grown in germ-free condition rather than normal chicks that grew to bacteria and viruses exposure had 15% higher growth rate (Klasing et al., 1987). As shown in Table 2 in this study Avilamycin treatment compared to the control treatment except of the growing season has

Table 3. Effects of different treatments on carcass weight, breast, thigh and abdominal fat percentage at the end of the period.

Source of variation (%)	Treatment	Antibiotic (avilamicin)	Probiotic (Gallipro)	Prebiotic (immunoval)	Phytobiotic (digestarom)	Symbiotic (Gallipro+ immunoval)	SEM
Carcass efficiency	63.27[b]	64.08[ab]	67.92[a]	67.31[a]	68.40[a]	67.93[a]	1.41
Breast	29.58[a]	30.37[a]	29.67[a]	29.88[a]	30.73[a]	30.04[a]	0.74
Thigh	28.24[a]	29.62[a]	29.60[a]	29.26[a]	28.06[a]	29.76[a]	0.56
Abdominal fat	2.11[a]	1.68[c]	1.74b[c]	1.87[b]	1.93[ab]	1.71[bc]	0.06

Means that have been shown in a row with dissimilar letters indicate statistically significant differences ($P < 0.05$

increased weight. However, in order to prevent antibiotic resistance in humans against pathogenic bacteria and also remove residual antibiotics in poultry products, the abuse of antibiotics in poultry production was prohibiting. The results of the present study showed that probiotic treated group showed greater weight gain than the control group. There is many evidence showing that the use of probiotics in poultry diets improves immune function, improved body weight, diarrhea decrease and feed conversion ratio (Reid and Friendship, 2002 ; Patterson and Burkholder, 2003). Two basic probiotics mechanisms are included; competitive removal and combination with beneficial bacteria. Competition for substrate, producing antimicrobial metabolites which inhibit the growth of pathogens, and the competition for binding sites is also. Probiotic Supplements, especially *Lactobacillus* species have positive effects on resistance to infectious agents such as Clostridium (Decroos et al., 2004) and Campylobacter populations (Stern et al., 2001). According to the normal intestinal microflora studies, supplementations with probiotics have highly variable results according to origin and species. Cecal population of coliform bacteria in the gut of chicks treated with *Lactobacillus* decreased significantly. However, other population of bacterial species was not affected (Watkins and Kratzer, 1984 and Jin et al., 1998). Murry and colleagues, (2006) reported that chickens treated with probiotics containing *Lactobacillus* had greater number of *Lactobacillus* and had fewer *Clostridium perfringens* than that of the control group. Received different answers may be very complicated because it has a strong bond with the environment also. For example, in heat stress condition, body weight gain of female poultry treated with the *Lactobacillus* probiotic increased by 12%. However, feed conversion and mortality rates were also increased; 4 and 29% (Zulkifli et al., 2000). Fritts et al., (2000) studied the use of probiotic products containing Bacillus subtilis (C-3102) in chickens feeding for 42 days and observed that their body weight gain and feed conversion ratio improved. Growth-promoting effects of specific species of probiotics compared to antibiotics have been fitted in several experiments (Cavazzoni et al., 1998; Zulkifli et al., 2000 and Mountzouris et al., 2007).

Conclusion

As a general conclusion it can be expressed that stimulant effects of probiotics depends on probiotic species, the using level of probiotics, age of birds and using method (through water or feed). Prebiotic have benefits in comparism with probiotics, because they stimulate the growth of bacteria that are present in the intestinal flora naturally, hence they are naturally adaptive to the intestinal environment (Snel et al., 2002). Most of prebiotic products derived from Fructo oligosaccharides (oligofructose, inulin) (Patterson and Burkholder, 2003), Gluco oligosaccharides, Stachyose, Oligocytoxan and Malto oligosaccharides, effects have been studied in Poultry diets (Zhan et al., 2003; Gao and Shan, 2004; Jiang et al., 2006 and Huang et al., 2007). Gibson and Roberfroid, (1995) reported that prebiotic can alter the metabolism of bacteria in mice from protolithic to be Sacccharolytic. The optimal dose for probiotics to exert its maximum stimulus activity still remains uncertain although higher levels (0.8%) of inulin and short-chain oligosaccharides reduces the growth performance, digestibility of amino acids and energy metabolism (Biggs et al., 2007). The results of this study showed that the use of probiotics (immunoval) causes more weight gain and feed conversion. However, it seems that probiotic amount is much less to exert their desired and absolute effects. Worldwide, extensive research on phytobiotics as a biological compound and as an alternative to antibiotics is done. Compared to synthetic antibiotics or inorganic chemical compounds, these products are mainly derived from plant origin, hence are natural products that are less toxic (Wang et al., 1998). Phytobiotics via two mechanisms of antimicrobial and immune system support have positive effects on growth performance and health of animals. Known photobiotic compounds have

antimicrobial properties (Cowan, 1999). Polysaccharides are known as a source of anti-microbial compounds (Xue and Meng, 1996). It has been demonstrated that the use of herbal compounds improve growth performance, reduce coliform population and improves blood and cellular immune responses in chickens infected with *Mycoplasma galiisepticum* or *Eimeria tenella* (Gao et al., 2004; Pangasa and Singla 2007; Pangasa et al., 2007 and Singla et al., 2007). Windisch and Kroismayr, (2006) reported that phytobiotic used as feed additives in poultry diets increases the secretion of digestive track. Despite the above, action mechanism of photobiotics as a complementary compounds is unknown. Four factors may have a role on photobiotic effect as a growth additive; part of the plant that are used, resource, time and compatibility rate with other dietary components (Yang et al., 2009). The results of the present study showed that in comparison with the control, treatment using phytobiotic increased performance (improved weight gain and feed conversion). However, the performance of probiotic treatment is lower than phytobiotic (Table 2). The results of this study showed that the use of Symbiotic (Gallipro + immunoval) in poultry diets significantly improved body weight gain and feed conversion ratio. Similarly Panda et al., (2000) study showed that during the trial period (1 to 42 days) chicks that their diets were contain *Lactobacillus Sporogenes* (as probiotics) have more daily gain and more appropriate feed conversion ratio. It was also reported that adding prebiotic such as fructo-oligosaccharides and Mannan oligosaccharide improve poultry performance (Iji et al., 2001; Yusrizal and Chen, 2003 and Yang et al., 2009). Basically, probiotic and prebiotic composition may have more advantages than any of them could have because prebiotics may increase growth and cloning of probiotic strains. In the present study, the improvement in growth performance observed in symbiotic treatment can be a proof of this assertion. According to the results obtained in present study, it can be found to have many benefits for broiler production by adding various growth stimuli. It is clear that adding probiotics and symbiotics to poultry diets caused positive effect on performance and carcass weight produced. However, the additives level used must be examined carefully because it will be influenced by many factors. Although further studies to confirm the present findings and other aspects of the growth drivers in poultry, are been examined.

Conflict of Interest

The authors have not declared any conflict of interest.

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Recovery times for dogs undergoing thoracolumbar hemilaminectomy with fenestration and physical rehabilitation: A review of 113 cases

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This study aimed to determine if physical rehabilitation, in the form of neuromuscular electrical stimulation, hospital/home exercises, and/or underwater treadmill therapy, improved recovery times for dogs undergoing thoracolumbar hemilaminectomy and fenestration for Type I intervertebral disc disease. The initial recovery time was established as time from surgery to 3 unassisted steps to fall. A modified Frankel score for stage of intervertebral disc disease was assigned at intake into physical rehabilitation and at release. The study also examined variables including age, sex, amount of time in rehabilitation, and duration of signs before surgery. Retrospective study design was used. A total of 113 dogs undergoing hemilaminectomy with fenestration for T3-L3 Type I intervertebral disc disease was used. Dogs exhibiting signs of Type I intervertebral disc disease underwent advanced diagnostics before hemilaminectomy with fenestration was performed. In hospital, physical rehabilitation included neuromuscular electrical stimulation, range of motion and sling walking. The owners received home care instructions for exercise, handling, sling walking, elimination management, and what was not allowed from the dogs. Underwater treadmill therapy was initiated 10 to 14 days postoperatively and done on a weekly basis. Additional exercises were progressively added to the program for strength and balance. The average recovery time in this study was 16 days and dogs spent an average of 40 days in formal physical rehabilitation. 23 dogs improved 1 full modified Frankel score (MFS) and 89 dogs did not have a full increase of 1 MFS. More time in formal rehabilitation ($P < 0.001$) and more underwater treadmill sessions ($P < 0.001$) increased the dog's chances of improvement. Physical rehabilitation improves the recovery in a portion of patients undergoing hemilaminectomy with fenestration for Type I thoracolumbar intervertebral disc disease.

Key words: Physical rehabilitation, neuromuscular electrical stimulation, intervertebral disc disease, underwater treadmill, modified Frankel score, nociception.

INTRODUCTION

Hansen type I intervertebral disc disease is typically associated with chondroid disc degeneration and has an

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acute onset. The disc extrudes through the dorsal annulus causing dorsal, dorsolateral, or circumferential compression of the spinal cord. Hansen type I is herniation of the nucleus pulposus through the annular fibers and extrusion of the nuclear material into the spinal canal. Acute disc extrusion is characterized by the presence of soft or firm fibrous disc material within the vertebral canal and extradural hemorrhage. The thoracolumbar junction accounts for the highest incidence of disc lesions (T12-13 to L1-L2). Clinical signs range from pain (hyperesthesia), postural deficits, to paraplegia of the pelvic limbs with or without nociception (Coates, 2004).

The two most common types of decompressive surgery are the dorsal laminectomy and hemilaminectomy. The hemilaminectomy is considered the superior technique because of decreased destabilization, improved visualization for removal of disc debris, less associated with a post-surgical compressive laminectomy membrane and decreased manipulation of the spinal cord. The dorsolateral approach allows for access to the disc spaces for prophylactic fenestration. One study reported retrieval of disc material in 93% of dogs that had hemilaminectomy compared with 40% with dorsal laminectomy (McKee, 1992).

Physical rehabilitation has many modalities that aid in the recovery of dogs that have undergone hemilaminectomy for Hansen's Type I intervertebral disc disease in the thoracolumbar vertebral column (T3-L3). These include neuromuscular electrical stimulation, exercises such as supported standing, range of motion, side bends, sling walking, underwater treadmill, and strengthening/balance exercises such as walks, circles, weaves and low step-overs. Neuromuscular electrical stimulation can make the muscles of the pelvic limbs go through an aerobic workout (to muscle fatigue) even though the muscles of the hind limbs are not getting the nervous impulses in order to flex and extend (Krauspe et al., 1992; Low and Reed, 2000; Crognale et al., 2013). Exercises that are started within 12 h of surgery and also done at home, such as range of motion, help to bring blood flow to the joints when the limbs are not moving during the recovery period (Brody, 1999; Shumway, 2007). Standing exercises utilize the upper motor neuron extensor tone and helps maintain the animal's extensor strength until the limb begins to sequence (Millis et al., 2004). Side bends aid the dog to keep flexibility in the paraspinal muscles as well as teaching early weight shifting (Millis et al., 2004). Underwater treadmill aids in gait training by providing buoyancy to make it easier to stand, resistance to help keep the animal's strength, warm water to help the tight UMN spastic muscles relax so that the handler or animal can sequence the hind legs in a series that mimics walking (Geigle et al., 1997).

Straight line walking aids the body in strength and gait. Circles and weavings allow for either subtle or direct weight shifting (Millis et al., 2004). Low height step-overs retrain proprioception using visual and audible clues such as toenails tapping on wood or rattling on hula hoops (Millis et al., 2004). Balance board exercises allow the dog an opportunity to shift weight and work on proprioception and balance (Millis et al., 2004). The goal of this study was to give an average recovery time (from surgery to 3 unassisted walking steps to fall or better) when a dog receives physical rehabilitation directly after surgery and the amount of time in formal rehabilitation. Comparisons were made based on age, weight, sex, modified Frankel score at time of surgery, disc(s) affected, presentation, days from surgery to therapy, number of therapy sessions, number of underwater treadmill sessions, and days in formal physical rehabilitation.

MATERIALS AND METHODS

One hundred thirty three cases from the January 2010 to December, 2014 period were reviewed for this retrospective study. Twenty dogs were not included in this study because they did not enter formal physical rehabilitation or information pertaining to when they walked was not obtained. The decision to go to surgery was based on physical exam by a board-certified veterinary surgeon in consultation with the owners. Dogs of any size, sex, age, breed, or any length of time with paresis/paralysis were allowed in the study. Dogs were categorized as either presented to the surgeon less than 24 h from onset of neurologic signs, between 24 to 48 h post onset of signs or greater than 72 h from onset of signs. Duration of clinical signs prior to surgery was not available in 16/113 dogs. Dogs either had a myelogram using iohexol as the contrast agent before surgery, computed tomography/myelogram or magnetic resonance imaging to determine the location of the ruptured disc(s). Diagnostics were performed either by a boarded veterinary neurologist or sugeon and the surgeries were performed by one of seven boarded veterinary surgeons.

The hemilaminectomies were performed by using a dorsal midline incision, elevating the epaxial muscle attachments off the lateral aspect of the dorsal spinous processes, lamina, articular facets and the pedicle to the level of the accessory process and keeping the muscles retracted with a Gelpi retractor. A high speed drill was used to make a rectangular area from the base of the dorsal spinous processes dorsally, the accessory processes ventrally, and the articular facet of the caudal vertebrae. The area was drilled cranial to caudal using a back and forth action once the cortical layer was reached to reveal the soft inner periosteum (Figure 1). The vertebral canal was carefully entered, and much of the herniated disc material was removed as possible with special care taken not to damage the spinal cord. Prophylactic fentestration (making a small rectangular window in the annulus fibrosis) using a number 11 scapel blade was also performed at the level of the affected disc, and at times, the cranial and/or caudal disc. Hemorrhage from soft tissue was controlled with cautery or Gel Foam[1]. A thin subcutaneous fat pad was placed over the hemilaminectomy site and the muscles and surrounding tissues were closed in three layers (Fossum et al., 2013).

Standard anesthetic protocols were followed for the dogs, an intraoperative antibiotic was given intravenously, and a continuous rate infusion of fentanyl (2 to 4 micrograms per kilogram per hour) was administered for a minimum of 18 h post surgery. Seventy five percent of the dogs were started on oral famotidine and sucralfate

[1]Gelfoam, Pharmacia, Kalamazoo, Mich.

as soon as possible after surgery. A fentanyl patch, tramadol, a non-steroidal anti-inflammatory drug (not given if a steroid was given before or during surgery), and/or gabapentin were included in the postoperative pain management.

Three quarters of the dogs had a Foley urinary catheter system placed at the time of surgery and removed when the dog showed signs of sequencing or when the bladder could be manually expressed with or without the aid of urinary drugs (such as diazepam, phenoxybenzamine or prazocin). One quarter of the dogs did not have a urinary catheter placed and were expressed by hospital staff with or without the aid of drugs that relax the urethral sphincter.

Physical rehabilitation for the dogs during their hospital stay was conducted by a DVM/CCRP, or rehabilitation assistant, and/or trained surgery technician. Each dog had a physical exam 1 day to 2 weeks after the surgery at the hospital where the surgery was performed by the same DVM/CCRP which included a body system exam, 70% thigh circumference, notes about any pain, postural deficits, nociception, ability to stand and if the animal could initiate volitional movement when supported. The DVM/CCRP then prepared a written set of home care instructions for the owner or rehabilitation assistant which included massage of the hind legs and feet, range of motion exercises, wide arc side bends, sling walks and standing exercises in addition to the home care instructions provided by the surgeons. Home care instructions from the surgeons emphasized range of motion exercises in the form of bicycles or on the hind legs or flexion and extension of each pelvic limb focusing on the stifle, towel/sling assisted walks, supporting spine when picking the dog up and keeping the spine level, continuation of medications, exercise restriction including confinement, no running, jumping, or explosive activity or playing with other dogs, and a recommendation for continued formal physical rehabilitation.

NMES was started at the first visit after the examination. One of two commercial hand held NMES machines was utilized[2,3]. The hair was clipped in specific muscle areas of the pelvic limbs of the dogs and cleaned with a paper towel moistened with tap water. For dogs less than 10 kg, 3.125 cm round pediatric disposable gel-lined electrodes were used. For dogs over 10 kg, 3.375 cm square electrodes were used. For channel one, one electrode was applied to two stifle extensor muscles – the sartorius and vastus lateralis. For channel two, one electrode was applied to one stifle flexor (caudal biceps femoris) and one electrode to a tarsal flexor (cranial tibial muscle). The electrodes were placed as close to the motor point as possible to achieve the best muscle contraction. An alternating current was used (15 s on channel one and fifteen seconds of rest for channel one while channel two was on for fifteen seconds). A 200 microsecond (µs) pulse width was utilized with a ramp of 3 s and 45 Hz of pulse rate was used. The fine adjustment for each animal was the amperage, which was 10 to 20 milliamperes or less for dogs less than 10 kg and 20 to 40 milliamperes for dogs over 10 kg.

Each pelvic limb was stimulated separately for fifteen minutes with the dog in lateral recumbency or as close as possible and good contractions were obtained as in Figure 2. Neuromuscular electrical stimulation was performed as close to every other day while in the hospital as possible. If the dogs did not have early volitional (motor) movement in the pelvic limbs at the time of discharge from the hospital, the owner or caretaker was rented and specifically demonstrated instructions on how to use the NMES machine at home. Home care instructions were given and demonstrated to the owner at the go home appointment. Each family was given a soft lined sling for sling walking to take out for elimination 4 times per day. Massage of the hind legs included gentle kneading down and up the legs as well as tickling of the toes. Hair brushing (staying away from the incision) for sensory stimulation was also recommended. Range of motion of the stifles either slowly in lateral recumbency or standing with hand support and bicycling one hind leg at a time for 10 to 25 repetitions was always recommended. Side bends included a treat offered from the nose to each side (about shoulder arc) which allowed for a good balanced stretch of the spine and weight shifting to each hind leg as in Figure 3. Having the dog stand to eat while offering a small amount of food at a time and watch TV with gentle hand support between the legs or propped up on a leg or pillow was the final exercise.

Underwater treadmill[4] therapy was initiated on the first appointment (minimum of 10 to 14 days post-op) when the dog began walking or sequencing when sling walked. The dogs were fashioned with a loose fitting harness and placed in the UT with the DVM/CCRP or her assistant (for the 1st two times on dogs >10 kg and every time with dogs < 10 kg). The water level was raised to the upper thigh. A minimal speed was set for the size of the dog (dogs < 20 kg at 0.48 kilometers per hour and dogs > 10 kg starting at 0.97 kilometers per hour) for the first minute. If a dog could sequence the pelvic limbs without help, the speed was then increased by 0.16 or 0.32 kilometers per hour. For dogs that sequenced on land, but not in the water, the hind limbs were manually sequenced trying to mimic natural gait as in Figure 4. The time of the first UT session was generally 3 to 5 min. Dogs were towel dried after the session.

At the end of each underwater treadmill session, a new exercise was shown to the owner to be performed daily and added to the home exercise program. These exercises were circles, weaves, step-overs and balance boards added in a progressive order. The number of repetitions was set at 5 to each direction for circles and 3 to 4 reps back and forth for weaves. Circles were to be wide (1.21 to 1.82 m in diameter) and the spaces between weaves were to be just slightly longer than the dog 30.5 to 45.7 cm for dogs < 10 kg as in Figure 5 and 0.6 to 1.21 m for dogs > 10 kg. Step-overs were to be objects 2.54 to 5.08 cm high (such as broomsticks, rolled towels or hula hoops) for dogs < 10 kg as in Figure 6, and 0.08 to 10.18 cm (boards or rolled blankets) for dogs > 10 kg. The height was chosen based on the ability of the dog to flex the stifle as the object was crossed. The step-overs were spaced at 30.48 cm for dogs < 10 kg and 60.96 to 91.44 cm for dogs > 10 kg. Appropriate balance boards were loaned out for 1 to 2 weeks according to patient size. Balance boards of 0.60 × 0.60 m were loaned out for dogs < 10 kg and 0.61 × 0.61 m or 0.91 × 0.91 m were loaned out for dogs > 10 kg. The dog was to walk across the board 10 times to get used to the wobble and then kept on for 10 reps at 10 s wobble intervals as in Figure 7. Two factors contributed to the release of the dog from the formal physical therapy program. The first was how well the dog was walking (near normal strides with little ataxia was the goal) and the second was client desire for the dog to stay in rehabilitation therapy.

A MFS (Levine et al., 2009) was assigned at the time the dog presented to surgery, when the dog started physical rehabilitation and when the dog finished physical rehabilitation. A zero score was paraplegia without nociception. A score of 1 indicated paraplegia without superficial pain sensation. A score of 2 indicated paraplegia with deep and superficial pain sensation. A score of 3.25 indicated no volitional movement and no weight bearing was present. A score of 3.5 indicated no volitional movement, but weight bearing was present.

This was a modification of the MFS made specifically for this study so that a progressive numerical value could be attached to the a and b subclasses of the 3 group. A score of 4 indicated volitional movement with ataxia. A score of 5 indicated volitional movement with spinal hyperesthesia only.

[2]Respond Select, EmpiCo., St. Paul, MN, 55126 USA
[3]Perfect Stim, Lead-lok Inc., Sandpoint, ID 83864 USA
[4]Ferno Underwater Treadmill, Ferno Pools, Wilmington, OH, 45177 USA

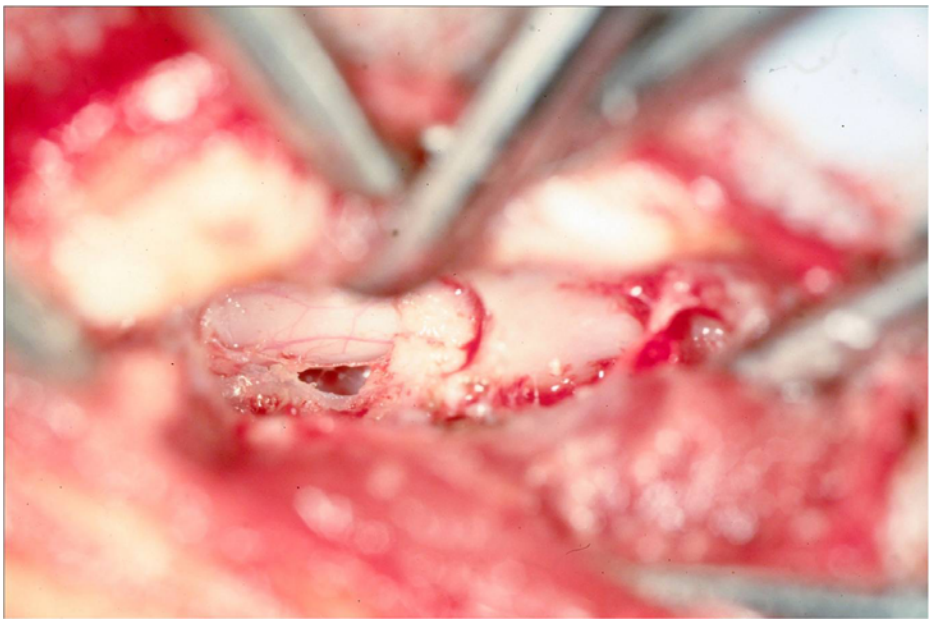

Figure 1. Photo of thoracolumbar hemilaminectomy procedure. Courtesy of KH Krause.

Figure 2. Photo of Dachshund cross dog receiving pelvic limb neuromuscular electrical stimulation.

Statistical analysis

Categorical data were described as proportions and 95% mid-P exact confidence intervals (CI). Quantitative data were described as medians and interquartile ranges (IQR). Clinic improvement during physical rehabilitation was defined as an increase of at least one modified Frankel score (MFS) unit from the post-surgery value. Categorical variables were compared between outcome groups using the chi-square or Fisher exact tests. Quantitative variables were compared between outcome groups using Mann-Whitney U

Figure 3. Side bending exercise on a Jack Russell Terrier. Note the paraspinal muscle stretch and weight shifting.

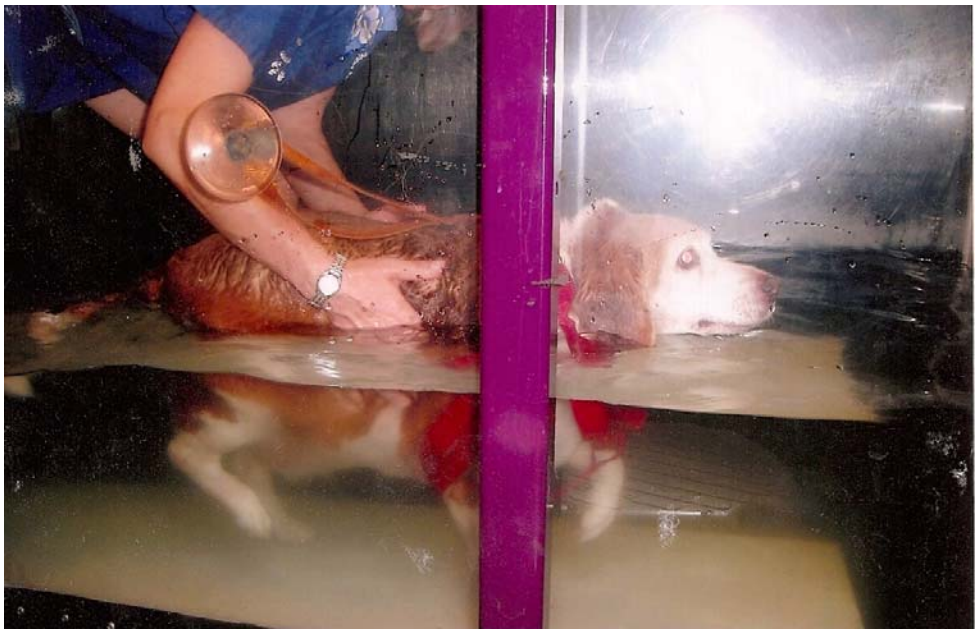

Figure 4. Beagle gaiting in underwater treadmill.

tests. Quantitative variables were categorized based on percentiles of the distribution when biologically relevant categories could not be determined. Univariate associations between successful physical rehabilitation and potential predictors were estimated using binary logistic regression. Univariate associations between time to successfully taking the first steps after surgery and potential predictors were estimated using Cox proportional hazards regression. Multivariable models were built using a backwards stepwise approach starting with all main effects that had P-value less than or equal to 0.20 in the univariate screening models. Variables were removed one-by-one based on the largest Wald P-values. Interaction terms were not evaluated in the multivariable models. The fit of the final logistic regression model was assessed using the Hosmer and Lemeshow test. Categorical data analysis

Figure 5. Chihuahua weaving between cones working on weightshifting and balance.

Figure 6. Jack Russell Terrier working on proprioception and gaiting.

was performed in available freeware[5] and other analyses performed using commercially available software.[6] Significance was set as $P < 0.05$.

RESULTS

One hundred and thirteen dogs met the criteria for

[5]Epi Info, version 6.04, Atlanta, GA, USA

[6]IBM SPSS Statistics Version 22. International Business Machines Corp., Armonk, NY, USA

Figure 7. Golden Retriever mix working on proprioception, balance and core strength on a wobble board.

inclusion into the study. Approximately 50% of the dogs were Dachshunds, 8.5% were Chihuahuas, and 4% each were Beagles, Corgis, Shih Tzus and terriers (or terrier crosses). The other breeds included in the study were both large and small breeds. Average body weight for this study was 10 kilograms (range 3 to 50 kg). Included were 53 spayed females (46%), 47 were neutered males (42%), females were sexually intact (4%) and 8 males were sexually intact (7%). Mean age was 6 years (range 2 to14 years). There were 16 lesion localization categories and some had a hemilaminectomy over more than one disc space. Twenty-four dogs had a T13 to L1 disc (21%), 20 dogs had a T12 to T13 disc (17.7%), 13 dogs had an L1 to L2 disc (11.5%), 11 dogs had a T11 to T12 disc (9.7%), 6 dogs had an L2 to L3 disc (5.3%), 3 dogs had a T10 to T11 disc (2.7%), and 3 dogs had an L3 to L4 disc (2.7%). Eleven dogs had two discs from T11 to T13 (9.7%) and the rest had a combination of either thoracic, lumbar (L1 toL3) or a combination of thoracolumbar discs.

Dogs took an average of 16.8 days (range of 1 to 270 days) to take 3 steps of ambulation by themselves and spent 40.7 days in formal physical rehabilitation (with visits every 1 to 7 days). Dogs received an average of 6.6 neuromuscular electrical stimulation sessions and 3.6 underwater treadmill sessions. Modified Frankel scores from intake to release from formal physical rehabilitation increased anywhere from a 0 (no nociception) to a 5 (ambulatory with spinal hyperesthesia only). Most of the increases were from a Modified Frankel score of 3.5 (no motor with non weight bearing) to a 4 (motor function to limbs with ataxia). Only 8/16 (50%) of dogs with loss of deep pain (nociception) improved with physical rehabilitation. Variables that were not affected by physical rehabilitation included breed, body weight and time to presentation to surgeon from the onset of signs. Spayed females had larger improvement (P = 0.054) than intact males (P = 1.0). Number of days in formal physical rehabilitation and number of therapy sessions each had a P-value of 0.001. Shorter days until successful steps also had a larger increase in MFS and a P-value of 0.001.

DISCUSSION

The present study gave a baseline recovery time for dogs undergoing thoracolumbar hemilaminectomy with fenestration and physical rehabilitation. Previous studiesreported that 90% of dogs were ambulatory 10, 12.9 and 10.8 days after hemi-laminectomy surgery alone (Davis and Brown, 2002; Ferreira et al., 2002; Ruddle et al., 2006). However, the dogs in the 10 day recovery were those that were ambulatory before surgery (Davis and Brown, 2002). One study where 90% of dogs walked 10 days after surgery did not include dogs with greater

Table 1. Descriptive statistics and comparison of categorical variables between dogs with improvement during physical therapy (increase of at least 1 modified Frankel score unit) and those without a substantial improvement post IVDD surgery.

Variable	Improvement		No improvement		P value*
	n	Proportion (95% CI)	n	Proportion (95% CI)	
Dachshund	23	0.43 (0.25, 0.64)	89	0.52 (0.41, 0.62)	0.483
Chondrodystrophic breed	23	0.61 (0.40, 0.79)	89	0.73 (0.63, 0.81)	0.254
Intact male	23	0.09 (0.01, 0.26)	89	0.08 (0.04, 0.15)	1.0
Neutered male	23	0.22 (0.08, 0.42)	89	0.46 (0.36, 0.56)	0.035
Intact female	23	0.04 (0.00, 0.20)	89	0.03 (0.01, 0.09)	1.0
Neutered female	23	0.65 (0.44, 0.82)	89	0.43 (0.33, 0.53)	0.054
T10-T11 affected	23	0.09 (0.01, 0.26)	89	0.04 (0.01, 0.10)	0.601
T11-T12 affected	23	0.22 (0.08, 0.42)	89	0.25 (0.17, 0.34)	0.766
T12-T13 affected	23	0.39 (0.21, 0.60)	89	0.36 (0.27, 0.46)	0.778
T13-L1 affected	23	0.43 (0.25, 0.64)	89	0.33 (0.23, 0.43)	0.328
L1-L2 affected	23	0.26 (0.11, 0.47)	89	0.21 (0.14, 0.31)	0.627
L2-L3 affected	23	0.09 (0.01, 0.26)	89	0.08 (0.04, 0.15)	1.0
L3-L4 affected	23	0.04 (0.00, 0.20)	89	0.03 (0.01, 0.09)	1.0
Presented < 24 h	18	0.56 (0.33, 0.77)	77	0.49 (0.38, 0.60)	0.635
Presented 24-72 h	18	0.33 (0.15, 0.57)	77	0.39 (0.29, 0.50)	0.658
Presented > 72 h	18	0.11 (0.02, 0.32)	77	0.12 (0.06, 0.20)	1.0

*Comparison between groups using chi-square or Fisher exact tests.

than 15 kilograms (Bush, et al., 2007).Also, dogs that did not enter formal physical rehabilitation were not included in this study, and so this may have skewed the time to ambulation when compared to other studies. One study reviewed 831 cases and placed dogs into recovery groups based on a neurologic grading scale of 0 to 5 with 0 being clinical normal dog and 5 representing paraplegia with absent nocicieption in both pelvic limbs and tail. Time to ambulation was reported for each grade along with the complications. 86.7% of dogs with grades 1 (thoracolumbar spinal pain without neurologic deficits) and 2 (ambulatory paraparesis) signs became ambulatory within 14 days. 81.9% of dogs became ambulatory within 14 days. 74.4% of dogs with grade 4a signs (paraplegia with intact nociception in both pelvic limbs and tail became ambulatory in 14 days. 69.7% of dogs with grade 4b signs (paraplegia with intact or decreased deep nociception in at least one of the pelvic limbs or tail) became ambulatory within 14 days. 36.4% of dogs with grade 5 signs (paraplegia with absent deep nociception in both pelvic limbs and tail within 14 days (Aikawa et al., 2012). However, the physical rehabilitation was limited to massage, passive range of motion, supported standing and encouragement of walking on non-slick surfaces by the owners when discharged.

Three factors could contribute to the longer recovery times in the present study. The first is if the dogs had volitional motor movement before surgery and having a grading scale is important to classify what the patient's clinical signs were prior to surgery, after surgery and before starting formal physical rehabilitation and at the end of formal physical rehabilitation. The scale can correlate the amount of spinal cord damage (Levine et al., 2009). However, it gives more objective data for comparison between groups of patients and research studies. The second is if the animal had deep pain perception (nociception) after surgery as this lowers the chance for becoming ambulatory (Levine et al., 2009). The last factor was the length of time between start of pain and loss of function until the time that the dog had surgery.

Four major findings were apparent from the data. The first is that fewer neutered males improved and that spayed females tended to have better recoveries (Tables 1, 2 and 3). The P-value was not less than 0.05, but equal to 0.054 (Tables 1 and 2). Older dogs were more likely to have an increased MFS (Table 3). More severe signs at presentation (a lower MFS) resulted in a larger increase in the MFS. The more affected number of discs also had a larger increase in the MFS (Table 2). More physical rehabilitation sessions, underwater treadmill sessions and longer amount of formal time in physical rehabilitation meant a better chance of improvement (Table 2). Dogs with more than 7 physical rehabilitation sessions were more likely to show improvement relative to fewer sessions (Table 4). Less severely affected dogs (larger MFS at start) were less likely to have a larger increase in improvement (Table 3). Those with a greater increase in MFS had longer days until taking steps, but this is a function of the fact that they were more severely

Table 2. Descriptive statistics and comparison of quantitative variables between dogs with improvement during physical therapy (increase of at least 1 modified Frankel score unit) and those without a substantial improvement post IVDD surgery.

Variable	Improvement		No improvement		P value*
	n	Median (IQR)	n	Median (IQR)	
Age (yrs)	23	7 (4, 9)	89	5 (4, 8)	0.178
Body weight (kg)	20	7.6 (6.4, 14.3)	88	7.0 (5.5, 9.9)	0.174
Modified Frankel score at presentation	23	1 (0, 2)	89	2 (2, 2.6)	0.004
Number of affected disks	23	1 (1, 1)	89	1 (1, 1)	0.042
Days post-op at therapy start	23	2 (1, 2)	89	2 (1, 3)	0.303
Days of physical therapy	23	41 (20, 79)	88	23 (13, 35)	0.006
Number of therapy sessions	23	7 (2, 25)	89	1 (1, 7)	<0.001
Number of treadmill sessions	23	3 (2, 7)	89	2 (1, 3)	0.023
Days until successful steps	23	21 (9, 50)	89	7 (4, 15)	0.001

MFS = modified Frankel score. *Comparison between groups based on Mann-Whitney U tests.

Table 3. Univariate logistic regression for the prediction of a successful physical therapy as defined as an increase of at least 1 modified Frankel score after surgery.

Variable	Parameter estimate ($\hat{\beta}$)	P-value (Wald)	Odds ratio (95% CI)
Age ≥ 6 yrs	0.984	0.049	2.68 (1.00, 7.14)
Weight < 10 kg	-0.693	0.181	0.50 (0.18, 1.38)
Female	0.984	0.049	2.68 (1.00, 7.14)
Neutered	-0.170	0.810	0.84 (0.21, 3.36)
Dachshund	-0.330	0.484	0.72 (0.29, 1.81)
Chondrodystrophic breed	-0.555	0.257	0.57 (0.22, 1.50)
MFS at surgery	-0.697	0.003	0.50 (0.32, 0.79)
More than 1 disk affected	1.027	0.034	2.79 (1.08, 7.21)
Presented < 24 h	0.249	0.636	1.28 (0.46, 3.60)
Days from surgery to therapy	-0.267	0.177	0.77 (0.52, 1.13)
Number of therapy sessions	0.080	0.001	1.08 (1.03, 1.14)
Number of treadmill sessions	0.063	0.103	1.07 (0.99, 1.15)
Days of physical therapy	0.004	0.183	1.00 (1.00, 1.01)

affected at intake and stayed in rehabilitation until they were walking (Tables 1, 5 and 6).

This author has found that two criteria that are important for a dog to walk again. First, the dog must be able to stand 10 to 30 s by itself or with gentle hand support. The dog must be able to paddle the hind legs in sequence when sling walked. Physical rehabilitation through electrical stimulation, exercises and underwater treadmill therapy helps to combine the two criteria so that the dog can gait by itself. In humans, 3 criteria must be present to achieve a normal or functional gait. The criteria are an adequate range of joint mobility, appropriate timing of muscle activation across the gait cycle, and unimpaired sensory input from the visual, somatosensory, and vestibular systems (Griggs et al., 2007). Although improvement for this study was considered to be an increase of one full MFS, the difference between a 3.25 or a 3.5 MFS is the difference between standing and walking.

Neuromuscular electrical stimulation provided in the early recovery period helped with muscle memory until the hind leg flexor muscles received innervation (Kanaya and Tajima, 1992). NMES may also decrease pain via the Gate Theory (Melzack and Wall, 1965). During NMES sessions, dogs were allowed to lie in a quiet room with music, have their hair coats brushed, and given lean turkey and water. For the dogs that appeared to have a slower recovery, daily electrical stimulation sessions with the owners were beneficial. Communicating with the owner after the surgery (within a day after surgery and a day after going home) about prognosis and recovery times was beneficial for the owners in what to expect and what was needed to be done as well as answering the many questions that they had in mind. Weekly follow-up

Table 4. Multivariable logistic regression for the prediction of a successful physical therapy (PT) as defined as an increase of at least 1 MFS after surgery.

Variable*	Parameter estimate ($\hat{\beta}$)	P-value (Wald)	Odds ratio (95% CI)
Female	1.246	0.038	3.48 (1.07, 11.3)
Male	Referent	-	-
Number of PT sessions	-	0.024	-
0 or 1 session	Referent	-	-
2 – 7 sessions	0.820	0.212	2.27 (0.63, 8.23)
> 7 sessions	2.080	0.006	8.00 (1.80, 35.6)
MFS at time of surgery	-	0.012	-
0-1	Referent	-	-
2	-1.938	0.003	0.14 (0.04, 0.52)
> 2	-1.291	0.097	0.28 (0.06, 1.27)

Hosmer and Lemeshow χ^2 = 5.357, df = 7, P = 0.617.

Table 5. Univariate Cox proportional hazard regression for the rate of taking first successful steps.

Variable	Parameter estimate ($\hat{\beta}$)	P-value (Wald)	Hazard ratio (95% CI)
Age ≥ 6 yrs	-0.118	0.542	0.89 (0.61, 1.30)
Weight < 10 kg	0.246	0.258	1.28 (0.83, 1.96)
Female	0.051	0.791	1.05 (0.72, 1.54)
Neutered	-0.245	0.409	0.78 (0.44, 1.40)
Dachshund	-0.175	0.369	0.84 (0.57, 1.23)
Chondrodystrophic breed	-0.195	0.353	0.82 (0.55, 1.24)
MFS at surgery	0.451	<0.001	1.57 (1.32, 1.86)
T10-T11 affected	-0.466	0.272	0.63 (0.27, 1.44)
T11-T12 affected	-0.202	0.370	0.82 (0.53, 1.27)
T12-T13 affected	-0.290	0.151	0.75 (0.50, 1.11)
T13-L1 affected	0.119	0.554	1.13 (0.76, 1.67)
L1-L2 affected	0.006	0.979	1.01 (0.64, 1.59)
L2-L3 affected	-0.294	0.424	0.75 (0.36, 1.53)
L3-L4 affected	0.195	0.704	1.22 (0.45, 3.32)
More than 1 disk affected	-0.461	0.032	0.63 (0.41, 0.96)
Presented < 24 hrs	-0.083	0.696	0.92 (0.61, 1.39)
Days from surgery to therapy	-0.011	0.737	0.99 (0.93, 1.05)
Number of therapy sessions	-0.090	<0.001	0.91 (0.88, 0.95)
Number of treadmill sessions	-0.121	<0.001	0.89 (0.84, 0.94)
Days of physical therapy	-0.017	<0.001	0.98 (0.98, 0.99)

appointments helped detect post-operative problems such as residual pain, regression in nerve function and bladder maintenance problems such as urine retention and infection.

Underwater treadmill therapy is useful for patterning gait and encouraging use of the limbs (Millis and Levine, 2014). A residual, temporary improved gait is often noted after treadmill therapy. At these appointments, dogs could be assessed in their improvement as they went on to learn a new exercise. Owners were encouraged to

Table 6. Multivariable Cox proportional hazard regression for the rate of taking first successful steps.

Variable*	Parameter estimate ($\hat{\beta}$)	P-value (Wald)	Hazard ratio (95% CI)
MFS at time of surgery	-	0.001	-
0-1	Referent	-	-
2	1.110	0.001	3.04 (1.55, 5.96)
> 2	1.331	<0.001	3.78 (1.82, 7.87)
Physical therapy sessions	-	<0.001	-
0-1	Referent	-	-
2-7	-0.559	0.022	0.57 (0.35, 0.92)
> 7	-1.828	<0.001	0.16 (0.07, 0.36)
Number of treadmill sessions			
0-3	Referent	-	-
> 3	-0.653	0.022	0.52 (0.30, 0.91)

keep activity to 5 min or less when the dog began walking. Running, jumping or sharp turns were discouraged. Crate rest was recommended when the owners were not home or in direct supervision of the dog.

Further studies need to be conducted to look at which groups of patients benefit the most from physical therapy. If a dog recovers within 3 days after surgery, recovery was almost completely to the surgery. Dogs that do not ambulate within a week of surgery or those that have lost deep pain perception would intuitively be the group that would benefit from physical rehabilitation the most. Although only 50% of the dogs that had no deep pain (nociception) at the time of intake improved, 10/16 (63%) presented to the surgeon less than 24 h from the onset of clinical signs which may indicate a more severe lesion. One study demonstrated that dogs with deep pain perception present at 2 weeks postoperatively had significantly higher success rate (8/12, 66.7% recovered) than dogs without deep pain perception at this time period (1/10, 10.0% recovered) (Laitinen and Puerto, 2005).

Four weaknesses are present in this study. The first is inconsistent long-term follow up (greater than 3 months) in all of the patients compared to a previous study (Aikawa et al., 2012). The second is trying to put a numerical value on the two subclasses of non-ambulatory paraparesis with or without weight bearing within modified Frankel spinal cord injury score. There may be a better overall scoring system such as the Texas spinal cord injury scale (Levine et al., 2009). Also, there is no control group within the study to compare dogs receiving hemilaminectomy and fenestration alone versus those that received formal physical rehabilitation. Finally, the current study did not look at electrical stimulation, underwater treadmill therapy or home exercise modalities separately or as separate combined groups. A previous study reported faster recovery times (5 days versus 12 days in the control group); however, both were also given polyethylene glycol (PEG), which has been shown to decrease recovery times (Draper, 2012; Laverty, 2004). Therefore, further controlled studies are needed to compare different rehabilitation modalities to see which one or combination is most effective at shortening and improving quality of recovery after thoracolumbar hemilaminectomy with fenestration.

Conflict of Interest

The authors have not declared any conflict of interest.

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Abbreviations: DVM/CCRP, Doctor of veterinary medicine/certified canine rehabilitation practitioner; **IVDD,** intervertebral disc disease; **MFS,** modified Frankel score; **NMES,** neuromuscular electrical stimulation; **UMN,** upper motor neuron; **UT,** underwater treadmill therapy.

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Full Length Research Paper

Resistance to 3rd generation cephalosporin of *Escherichia coli* isolated from the feces of healthy broilers chickens in Algeria

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High resistance of *Escherichia coli* have been demonstrated to 3rd generation cephalosporin in livestock, especially in broiler chickens; however, data on emission sources of these bacteria into Algeria are still rare. From January to March 2014, a preliminary epidemiological study of *E. coli* contamination in healthy broiler chicken flocks was carried out in the regions of Tlemcen, Algeria. 21 *E. coli* resistant isolates were examined to 3rd generation cephalosporin antibiotics (ceftriaxone, ceftazidime and cefotaxime). The antimicrobial susceptibility was determined by disk diffusion, and the MICs were determined by agar dilution method with Antibiogram Committee of the French Society for Microbiology (CA-SFM) 2013 guidelines. All strains were resistant to ceftriaxone and cefotaxime referring to CA-SFM, Antibiogram Committee of the French Society for Microbiology (EUCAST), and Clinical and Laboratory Standards Institute (CLSI). However, the resistance rate of ceftazidime is different according to the breakpoints criteria used; the susceptibility result of CA-SFM and EUCAST is similar for each farm. Farm B 50% of *E. coli* was resistant and 50% was susceptible, and 21% was susceptible and 79% was resistant for the farm C. However, comparing these two with CLSI, all strains were susceptible to ceftazidime.

Key words: *Escherichia coli*, antimicrobial resistance, 3rd generation cephalosporin, feces, broilers chicken.

INTRODUCTION

A large number of antimicrobial and anticoccidial agents are used in modern food animal production including broiler production resulting in the emergence of antimicrobial resistance, which is a cause of concern worldwide (Aarestrup et al., 2008; Pangasa et al., 2007). In recent years, antimicrobial resistance and especially multi-drug resistance, has become very common in clinical isolates, including *Escherichia coli* isolates of animal origin (Dolejská et al., 2008). *E. coli* strains are a part of intestinal normal microflora of many animals, including humans and birds (Brzuszkiewicz et al., 2001). *E. coli* is considered to be an excellent indicator of antimicrobial resistance for a wide range of bacteria (Bogaard and Stobberingh, 2000; McEwen and Fedorka-

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Cray, 2002). Resistant *E. coli* can be transmitted to humans from animals. A large proportion of resistant isolates causing human infections are derived from food animals (Jakobsen et al., 2010).

Collingnon et al. (2013) have extrapolated values from all over Europe they have found if 56% of the 3rd generation cephalosporin-resistant *E. coli* (G3CREC) were derived from poultry. In addition, Depoorter et al. (2012) showed that acquired resistance of *E. coli* to 3rd generation cephalosporin antimicrobials is a relevant issue in intensive broiler farming. G3CREC can be transferred from broiler to humans, not only through direct contact but also indirectly. This indirect transfer involves mainly consumption of broiler meat or contact with surface water or vegetables contaminated with broiler excreta (Blake et al., 2003).

In Algeria, since the 1980s, the emergence of the poultry industries increased the consumption of animal proteins at a much affordable cost (Ferrah et al., 2003). The recent study of Aboun (2012) showed that from 989 *E. coli* isolated from four veterinary laboratories, the percentages of extended-spectrum beta-lactamases (ESBL) production were 1.3, 20 and 85% in three laboratories: Pasteur Institute of Algeria, Constantine Regional Veterinary Laboratory and the Regional Veterinary Laboratory Laghouat, respectively. The antibiotics β-lactams used in Algeria are ampicillin, amoxicillin, oxacillin, penicillin, amoxicillin-clavulanate, cephalothin and ceftiofur. Growth factors antibiotics are not incorporated into animal feed and are banned from use since April 2007 (Kechih-Bounar).

Our objectives of this study were to estimate the frequency of resistant *E. coli* in feces samples of healthy broiler chickens in the regions of Tlemcen, during the rearing of broilers period and to identify the antimicrobial resistance of isolates to 3rd generation cephalosporin antibiotics and compared to CA-SFM 2013 and new version Antibiogram Committee of the French Society for Microbiology (CA-SFM) 2014, European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2014 and Clinical and Laboratory Standards Institute (CLSI) 2014 breakpoints criteria.

MATERIALS AND METHODS

Study area

Tlemcen is a town in Northwestern Algeria and the capital of the province (Wilaya) of Tlemcen (Figure 1). It is open to all midfield: Mediterranean sea in the north; Morocco frontiers in the west, a land place of international exchanges.

This study was conducted in farms around a radius of 40 km from the city of Tlemcen. Each farm is situated in a distance to another.

Sampling

All materials needed for sampling were prepared before leaving the laboratory: a cooler filled with ice, latex gloves, spatula, lighter, sterile tubes, labels, boots, and blouse. For the samples, latex gloves were used to prevent direct contact with the samples; the tubes were marked and labeled before taking the samples not be mixed. For each broiler flocks, the maximum number of samples is 60 feces during the rearing of broilers for 50 to 55 days. Each sample of fresh feces (approximately 10 to 15 g) of broilers was randomly collected soil along sheds (one in each 2 m, and this ensures that fecal samples are representative of the group) with a sterile spatula before soaring with a lighter, and is placed in a sterile tube (60 ml). The tubes were then placed in a cooler, returned to the laboratory within two hours, and analyzed for 6 h after collection.

Isolation/Identification of *E. coli*

Approximately 1 g of each fecal sample was inoculated into tubes, containing 9 ml vice Brain Heart Infusion Broth (Fluka BioChemika, Spain), and incubated aerobically at 37°C for 18 to 24 h without shaking. Platinum loopful of the broth was subcultured on MacConkey agar medium (Fluka BioChemika, Spain) supplemented with cefotaxime 2 mg/ml and incubated aerobically for 18 to 24 h at 37°C. A single colony morphology typical *E. coli* large pink to red was selected and identified by conventional biochemical methods, the Tryptic Sugar Iron Agar, the Oxidase test and API 20E test (bioMérieux, Marcy-l'Étoile, France). One isolate per feces per broiler chicken was accepted. Isolates of *E. coli* were retained between 2 and 6°C.

Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) were determined by agar dilution method in Mueller-Hinton medium (Fluka BioChemika, Spain), in accordance with the Comité de l'Antibiogramme de la Société Française de Microbiologie (CA-SFM) 2013 guidelines. The method of serial dilution in a solid medium was used to determine the bacterial susceptibility to antibiotics. It consists in a standardized bacterial inoculums contact with increasing concentrations of antibiotics. *E. coli* strain ATCC 25922 was used as a control.

RESULT

E. coli in tested poultry farms

From January to March 2014, 160 feces samples were collected from housing of healthy broiler chicken flocks at 4 different rearing sites (farms A, B, C and D) in Wilaya of Tlemcen (Table 1) during fattening in the rearing period for each farm. Each of the four flocks comprised between 800 and 4000 birds per house. The specimens were collected by walking through the housing. Overall 21 non-duplicate strains of *E. coli* were isolated from two farms. The frequency of contamination for the period of study is 13.12% (21/160).

Antibiotic susceptibility of isolated strains

The CA-SFM 2013 is based on three clinical categories which was selected for the interpretation of *in vitro* sensitivity: Sensitive (S), Resistant (R) and Intermediate

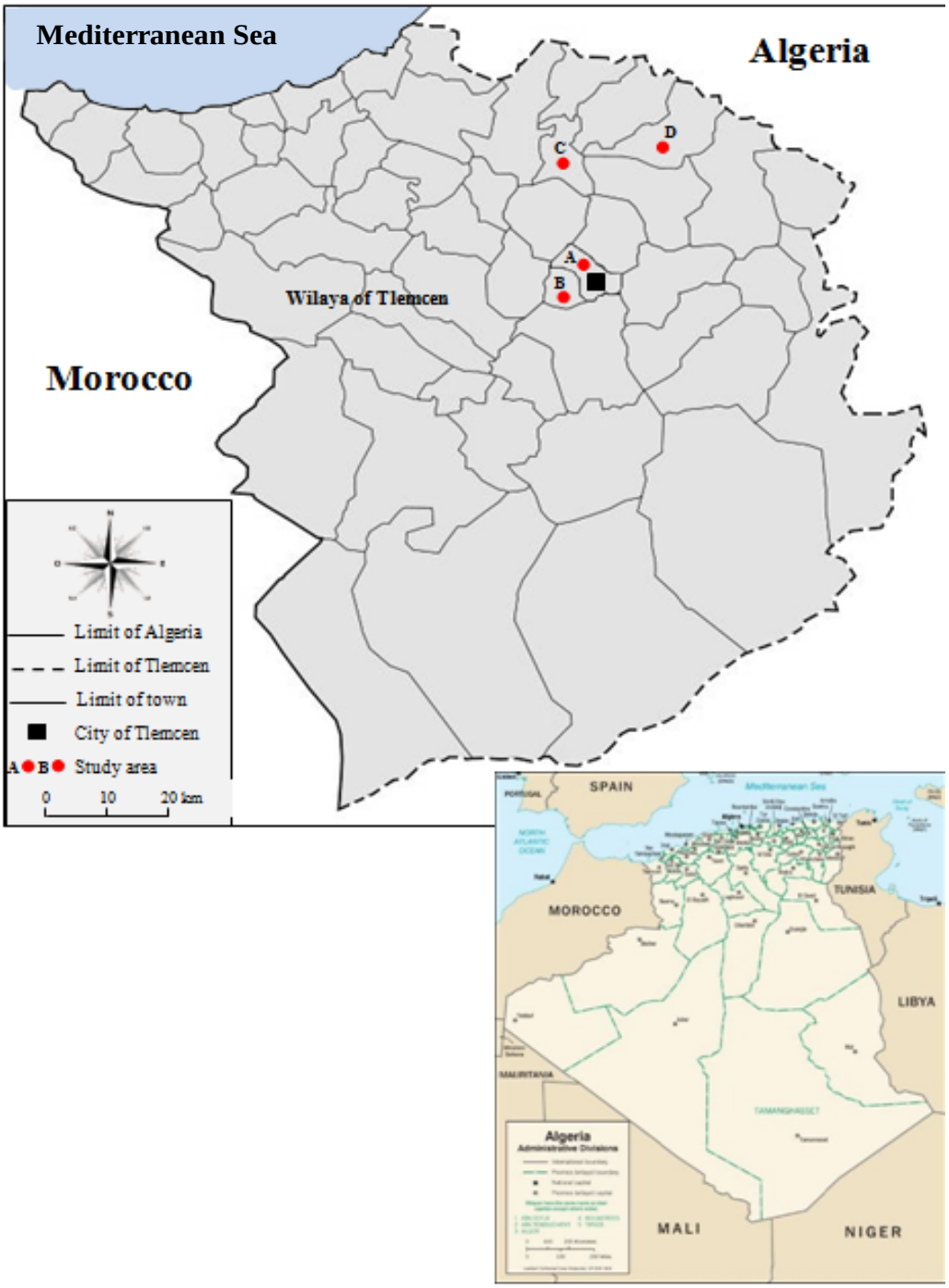

Figure 1. Map of the Wilaya of Tlemcen. Areas shown in circles represent locations of broilers farming activities.

(I). However, in this study the isolates were classified as susceptible (S) or resistant (R) using the zone diameter interpretative standards recommended by CA-SFM 2013. Isolates with intermediate susceptibility were considered susceptible. The results were categorized as very high rate of resistance (>75% of isolates resistant); high rate (>50 to 75%); moderate rate (>30 to 50%); low rate (>10 to 30%), and very low resistance rate (0 to 10%) (Knezevic and Petrovic, 2008).

Table 2 shows that all *E. coli* strains were resistant to ceftriaxone and cefotaxime referring to CA-SFM 2013. The same result observed using CA-SFM 2014, EUCAST

Table 1. Distribution of *E. coli* isolates per farms.

Farm	Number of broilers	Number of samples	Number of *E. coli* isolates
Farm A	4000	40	00
Farm B	4000	60	02
Farm C	4000	20	19
Farm D	800	40	00
Total 04 farms	12800	160	21

Table 2. Susceptibilities rate of isolates of *E. coli* to ceftazidime, ceftriaxone and cefotaxime using CA-SFM 2013 and CA-SFM 2014, EUCAST table v 4.0 and CLSI M100-S24 breakpoint criteria.

Farm	Ceftazidime (CAZ)					Cefotaxime (CTX)			Ceftriaxone (CXM)		
	CA-SFM		EUCAST		CLSI	CA-SFM	EUCAST	CLSI	CA-SFM	EUCAST	CLSI
	S	R	S	R	S	R	R	R	R	R	R
Farm B	50	50	50	50	100	100	100	100	100	100	100
Farm C	21	79	21	79	100	100	100	100	100	100	100

S: Susceptible; R: Resistant.

2014, and CLSI 2014 breakpoints criteria. However, the ceftazidime indicate the resistance of some strains. Comparing the resistance rate of ceftazidime, the susceptibility result of CA-SFM, and EUCAST is similar for each farm; for farm B 50% (*n*=1), *E. coli* was resistant and 50% (*n*=1) was susceptible, and 21% (*n*=4) of *E. coli* was susceptible and 79% (*n*=15) was resistant for farm C. However, comparing these two with CLSI, it was noted that there was great difference; all strains were susceptible to ceftazidime.

DISCUSSION

E. coli in tested poultry farms

To our knowledge, this is the first epidemiological study of 3rd generation cephalosporin-resistant *E. coli* in broiler chicken flocks ever carried out in the Tlemcen region of Algeria. Prior to this research, very little information was available on G3CREC in Algerian poultry farms. The Algerian researchers are much more interested in the study of *Salmonella* (Bouzidi et al., 2012) and the study of *Campylobacter* (Messad et al., 2014).

The presence of cephalosporin-resistant *E. coli* in the intestinal tract of food-producing animals is extensively described in many reports (Hasman et al., 2005; Kojima et al., 2005; Riano et al., 2006; Cloeckaert et al., 2007; Liu et al., 2007). The result of frequency contamination is in accordance even with the rates detected until recently in some countries. Low levels of resistance to cefotaxime, ceftazidime, and cephalothin were observed throughout the study period (18.0 to 27.2%), probably because these antimicrobials are prohibited from use as veterinary products in China (Xiang et al., 2014). Another studies conducted in other countries reported similar results in USA (Tadesse et al., 2012) and in Spain (Blanco et al., 1997). In Belgium, about 35% of the *E. coli* strains isolated from live broilers are resistant to 3rd generation cephalosporins, while over 60% of the broilers are found to be carrier of these 3rd generation cephalosporin resistant *E. coli* after selective isolation (Depoorter et al., 2012).

In this study, two farms were contaminated. A low rate observed in farm B 3.33% (2/60), however, in farm C, a high rate 95% (19/20) and 0% in two farms: A and D.

Antimicrobial resistance

MIC results were interpreted following four sets of guidelines: those published in 2013 and 2014 by the CA-SFM, those published in 2014 by EUCAST, and the CLSI guidelines published in 2014.

The CA-SFM 2013 and new version 2014 susceptibility breakpoints for ceftriaxone, ceftazidime and cefotaxime were ≤1 µg/ml and resistance breakpoints were >2, >4 and >2 µg/ml, respectively. EUCAST 2014 susceptible breakpoints for cefotaxime, ceftazidime and ceftriaxone were ≤1 µg/ml and resistance breakpoints were as CA-SFM. CLSI 2014 susceptibility breakpoints were ≤1, ≤4 and ≤1 µg/ml and resistance breakpoints were >4, >16 and >4 µg/ml, respectively.

There was no difference of resistance rate to these antimicrobials (cefotaxime and ceftriaxone) when compared. For example, the resistance rate of all isolate was 100% to cefotaxime when determined using CA-SFM 2013 and 2014, EUCAST 2014 and CLSI 2014 criteria. However, the susceptibilities of ceftazidime were irrespective of the breakpoints used. In farm B, the

isolated *E. coli* were 50% susceptible to ceftazidime for CA-SFM 2013 and 2014 and EUCAST 2014; and 100% for CLSI 2014. For farm C, the strains susceptible to this antibiotic were 21% for CA-SFM 2013 and 2014 and EUCAST 2014; and 100% susceptible for CLSI 2014.

Compared with CA-SFM 2013 and 2014, EUCAST 2014 breakpoints successfully designated a larger number of isolates as cephalosporin-resistant. Furthermore, CA-SFM 2013 susceptibilities were similar to those using CA-SFM 2014 and EUCAST 2014 guidelines. However, CLSI 2014 susceptibility is very different to CA-SFM and EUCAST. The difference of percentage noted between the two farms return to the numbers of strains isolated from each farm.

CLSI breakpoints may differ from those approved by various regulatory authorities for many reasons, including the following: different databases, differences in interpretation of data, differences in doses used in different parts of the world, and public health policies. Differences also exist because CLSI proactively evaluates the need for changing breakpoints (CLSI, 2014).

The result in this study is in accordance with that released in Spain in 2003, which reported 10.1% cefotaxime resistant *E. coli* isolated from poultry. These levels were gradually rising to 23 and 30% between 2005 and 2008 and they have decreased to 20.8% in 2011. Same dynamics were observed in the Netherlands, with increasing numbers of resistance to 3rd generation cephalosporins from 14.1 to 17.5% between 2005 and 2009 and a decrease to 8% in 2011 (Garcia-Migura et al., 2014).

Conclusion

The data of this survey indicate that *E. coli* isolated from healthy broiler chickens in some farms were resistant to 3rd generation cephalosporin, especially ceftriaxone and cefotaxime, which originated from feces during the rearing of broilers 50 to 55 days. The ceftazidime remains effective for some strains. The result mentions the utility to choose a good interpretation of the results of antimicrobial susceptibility and MICs according the guidelines CA-SFM, EUCAST and CLSI.

The widespread resistance of *E. coli* isolates should raise concerns about imprudent use of antibiotics in veterinary medicine. These observed differences should be further investigated in new prevalence studies.

Conflict of Interest

The authors have not declared any conflict of interest.

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