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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Effect of Sulfanomides Residues on Egg Quality Traits

Huwaida E.E. Malik[1], Julnar E. Omer[1] and Khalid M. Elamin[2]
[1]Department of Poultry Production, Faculty of Animal Production, University of Khartoum, Khartoum, Sudan
[2]Department of Animal Breeding, Faculty of Animal Production, University of Gezira, Gezira, Sudan

Abstract: This study was conducted to detect the effects of Sulfanomide drug on table egg quality and to determine the withdrawal time after which eggs are save for human consumption. Sulfanomide (Coccidiofan) was administrated to 36 layer hens, at a dose of 1g/liter in the drinking water for three 3 consecutive days. Traits studied were egg quality traits and the presence of drug residues. Results showed that treatments were significantly ($P<0.05$) different in Egg shell weight and Eggshell thickness, estimates were 6.61±0.10, 6.61±0.10 and 7.09±0.23 for the first trait and 0.22±0.01, 0.26±0.012 and 0.28±0.012 for the second trait in the control, treated and withdrawal groups respectively. For internal egg traits, yolk height, Yolk width and Yolk index were significantly ($P<0.05$) affected. The control group recorded 16.02±0.37, 34.79±0.28 and 46.19±1.30 for the three traits, treated group estimated 15.64±0.19, 38.11±0.30 and 39.69±0.55 while the group in the withdrawal period recorded 15.10±0.21, 39.69±0.55 and 42.47±0.62 respectively. The percentages of mottled, blood spotted, misshaped and rough shelled eggs were high in the layers in the withdrawal period compared to layers in the control group which reported 0.0% for all traits and layers in treated group which recorded lower percentages than those in the withdrawal period. Results also showed that the drug residues appeared in eggs in second day and third of treatment at 1.80 level and the level increased to 2.22 in the first and second day of the withdrawal period then decreased at increasing rate to reach 0.22 on the fifth day of the withdrawal period. So it is better to avoid eggs consumption during treatment and withdrawal periods.

Key words: Blood spots, furazolidone, shell, yolk mottling

INTRODUCTION

Poultry egg emerged as a good substitute of animal protein source but Due to lack of bio-security measures, prevalence of infectious diseases and subsequently indiscriminate use of drugs without observing withdrawal period has made the poultry products (meat and eggs) unsafe for human health. In developed countries, federal agencies ensure the supply of residues free and wholesome products for human consumption (Cochrane *et al*., 1995). The presence of drug residues in meat and eggs above MRL (maximum residues limit) is injurious to human health and considered illegal (Booth, 1988).

Sulfanomides, a class of antimicrobial drugs, are widely used for therapeutic and prophylactic purposes in both human beings (Kim and Park, 1998) and animals (Schwarz and Dancla, 2001). Sometimes the drug is used as additives in animal feed because prolonged ingestion of Sulfanomides may have a growth-promoting effect (Long *et al*., 1990). At present, sulfanomides and other drugs (chlortetracycline, penicillin and several ionophores) are the most common contaminants human feed of animal origin, generating potentially serious problems in human health, such as allergic or toxic reactions. Furthermore, the main risk from the excessive use of antimicrobials in animals is that bacteria may develop resistance (Salem, 1998). In addition, some sulfanomides have been found to be potentially carcinogenic (Nue, 1992) and this fact has become a cause for considerable debate in food safety. It has been estimated that approximately five percent of human patients medicated with sulfanomides received unwanted effects from the drugs (Bevill, 1984). As a result, the presence of Sulfanomide residues in food is considered to be harmful to consumers.

Sulfanomides in poultry are widely used for the treatment of many Infectious diseases (Giguere *et al*., 2006). As a result, there is concern that the residues may be retained in the meat (broiler) and in the eggs (layers) and present a potential risk to human health (Sutiak *et al*., 2000). These drugs or their metabolites left over in the body after their administration for long time are termed as residues (Booth, 1973). After the treatment of infected hen with drugs, the residues of drugs are present at some level in edible products like eggs and meat of treated hens (Booth, 1973).

Drug residues concentrations vary considerably from tissue to tissue and are generally observed at higher levels in tissues of storage such as body fat or in organs that actively metabolize and excrete them. Therefore, residues may be highest in liver and kidneys (Booth, 1973).

Corresponding Author: Khalid Mohammed Elamin, Department of Animal Breeding, Faculty of Animal Production, University of Gezira, Gezira, Sudan

In order to decrease the potential risk to the consumer's health and to ensure that eggs contain no residues, these substances must be administered only in recommended concentrations and their respective withdrawal times must be observed (Kozarova *et al*., 2004). There are various methods for the detection of drug residues and each test format has its own attribute. At present, high-performance liquid chromatography (Agarwal, 1992), gas chromatography (Tarbin *et al*., 1999), liquid chromatography-mass spectrometry (LC-MS), thin-layer chromatography (Reimer and Suarez, 1991) are the common analytical methods used for the detection of Sulfanomide residues in poultry meat and eggs. However, High Performance Liquid Chromatography (HPLC) has become the most widely adopted confirmatory technique for the determination of Sulfanomide residues in foods of animal origin. (Agarwal, 1992). Sulfanomide residues decreased egg production and egg shell thickness but increased the incidence of misshapen eggs and eggs with blood spots (Kan *et al*., 2000 and Jacob *et al*., 2000).

The objective of this study is to investigate the Effect of sulfanomides residues on table egg quality and to assess the residual level of sulfanomides in poultry table egg.

MATERIALS AND METHODS

Study location: This experiment was conducted in Hillat Kuku, Khartoum north, Animal Production Research Centre where birds were housed and data collected. Drug residues in the eggs were determined in Faculty of Agriculture, University of Khartoum.

Experimental hens and housing: A total of 36 imported leghorn layers at 51 weeks of age were used. The layers were reared in a naturally ventilated, open-sided, deep litter poultry house, with a concrete floor and corrugated sheet roof. The house extended east-west and constructed from 0.5 meter bricks walls, iron posts and wire netting. The layer hens were accommodated in battery cages.

Experimental diets: The experimental diet was formulated according to National Research Council (1994) shown in Table 1.

Experimental procedures: The experimental birds were treated orally with Sulfanomide (Coccidiofan) at a dose of 1g/ litre in the drinking water. This drug is composed of 150mg of sulfaquinoxaline, 70mg of sulfamethazine sodium, 70mg of sulfadiazine sodium, 2mg of vitamin K_3 and 8000 I.U of vitamin A. The drug was administered for three consecutive days. After 10 withdrawal period vitamin K_3 (1g/10 L) was given for three days to stop blood spots occurrence.

The study was conducted at two phases. In the first phase eggs were collected from the flock and subjected for physical examination to evaluate quality traits. In the second phase, five eggs were taken to determine the drug residues level and this was done in the microbiology lab, faculty of Agriculture, University of Khartoum. The data were collected three times (one day before treatment, three days during treatment and five days during withdrawal period) to detect Sulfanomide residues levels. The sulfanomides residues were detected after 7, 8 t and 11th days withdrawal period to insured that eggs were free of Sulfanomide drug and safe for human consumption.

Table 1: The calculation of experimental diet

Ingredient	%
Sorghum	64
Ground nut cake	21
Wheat bran	2.20
Super Concentrate	2.20
Lime	9
DCP	0.75
Salt	0.10
Methionine	0.10
Lysine	0.15
Choline chloride	0.10
Premix	0.20
Anti toxins	0.10
Biotronik	0.10

Table 2: Chemical analysis of the experimental diet

Ingredient	%
Dry matter	93
Crude protein	20.32
Crude fiber	8.8
Ether extract	6.2
Ash	6.67
Nitrogen Free Extraction (NFE)	51.01
Calcium	3.67
Av. Phosphorus	0.34

Equipments used in the experiment: The equipment used in this study sensitive balance, vernia, micrometer and yolk colour fan to estimate egg quality traits, Petri dish, ampoules, nutrient agar media and *Bacillus subtilis* were used to determine the drug residues.

Measurement of external egg quality: The fresh eggs were randomly collected from cages and immediately sent to the laboratory in the Department of Poultry Production. All samples were weighed using digital sensitive balance with accuracy (0.001g). Egg Length (EL) and Egg Width (EW) were measured by a pair of vernier callipers calibrated (precision 0.1). Obtained values of the EL and EW were used to determine the shape index. Each egg was later carefully broken for determination of egg shell weight (gm). Then thickness of each shell was measured using a micrometer with an accuracy of 0.2 mm. For the accuracy of shell thickness measurement egg shell cleaned and left over night to dry then egg shell was measured at the broad end, middle part and narrow end of the shell. Then the average shell thickness was recorded in (mm).

Measurement of internal egg quality: The yolk and albumen was carefully separated and weighed by sensitive balance to determine their respective weights (gm). The albumen height, yolk height and yolk width were determined using vernier calliper.

The Haugh unit was calculated based on the records of albumen height and egg weight (Nesheim *et al*., 1979) using the formula:

$$HU = 100 * Log [H - (1.7W^{0.37}) + 7.6]$$

Where:
HU = Haugh unit
H = Albumen height (mm)
W = Egg weight in grams

Level of yolk colour was determined using Roche yolk colour fan which comprise a range of graded yolk colours from one to fifteen degree.

Antimicrobial residues detection: The residues of sulfanomide in egg was detected by microbial method using agar well diffusion method. In this method, the nutrient agar with four wells was prepared and B. subtilis was uniformly streaked. Then 0.1ml of the egg content was added in the wells and incubated at 37°C for 24 h. After the incubation period, the cultures were examined and the diameters of the inhibition zones were measured with slipping calipers in the positive samples (Franek *et al*., 1999).

Experimental design and statistical analysis: The statistical analysis for the recorded data for both egg quality and the level of Sulfanomide residues was carried out using one-way analysis of variance (one-way ANOVA) according to Statistical package for Social Sciences (SPSS version 9). Means were compared using Duncans multiple range tests. Chi-squared tests was used to evaluate the level of yolk color using (SPSS version 9). The values were expressed as means and standard Error. The means were considered significantly different at P<0.05.

RESULTS AND DISCUSSION

Table 3 shows the estimated result of Egg weight. The estimated result was 51.04. The result reflected no significant (p<0.05) differences in the egg weight among different treatments. Egg weight in this study is in accordance to Nakano *et al*. (1998) and Yasmeen *et al*. (2008) but lower than the results estimated by Daneshyar *et al*. (2007).

The estimation of egg length was found to be 55 mm and egg width was 41 mm was presented in Table 3, The result demonstrated no significant (P<0.05) differences in the egg length and egg width among different treatments.

The estimation of shape index was found to be ranged between 74-75 percent was presented in Table 3. The result showed no significant (P<0.05) differences in the shape index among different treatments.

The estimation of egg shell weight was found to be in the range of 7.61-7.09 (Table 3). The result showed significant (P>0.05) differences in the egg shell weight among different treatments. The less measures were recorded during the withdrawal period. This result in consistent with that obtained by Jacob *et al*. (2000) and Beyer (2005) where sulfanomide drug increased the egg shell thinning. This situation could be justified by impact of anemia leading to decrease level of oxygen in the blood stream which effected the synthesis of calcium carbonate in a rate sufficient 94-97% (Burley and Vadehra, 1989).

Table 3 shows the estimation of egg shell thickness. The recorded figures ranged between 0.28-0.22 mm. The result reflected significant (P>0.05) differences in the egg shell thickness among different treatments. The lowest estimates were recorded during the withdrawal period. This result in consistence with that obtained by (Jacob *et al*., 2000 and Beyer, 2005) who reported that sulfanomide drug decreased egg shell thickness. This situation could be justified by the fact that Sulfanomide inhabit folic acid synthesis (Botsoglou and Fletouris, 2001), leading to anaemia (Brown, 1962), anaemia in turn cause decreased the level of oxygen in the blood stream which hindering the calcium carbonate synthesis in a sufficient rate 94-97% (Burley and vadehra, 1989).

Table 4 shows that the estimation of albumen weight ranged from 28.29-30.21 gm, albumen height ranged

Table 3: Means and standard errors of external egg quality traits

Treatment→	Control	Treated	Withdrawal period
Parameter↓			
EW (g)	51.04±0.87	51.50±0.62	51.49±0.53
EL (mm)	55.02±0.48	55.31±0.37	55.77±0.26
EW (mm)	41.26±0.27	41.6 2±0.16	41.38±0.26
SI	74.74±0.59	75.38±0.48	74.27±0.54
ESW (g)	7.09±0.23^{a}	6.61±0.10^{ab}	6.61±0.10^{b}
EST (mm)	0.28±0.012^{a}	0.26±0.012^{ab}	0.22±0.01^{b}

^{a,b,c}Means values within the same row with different superscripts letter are significantly (P<0.05) different. EW: Egg weight, EL: Egg length, EW: Wgg width, SI: Shape index, ESW: Egg shell weight, EST: Egg shell thickness

Table 4: Means and standard errors of internal egg quality traits

Treatment→	Control	Treated	Withdrawal period
Parameter↓			
Albumen weight	30.21±1.14	28.29±0.43	29.61±0.59
Albumen height	6.02±0.35	6.60±0.24	6.46±0.15
Hough unit	91.81±1.87	94.58±1.18	94.20±0.75
Yolk weight	14.60±0.54	14.38±0.19	14.37±0.14
Yolk height	16.02±0.37^{a}	15.64±0.19^{ab}	15.10±0.21^{b}
Yolk width	34.79±0.28^{c}	38.11±0.30^{a}	36.29±0.23^{b}
Yolk index	46.19±1.30^{a}	39.69±0.55^{c}	42.47±0.62^{b}

^{a,b,c}means values within the same row with different superscripts letter are significantly (P<0.05) different

Table 5: The percentages of mottling, blood spotting misshaped egg and rough shell texture

Treatment	Day	Mottling (%)	Blood spotting (%)	Misshaped egg (%)	Roughly shell texture (%)
Control	1st	0	0	0	0
Treatment period	2nd	23.53	5.88	0	5.88
	3rd	16.67	5.56	0	16.67
	4th	0	7.14	0	0
Withdrawal period	5th	16.67	16.67	0	16.67
	6th	17.65	0	0	17.65
	7th	36.36	18.18	18.18	18.18
	8th	80	70	20	20
	9th	27.78	16.67	16.67	5.56
	10th	10	5	10	5
	11th	8	8	4	8
	12th	90	0	15	15
	13th	47.83	8.70	8.70	8.70
	14th	28	4	4	12
	15th	52.17	0	13.04	8.70
	16th	15	0	5	0
	17th	0	0	4	0
	18th	0	0	0	0

from 6.02-6.60 mm and Haugh unit, 91.81-94.48 percent. The result indicated no significant ($P<0.05$) differences among the different treatments. Haugh unit estimates were higher than the estimates reported by Yasmeen *et al.* (2008) and Kucukersan *et al.* (2005). Albumen weight reported in this study is lower than result reported by Wolanski *et al.* (2007). Albumen height is lower than the estimate reported by Yasmeen *et al.* (2008).

The estimation of yolk weight was found to be in the range of 14.37 to 14.61 gm and this result agreed with Yasmeen *et al.* (2008) but lower than the result depicted by Wolanski *et al.* (2007). The result indicated no significant ($P<0.05$) differences among the different treatments. The estimations of yolk height was found to be ranged from 15.10-16.02 mm, yolk width was found to be ranged from 34.79-38.11 mm and yolk index was found to be ranged from 39.69 to 46.19 percent and this study revealed significant differences ($P>0.05$) between the treatment. However, obtained results for yolk index were lower than those reported by Yasmeen *et al.* (2008). This situation could be attributed to the fact that, when hen is treated with Sulfanomide may suffer from a decreasing carbon dioxide (CO_2) level in the egg and if carbon dioxide (CO_2) level decreased in the eggs the egg content become more alkaline, causing more watery albumin (Jones, 2006), resulting in occurrence of absorbability of the liquid from albumen to the yolk during egg formation in the 18th h in uterus and gives the yolk a flattened shape (Jacob *et al*., 2000).

Incidence of yolk mottling is shown in Table 4. Mottled eggs appeared on the second day then increased and disappeared completely on the 15th day. Mottled yolks was suggested to be due to decreased the levels of oxygen and carbon dioxide (CO_2) (Jones, 2006). Those lead to the egg interior become more alkaline as a result the albumen is watery so more fluids migrate to the yolk (Jones, 2006).

The incidence of blood spots in different days was presented in Table 5. Bloody eggs appeared on the 2nd day reached the peak in the 8th day then decreased and disappeared in the eggs of the 15th day of the experiment. This result agreed with Jacob *et al.* (2000). Extravasation of blood in the ovarian region may be due to the secretion of Sulfaquinoxilate which cause haemolytic anaemia (Jacob *et al*., 2000). This might be caused by decreased amount of folic acid. Brown (1962) reported that folic acid deficiency is not recognized until anti-microbial agents such as Sulfanomide were employed. As a result macro-cytic anaemia appear which lead to occurrence of blood spotting (Hunnekens, 1968). On the other hand, Bains (1999); (Frazier *et al*., 1995); Botsoglou and Fletouris (2001) suggested that this phenomenon could be due vitamin k_3 deficiency caused Sulfanomide therapy. The occurrence of blood spots leads to low egg grading as the consumer dislike theses eggs thinking of fertile eggs.

Table 4 show the percentages of the misshaped and rough shelled egg occurrence. Both traits increased due to drug administration. Sulfanomide inhabit folic acid bio- synthesis (Botsoglou and Fletouris, 2001), leading to anemia (Brown, 1962), followed by low oxygen level in the blood stream which affect the calcium carbonate synthesis. This is in line with that of (Beyer, 2005).

Table 6 showed that Sulfanomide residues appeared in eggs the first day post treatment. This result agreed with that obtained by Romvary and Simon (1992). Kan and Petz (2000) suggested that two days are required to achieve constant level of the drug in the plasma and hence in the albumin. The highest level of Sulfanomide

Table 6: Sulfanomide residues during the treatment and withdrawal periods

Level of Sulfanomide residues Mean±SE	Treatment
1.80±0.04[bc]	2nd day treated
1.80±0.05[bc]	3rd day treated
2.02±0.06[b]	1st day withdrawal period
2.40±0.16[a]	2nd day withdrawal period
1.66±0.04[c]	3rd withdrawal period
0.28±0.12[d]	4th withdrawal period
0.22±0.09[d]	5th withdrawal period

Mean±SE= the mean±standard error.
[a,b,c,d]Means values in with the same column with different superscripts letter are significantly (P<0.05) different

residues detected in the 2nd day of the withdrawal period. This result is in accordance of that reported by Pensabene *et al.* (1998). Sulfanomide residues completely disappeared after the 11th day withdrawal period.

Conclusion: Sulfanomide drug affected egg external and internal quality by decreasing egg shell weight, egg shell thickness, yolk height, yolk width and yolk index. So it is advisable to avoid eggs selling and consumption during treatment and withdrawal periods. The presence of Sulfanomide residues necessitate the application of bio-security measures at poultry farms level.

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