

Research Article



The Effect of Using Organic Acid as an Alternative to Antibiotics Drugs on Productive and Physiological Performance of Broilers Ross – 308

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Abstract | An experiment was conducted to study the effect of graded levels of butyric acid (butyrate) on performance, gastrointestinal tract health and carcass characteristics in young broiler chickens. Control starter (0-3 wk.) and finisher (4-6wk.) diets were formulated to contain 2,900 kcal ME/kg and 23% CP, and 3,100 kcal ME/kg and 21% CP, respectively. Subsequently, four groups of diets were formulated as following (T1: Positive Control contain 0.05% antibiotic maudramycin), (T2 : Negative Control without maudramycin), (T3 : T2 + 0.3% butyric acid) and (T4 : T2 + 0.6% butyric acid). Each diet was fed at random to 4 replicates of 30 chicks each throughout the experimental period (0-6wk). The results showed that 0.3% and 0.6% butyrate in the diet was improvement the body weights more than other treatments, and superior for feed conversion ratio. Feed intake were not influenced by the dietary treatments. A reduction in pH of the upper GI tract (crop, proventriculus and gizzard) was observed by inclusion of butyrate in the diets of broilers compared to either control or antibiotic-fed group. Butyrate at 0.6% was more effective in reducing the pH than 0.3%. Within the lower GI tract, 0.6% butyrate was effective in lowering pH in the jejunum, but no effect was found in either the duodenum or ileum. Dressing percentage was higher in all the butyrate treatment groups compared to the positive control or negative group also Increasing of histomorphological response. The best rate of villi length was recorded in the fourth and third treatments compared to the lowest length of treatment in the second and first treatment. From these findings, it is concluded that 0.3 and 0.6% butyric acid supplementation lead to more high villi and mucosal thickness at small intestine, and increasing dressing percentage in broiler chickens.

Keywords | Organic Acid, Antibiotics, Performance, Dressing percentage, Broiler chickens

Editor | Kuldeep Dhama, Indian Veterinary Research Institute, Uttar Pradesh, India.

Received | January 29, 2018; **Accepted** | July 08, 2018; **Published** | August 17, 2018

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Citation | Ali N, Alkassar S, Alkassar A (2018). The Effect of Using Organic Acid as an Alternative to Antibiotics Drugs on Productive and Physiological Performance of Broilers Ross – 308. *Adv. Anim. Vet. Sci.* 6(9): 359-365.

DOI | <http://dx.doi.org/10.17582/journal.aavs/2018/6.9.359.365>

ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

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INTRODUCTION

High levels of production and efficient feed conversion are the need of the modern poultry industry, which to a certain extent could be achieved by the use of specific feed additives. Antibiotic feed additives as growth promoters have long been supplemented to poultry feed to stabilize the intestine microbial flora, improve the general performances and prevent some specific intestinal pathology (Hassan et al., 2010). However, due to the emergence of microbes resistant to antibiotics which are used to treat human and animal infections, the European Commission (EC) decided to phase out, and ultimately ban (1 January

2006), the marketing and use of antibiotics as growth promoters in feed (EC Regulation No.1831/2003. Consumer pressure is pushing the poultry industry to rear birds without antibiotics (Castanon, 2007). Such a situation has compelled the researchers to explore the utility of other non-therapeutic alternatives like organic acids, enzymes, probiotics, prebiotics, herbs, essential oils and immune stimulants as feed additives in poultry production. The European Union allowed the use of organic acids and their salts in poultry production because these are generally considered safe (Adil et al., 2010). The use of organic acids has been reported to protect the young chicks by competitive exclusion (Mansoub et al., 2011), enhancement of nutrient

utilization, growth and feed conversion ratio (Lückstädt and Mellor, 2011). The addition of organic acids in diet can have a beneficial effects on the performance of poultry by decreasing pathogenic bacteria. Most common bacteria that affect the intestinal health of poultry are Salmonella, Campylobacter and *Escherichia coli* which can be controlled by supplementation of an organic acid in diet (Naseri et al., 2012).

(Byrd et al., 2001; Açıköz et al., 2011; Hamed and Hassan 2013; Gheisari et al., 2007) found that supplementation of 0.2% encapsulated organic acids to the diet might improve the proliferation of useful micro flora (*Lactobacillus* spp.) and diminish the population of harmful bacteria (*Clostridium perfringens*, *E. coli* and *Salmonella* spp.) in poultry gut contents. Leeson et al. (2005) and Panda et al. (2009) reported that butyrate, irrespective of concentrations (0.2%, 0.4% or 0.6%) in the broiler's diet, improved the villus length and crypt depth in the duodenum. Thus, butyrate supplementation could be highly helpful to young birds for intestinal development. Houshmand et al. (2012) found that at 21 days of age of the broiler, dietary addition of organic acids 0.15% in a starter diet resulted in significant increases in antibody titers against Newcastle disease. However, at 42 days of age, a non-significant difference was noticed between treatments. The aim of this study was to evaluate the effects of different levels of butyric acid in broiler diets on performance, histomorphological traits of small intestine, internal organ weights, dressing percentage compare it with or without added antibiotics..

MATERIALS AND METHODS

EXPERIMENTAL PROCEDURE

Each experimental group was fed ad-libitum with its own diet for 42 d. Feed intake, gain weight and feed conversion ratio were determined in each period weekly. The study was conducted according to the International Guidelines for research involving animals (Directive 2010/63/EU), specially slaughtering birds according to the Islamic procedures.

BIRDS AND PLANE OF NUTRITION

A total of 480 one-day-old mixed-sex Ross 308 broiler birds were obtained from commercially hatched eggs (Green World company-Najaf). They were raised from day old at the Poultry farm of the Animal Production Department.

Birds with one day-old-age were randomly allocated to 16 floor pens (2 × 1.5 m) with wood shavings (30 birds per pen). The floor pens were located in an open-sided house, and each pen was equipped with an automatic bell drinker and 1 tube feeder. The pen was considered as experimen-

tal unit for performance measurements. The birds were randomly allocated to four dietary treatments of 30 birds per replicate and four replicates per treatment in a randomized completely block design. The experimental design is shown in Table 1. Experimental diets were : T₁ (negative control without drugs or organic acids), T₂ (positive control with drug and without organic acid), T₃ (T₁+ 0.3% butyric acid), T₄ (T₁+0.6% butyric acid). The used BA (product of BABY-C4) contained 25 – 30% monoglycerides in the 1 or 3 positions, 50 – 55% diglycerides in the 1 or 3 position and 15 – 25% triglyceride. The ME of BABY – C4 was assumed to be 3600 Kcal Kg⁻¹. Feed and water were available ad libitum. These diets were formulated to be iso energetic and iso nitrogenous Table (2,3) according to NRC, (1994). The birds were reared and grown to market age 6 weeks. The birds were also given standard medication and prophylactic treatments as recommended by the Iraqi Veterinary Medical Association for this region. Birds were provided free access to feed and water, with constant illumination of 23 h of light and 1 h of dark per day during the entire growing period. Feed intake and mortality were recorded daily and BW was recorded at 0, 21, 42 d of age, by pen (average BW of all birds), to determine the FCR and ADG.

Table 1: Experiment design

Treatment	P. Control	N. Control	N.C+ 0.3% PSB	N.C+ 0.6% PSB
	T1	T2	T3	T4
Antibiotics*	+	--	--	--
PSB**, mg ⁻¹ kg	--	---	3gm ⁻¹ kg	6gm ⁻¹ kg
N***	40	40	40	40

*Maduramycin(50mg/kg) as label of plant protein concentrate.

**PSB:Mean Powder Sodium Butyric Acid.

***N:Mean number of chicks in treatment.

PERFORMANCE TRAITS

Feed intake (FI:g/bird/period) and body weight gain (BWG, g/bird /period) were recorded at the beginning of the experiment (day 1) until the end of the starter period 21th d of age, finisher period 22th-42ndd of age and total period 42 d of age (Alkassar, 2012). Feed conversion ratio-FCR) was calculated by dividing feed intake / body weight gain (Alkassar, 2010). On the final day of the experiment, (42 d-of-age), two bird from each replicate (eight from each treatment) were randomly selected slaughtered and dissected manually, plucked and eviscerated. Chickens heads and Legs were removed, and then internal organs (liver, gizzard and heart) were removed, weighted and calculated as percentage of carcass weight. The following internal organs were separated and weighed to the nearest 0.001 g on a Medicate M160scales: gizzard (without digesta), liver, (without gallbladder), heart. Small intestine of birds was opened immediately after killing and then ap

Table 2: Ingredient composition of starter diets

Ingredient %	Positive Control	Negative Control	NC+ BA	NC+ BA
	T ₁	T ₂	T ₃	T ₄
Corn	40.0	41.0	41.0	41.0
Wheat	22.0	22.2	21.9	21.6
Soybean meal (48% CP)	31.0	35.0	35.0	35.0
Plant protein concentrate*	5.0	-----	-----	-----
Premix (Vit&Min) **	----	0.5	0.5	0.5
Corn oil	1.0	0.8	0.8	0.8
Salt	0.3	0.3	0.3	0.3
Dicalcium phosphate	0.7	0.7	0.7	0.7
Butyric acid	-----	-----	0.3	0.6
Antibiotics	+	-	-	-
Total	100.0	100.0	100.0	100.0
Calculated analyses				
Metabolizable Energy (kcal/kg)	2913.00	2915.00	2917	2919
Crude protein (%)	23.10	23.14	23.10	23.06
Calcium (%)	1.00	1.00	1.0	1.0
Available phosphorus (%)	0.44	0.44	0.44	0.44
Lysine (%)	1.12	1.12	1.12	1.12
Methionine (%)	0.47	0.47	0.47	0.47
Methionine + cystine (%)	0.88	0.88	0.88	0.88
Calorie :protein ratio	126.10	125.90	126.2	126.5

*:Animal protein concentrate(3007-provimi-Jordan),chemical analysis: ME, 2200 Kcal⁻¹kg; crude protein(Min 40%); Fat(Min 8%); Fiber (Min 3%); Ash 2.5%; Calcium 6%; Cl (1.5%); Na(1.5%);Aval.P3%;Lysine3%;Methionine2%;Meth+cyst(2.5%)V.K(5mg⁻¹kg); V.E(500mg⁻¹kg); V.D₃(30000iu); V.A(130000iu); Niacine(400mg⁻¹kg); Pantothenic acid(120mg⁻¹kg); B₂(75mg⁻¹kg); B₁(3mg⁻¹kg); Folic acid(200µg⁻¹kg); Biotin(15mg⁻¹kg); B12(60mg⁻¹kg); V.C(1500 µg⁻¹kg); cholin chloride(5000mg⁻¹kg); Fe(450mg⁻¹kg); cu(70mg⁻¹kg); Zn(600mg⁻¹kg);Mn(600mg⁻¹kg); I(5mg⁻¹kg); Co(1mg⁻¹kg); Se(1mg⁻¹kg); Contain Maduramycin(50mg⁻¹kg); Ethoxyquin, f ree of meat meal or meat & bonemeal; Anticoccidia 10%.

Premix², provided⁻¹kg of diet: **vitamin A 11,000 IU, vitamin D₃ 5,000 IU, vitamin E 75 IU, vitamin K₁ 3 mg, vitamin B₁ 3 mg, vitamin B₂ 8 mg, niacin 60 mg, pantothenic acid 15 mg, pyridoxine 4 mg, folic acid 2 mg, biotin 0.15 mg, choline 1,600 mg, vitamin B₁₂ 0.016 mg, Mn 120 mg, Zn 100 mg, Cu 16 mg, Selenium 0.30 mg, I 1.25 mg, Fe 40 mg.

aproximately 1 g of ileal content per chicken was collected and transferred to 2 ml of distilled water and PH of ileal content was measured using PH meter (Chaveerach et al., 2004).

Table 3: Ingredient composition of finisher diets

Ingredient %	Positive Control	Negative Control	NC+ BA	NC+ BA
	T ₁	T ₂	T ₃	T ₄
Corn	42.5	43.0	42.6	42.4
Wheat	22.0	22.0	22.0	22.0
Soybean meal(48% CP)	26.0	30.0	30.0	30.0
Plant protein concentrate*	5.0	-----	-----	-----
Premix(Vit&Min) **	----	0.5	0.5	0.5
Corn oil	3.5	3.5	3.5	3.5
Salt	0.3	0.3	0.3	0.3
Dicalcium phosphate	0.7	0.7	0.7	0.7
Butyric acid	-----	-----	0.3	0.6
Antibiotics	+	-	-	-
Total	100.0	100.0	100.0	100.0

Calculated analyses				
Metabolizable Energy (kcal/kg)	3110.00	3112.00	3114	3116
Crude protein (%)	20.94	20.98	20.94	20.94
Calcium (%)	1.00	1.00	1.0	1.0
Available phosphorus (%)	0.44	0.44	0.44	0.44
Lysine (%)	1.12	1.12	1.12	1.12
Methionine (%)	0.47	0.47	0.47	0.47
Methionine + cystine (%)	0.88	0.88	0.88	0.88
Calorie :protein ratio	148.50	148.30	148.7	148.8

*: **Animal protein concentrate(3007-provimi-Jordan),chemical analysis:ME,2200**

Kcal⁻¹kg; crude protein(Min 40%); Fat(Min 8%); Fiber (Min 3%); Ash 2.5%; Calcium 6%; Cl (1.5%); Na(1.5%);Aval. P3%;Lysine3%;Methionine2%;Meth+cyst(2.5%)V.K(5mg⁻¹kg) ;V.E(500mg⁻¹kg) ;V.D₃(30000iu) ;V.A(130000iu) ;Niacine(400mg⁻¹kg) ;Pantothenic acid(120mg⁻¹kg) ;B₂(75mg⁻¹kg) ;B₁(3mg⁻¹kg) ;Folic acid(200µg⁻¹kg) ;Biotin(15mg⁻¹kg) ;B12(60mg⁻¹kg) ;V.C(1500 µg⁻¹kg) ;cholin chloride(5000mg⁻¹kg) ;Fe(450mg⁻¹kg) ;cu(70mg⁻¹kg) ;Zn(600mg⁻¹kg);Mn(600mg⁻¹kg) ;I(5mg⁻¹kg) ;Co(1mg⁻¹kg) ;Se(1mg⁻¹kg) ; Contain Maduramycin(50mg⁻¹kg);Ethoxyquin,free of meat meal or meat&bonemeal;Anticoccidia 10%.

Premix, provided⁻¹kg of diet: **vitamin A 11,000 IU, vitamin D₃ 5,000 IU, vitamin E 75 IU, vitamin K₁ 3 mg, vitamin B₁ 3 mg, vitamin B₂ 8 mg, niacin 60 mg, pantothenic acid 15 mg, pyridoxine 4 mg, folic acid 2 mg, biotin 0.15 mg, choline 1,600 mg, vitamin B₁₂ 0.016 mg, Mn 120 mg, Zn 100 mg, Cu 16 mg, Selenium 0.30 mg, I 1.25 mg, Fe 40 mg.

Table 4: Effects of Sodium Butyrate on Performance of Broilers at all periods

Days	(T1) P.C	(T2) N.C	T3 (T2+0.3%BA)	T4 (T2+0.6%BA)	SEM
1-21 d					
Initial weight(g)	43.0	43.0	43.0	43.0	
Final weight(g)*	980.83a	920.67b	952.67ab	972.83b	7.327
ADG (g/birds)	44.43a	41.57b	43.09ab	44.05a	0.349
ADFI (g/birds)	67.08a	62.26b	62.80ab	64.52ab	0.684
FCR (g/g)	1.51	1.50	1.46	1.46	0.016
22-42 d					
Initial weight (g)	980.83a	920.67b	952.67ab	972.83b	7.327
Final weight (g)	2815.96	2782.18	2914.19	2937.12	26.346
ADG (g/birds)	87.39	88.64	93.41	93.54	1.253
ADFI (g/birds)	182.16	179.3	189.65	181.99	2.840
FCR (g/g)	2.08	2.02	2.03	1.95b	0.011
1-42 d					
Initial weight (g)	47.75	47.75	47.75	972.83b	
Final weight (g)	2815.96	2782.18	2914.19	2937.12	26.346
ADG (g/birds)	65.91	65.11	68.25	68.79	0.627
ADFI (g/birds)	124.62	120.78	126.22	123.26	0.012
FCR (g/g)	1.89a	1.86ab	1.85ab	1.79b	1.546

*Means in the same rows with different superscripts were significantly (p<0.05) Different

STATISTICAL ANALYSIS

Statistical analysis were conducted using SAS(Version 6, SAS Institute, Cary, NC, USA) (SAS, 2001). Data collected were subjected to analysis of variance (ANOVA) by means of the General Linear Models (GLM) procedure, based on the Randomized Completely Block. Means were compared using the Duncan’s Multiple Range Test (Dun-

can,1955).

RESULTS

The performance of the birds fed Maduramycin or butyric acid (butyrate) are presented in Table 4. Body weight gain and feed conversion ratio was influenced by dietary treatm-

Table 5: Effects of Sodium Butyrate on pH values

Days	P.C (T1)	N.C(T2)	T3(T2+0.3%BA)	T4(T2+0.6%BA)	SEM
42 d					
Glandular stomach	4.28	4.16	4.19	4.19	
Gizzard	4.46	4.36	4.33	4.36	7.327
Duodenum	5.81	5.74	5.80	5.78	0.349
Jejunum	*6.03a	5.81b	5.94ab	5.96ab	0.684
Ileum	6.04	5.87	5.90	5.92	0.016

*Means in the same rows with different superscripts were significantly ($p < 0.05$) Different

Table 6: Effects of Sodium Butyrate on dressing percentage , carcass weight and edibles for all treatments at 42 day of age

Days	P.C (T1)	N.C(T2)	T3(T2+0.3%BA)	T4(T2+0.6%BA)	SEM
42 d					
Final body weight(g)/bird	2815.96	2782.18	2914.19	2937.12	26.346
Dressing percentage (% LW)	73.0	72.0	75.0	76.0	0.850
Cleaned carcass weight (g) without edibles	2055.65	2003.16	2185.64	2232.21	21.118
Heart weight(g)	9.2	9.0	9.3	9.3	0.765
Liver weight(g)	68.0	65.0	70.0	72.0	0.016
Gizzard weight(g)	58.0	57.0	60.0	60.0	0.012
Abdominal fat(g)	*50.10 a	51.70 a	46.10 b	45.20 b	0.014

*Means in the same rows with different superscripts were significantly ($p < 0.05$) Different

ents during both the starter and finisher period. The body weight of birds in T1,T3 were recorded the highest significant ($P \leq 0.05$) value with average 980.83,952.67 g/bird comparable with the lowest value at T4,T2 with average 972.83,920.67 g/bird at starter period (0-3wk) .The same trends at finisher period (4-6wk). Feed intake were significantly different among treatments ($P \leq 0.05$), T1 recorded the highest value vs T2,T3,T4. At starter and finisher periods respectively, but there was not significant differences in the total periods between all treatments. Feed conversion ratio didn't significant differ at starter period among all treatments, but different significantly ($P \leq 0,05$) at the finisher, total period, T4 recorded the best value versus T1,T2,T3 respectively.

The pH of the upper but not lower gastro-intestinal tract (except jejunum) was influenced by the butyric acid treatment in the present study (Table 5). The pH of crop, proventriculus and gizzard reduced in the entire butyrate treatment groups compared to control and Maduramycin group. Amongst the butyrate groups, pH of proventriculus and gizzard was further reduced in 0.3 compared to 0.6% butyrate. Duodenal pH was comparatively lower in the 0.3 and 0.6% butyrate group compared to control, antibiotic or 0.3% butyrate group. However, no such effect was found in subsequent lower tract. The pH of jejunum and was significantly decreased ($p \leq 0.05$) comparable among all the dietary treatments.

The dressing percentage was influenced by the butyric acid treatments employed in the present study (Table 6). Dressing percentage increased significantly in all the butyrate treatment groups compared to either control or Maduramycin group. The weights of heart, liver, gizzard were not influenced by the dietary treatments, however abdominal fat weight decreased significantly at supplementation of butyric acid. Intestinal morphology traits showed in table 7 that T4,T3 recorded the best significant ($P \leq 0.05$) value in villus height and crypt depth comparable with T1,T2 which free of butyric acid.

DISCUSSION

The results of the present study suggested that organic acid could replace antibiotics in broiler chicken's diet for realizing optimum performance. Butyric acid at 0.3% was not sufficient to maintain the performance. Higher concentration of butyrate i.e. 0.6% in the diet was adequate for optimum body weight gain and feed conversion ratio. Contrary to the findings of the present study, Leeson et al. (2005) and Antongiovanni et al. (2007) suggested a lower level i.e. 0.2% butyrate to maintain performance of broiler chickens. It is noteworthy to mention here that both the above workers used butyrate, which is composed of mono and diglycerides with approximately 75% by weight of butyrate. However, in the present study laboratory grade butyric acid (CHINA Research Laboratory) was used. From

this, it could be inferred that the concentration of butyrate in the diet depends on the form in which it is to be used. In the current study, butyrate up to 0.6% had no adverse effect on feed intake. Similar findings are also available in literature (Antongiovanni et al., 2007). Dibner and Putin (2002) suggested that organic acids improve protein and energy digestibility by reducing microbial competition with the host for nutrients and endogenous nitrogen losses, by lowering the incidence of sub-clinical infections and secretion of immune mediators, by reducing the production of ammonia and other growth depressing microbial metabolites. Probably these could be the reasons that butyrate improved feed utilization leading to better performance in the birds. A few studies are available in literature with respect to the effect of butyrate in broiler chickens (Leeson et al., 2005; Antongiovanni et al., 2007) but none of the study has reported the pH of the individual segments of the GI tract as in the present study. In our study, a reduction in pH of the upper GI tract (crop, proventriculus and gizzard) was observed by inclusion of butyrate in the diets of broilers. 0.3% butyrate was more effective in reducing the pH than 0.6% butyrate. Amongst the lower GI tract, 0.6% butyrate was only effective in lowering the pH in jejunum, but no effect was found in either duodenum or ileum. Bolton and Dewar (1965) indicated that free butyrate absorbed quickly in the upper digestive tract, and while almost 60% of the feed source was intact in the crop, less than 1% is recovered from the upper small intestine. This could be the reason that butyrate was more effective in reducing the pH in the upper GI tract and only in duodenum in lower GI tract. One of the strategies to eliminate the clostridia from the gastrointestinal tract is by maintaining a lower pH, which is unsuitable for the growth of the organism. Kwan and Ricke (2005) showed that amongst the SCFA, butyrate has the highest bactericidal efficacy against the acid-intolerant species such as *E. coli* and *Salmonella*. In the present study dietary inclusion of organic acid such as butyrate reduced the pH of gizzard, proventriculus and small intestine. Thus, it can be suggested that butyrate could replace antibiotic totally in practical broiler diets. In addition to bactericidal activity, butyrate appeared to have a role in development of the intestinal epithelium in this study. Butyrate, irrespective of the concentrations (0.3, 0.6%) in the diet in our study may be improved the villus length and crypt depth in the duodenum. Thus, butyrate supplementation will be much helpful to young birds for intestinal development, especially when there is no protection from antibiotics. Leeson et al. (2005) reported higher crypt depth in duodenum of broiler chicks fed 0.2% butyrate compared to those fed bacitracin in the diet. It could be suggested here that young chicks are therefore the best candidate for diet supplementation of organic acid especially butyric acid because of its both bactericidal and stimulant of villi growth property. Another two important findings of the present study were the improvement in dressing percentage and

reduction in abdominal fat content by supplementation of butyrate to broilers diet. Similarly, Leeson et al. (2005) reported higher carcass yield in broilers fed 0.2% butyrate in the diet. Though no information on literature is available on the role of butyrate on abdominal fat content of broilers, Izat et al. (1990) reported significant reduction in abdominal fat content in male broiler chickens by dietary supplementation of propionic acid. Thus it can be inferred that organic acid supplementation in broiler diet not only maintains performance but also higher carcass yield. In the present study, 0.3% butyric acid was on par with antibiotic in maintaining body weight gain, and found superior for feed conversion ratio. Several additional effects that go beyond those of antibiotics such as stimulating the villi growth of intestine, higher carcass yield and low abdominal fat content were also observed by dietary addition of butyrate. From the findings of the present study, it is concluded that 0.3% butyric acid could totally replace antibiotics in broiler chicken diet.

ACKNOWLEDGEMENTS

We would like to thank The College of Agriculture, Kufa University for giving us this opportunity to express our science.

CONFLICT OF INTEREST

This research is a personal non-profit work and there is no conflict of interest.

AUTHORS CONTRIBUTION

Both of Nihad ali and Saif alkassar are responsible for animal work and samples collection. Ali alkassar is responsible for data analysis, writing correction and proof reading.

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