

## Echocardiographic Assessment of Prescribed Nanosilver on Cardiac Structure and Function Indices in Broilers

<sup>1</sup>Ezatollah Fathi, <sup>2</sup>Mehrdad Yadegari, <sup>1</sup>Majid Gholami-Ahangaran and <sup>3</sup>Esmail Hosseini

<sup>1</sup>Department of Poultry Diseases, Veterinary Medicine Faculty, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

<sup>2</sup>Department of Radiology, Veterinary Medicine Faculty, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

<sup>3</sup>Language Faculty, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

**Abstract:** Nanocid is a nanosilver base disinfectant commonly used in poultry production. Excessive consumption of this disinfectant can risk poultry health. The present study embarked on examining the effect of the disinfectant nanosilver (nanocid) on some functional and structural indices of the heart using echocardiography on 270 Ross broiler chickens. In this study the internal diameter of the left ventricle at the end of systole (LVIDs) and diastole (LVIDd), the left ventricle fractional shortening (LVFS), the ejection fraction (EF), the stroke volume (SV) and the inter-ventricular septum at the end of systole (IVSs) and diastole (IVSd) were evaluated. nanosilver was added to the consumption water for 6 days in 5 different dose. Chicken echocardiograms were taken using a 10 MHz linear probe in dorsal recumbent position. In chickens received 7 ppmnanosilver, the internal diameter of the left ventricle at the end of systole and diastole and the stroke volume had the highest measure. The fractional shortening, ejection fraction and inter-ventricular septum at the end of diastole had the lowest measures among the groups. The present study showed that nanosilver consumption leads to a change in cardiovascular function and forming different degree of ascites in broiler chickens.

**Key words:** Echocardiographic • Cardiac Structure • Nanosilver • Heart Indices • Broiler Chickens

### INTRODUCTION

Echocardiography is a nonaggressive technique utilized to assess the functional and structural features of the heart and since it is regarded as a noninvasive and hazardless imaging approach, it can in many cases substitute other imaging methods [1, 2].

Various modes of echocardiography (one-dimensional, two-dimensional and Doppler) have thus far been applied in different animal species to determine the heart anatomy, to appraise the functional structural characteristics of the heart and to measure the heart cavities and blood flow patterns [3, 4]. The first application of echocardiography in birds took place by Einzing *et al.* [5] who succeeded in exhibiting the capabilities of this technique in non-aggressive observations of cardiovascular functioning in different

bird species. In recent years, there has been a growing concern over the widespread utilization of nano-products in poultry industries and the threats it might pose to the environment and health. Moreover, attention has been paid to the far-reaching use of nano elements in manufacturing various disinfectants and in the control and prevention of bird infectious diseases. Nanosilver is one type of the nano particles widely exploited in recent times for its antibacterial effects [6]. It has been argued, however, that overusing nanocids with differing densities leads to side effects involving various organs of the body.

Nanocids impact mechanism may appear in four ways: 1) It can generate oxygen-free radicals by silver, which may augment the microorganism cell wall destruction [6] 2) It can destroy the microorganism cell walls [7]. 3) It may degenerate cell walls and convert the

wall sulfa bonds to silver sulfides in the microorganism [7]. 4) It may destroy the microorganism cell walls due to the elevation of silver positive charge in nano-scale in bond with microorganisms carrying negative charges [6]. This may, in turn, result in the annihilation of the breathing enzymes and the death of the microorganism [6, 7]. In poultry industry the nanosilver is extensively used as an antimicrobial drug or a disinfectant substance. Recently, there have been some reports that have stated the health hazards of nanoparticles in humans and animals. In this study, the effect of different doses of nanocid disinfectants on the functional and structural indices of the heart was addressed by employing echocardiography technique in Ross broilers.

## MATERIALS AND METHODS

**Experimental Design:** This study was done in the chicken house of Islamic Azad University-Shahrekord Branch, Iran in 2011. For achieving the intended purposes of this study, 270 Ross 308 broiler chickens in 6 groups and 3 replicates were selected and randomly assigned into groups. All groups, in the entire course of the study, were under accurate scrutiny to be in identical conditions as meticulously as possible in terms of drinking water, light, food, ventilation and vaccination. The prescribed nanocid (Nano-nasb com., Iran) amount was supplied through the drinking system and prepared daily in different groups as follows: the first group, serving as the control group, took no amount of nanocid. The second group took a 1 ppm dose. The third one had a 2 ppm dose. The fourth one was administered a 3 ppm, the fifth one with a 5ppm and the sixth one with a 7 ppm dose. All the chickens intake the same amount of water. All the broilers in the various groups were exposed to the determined doses from the 30<sup>th</sup> day after birth.

**Nanocid:** The consumed nanosilver compound was ordered by Iran Nasb Pars nano company under the commercial name of Nanocid manufactured in Behbahan Shimi company in Iran. This product contains 4000 ppm nanoparticles.

**Echocardiography:** After a 6-day period of nanosilver consumption, 3 broilers from every replicate were randomly selected, weighed, registered by their general and clinical characteristics and eventually echocardiographed. The echocardiography device used in the study was Dupler EX 8000 Madison model made in South Korea.

To perform the echocardiography, the feathers in and around the chest area (the exact place of the echocardiography probe) were gently and cleanly removed and attempt was made to lower the stress conditions of the whole atmosphere as much as possible. For this study the 10MHZ linear probe was employed. The probe was placed in the right parasternal region across from the Stifle joint.

In the present study, by exploiting echocardiography in the bright and movement modes, the internal diameter of the left ventricle at the end of systole and diastole, the fractional shortening of the left ventricle, the ejection fraction and the stroke volume, the inter-ventricular septum at the end of systole and diastole and pericardial effusion were evaluated and measured.

To measure the fractional shortening of the left ventricle, the attained numbers associated with the left ventricle at the time of systole and diastole can be put in the formula  $FS = \frac{LVIDd - LVIDs}{LVIDd} * 100$  [7, 8]. The outcome figure illustrates the left ventricle fractional shortening percentage. This is considered as the most outstanding parameter of the left ventricle functioning that is used to estimate the myocardium contraction pressure.

**Statistical Analysis:** The ultimately obtained data were analyzed by means of Analysis of Variance test (ANOVA), using Scheffe test for quantitative data and Chi-square test for qualitative data using SAS software (SAS Institute, 2001).

## RESULTS

The comparison of heart indices showed increasing of nanosilver concentration can change heart function higher than low doses. Chickens that were fed high doses of nanosilver showed ascites in clinical examination (Table 1). Results indicates that the mean of left ventricle internal diameter in group 6 (that took 7 PPM nanocid) are the highest and the internal diameter of the left ventricle at the end of diastole in group 6 has a meaningful significance in comparison to groups 3 and 4 ( $p < 0.05$ ).

The attained results indicated that the left ventricle fractional shortening in group 6 seemed to be significantly different from those in groups 1, 2 and 3 ( $p < 0.05$ ). In the next step the inter-ventricular septum at the end of systole and diastole was measured and the statistical analysis seemed to determine that the inter-ventricular septum at the end of diastole in group 6 gained the lowest amount and manifested a significant difference compared to groups 1, 4 and 5 at the significance level of  $P < 0.05$ .

Table 1: The mean of the measured heart indices in echocardiography

Indices Group	LVFS (%) (Mean±SD)	EF(%) (Mean±SD)	SV(cm) (Mean±SD)	IVSs(cm) (Mean±SD)	IVSd(cm) (Mean±SD)	LVIDs(cm) (Mean±SD)	LVIDd(cm) (Mean±SD)
1	47.82±0.92a	83.77±0.98ab	2.19±0.11a	0.42±0.02ab	0.34±0.02b	0.56±0.02ab	1.08±0.03ab
2	51.29±1.30a	85.36±1.62b	2.02±0.15ab	0.43±0.03ab	0.32±0.01ab	0.51±0.02b	1.04±0.02ab
3	50.77±3.57a	85.48±2.41b	1.88±0.09ab	0.43±0.01b	0.32±0.01ab	0.50±0.03b	1.02±0.01b
4	47.70±0.52ab	83.05±0.54ab	1.80±0.16b	0.40±0.03ab	0.36±0.01b	0.53±0.01b	1.01±0.02b
5	46.42±5.75ab	81.61±5.15ab	1.84±0.07b	0.36±0.04a	0.33±0.03b	0.55±0.06ab	1.03±0.02ab
6	42.32±1.82b	79.40±3.95a	2.09±0.30ab	0.38±0.01ab	0.29±0.01a	0.62±0.05a	1.09±0.06a

Data presented as Mean±SD, The different superscript shown significant differences between groups (P<0.05). (LVIDd: Left ventricular diameter diastole, LVIDs: Left ventricular diameter systole, IVSd: Inter ventricular septum diast, IVSs: Inter ventricular septum systole, SV: stroke volume, EF: ejection fraction, LVF Left ventricular fraction shortening)

This parameter at the end of systole, however, manifested no significant difference among the groups. Eventually, the stroke volume and fractional shortening as the other two critical parameters for cardiovascular disorder diagnosis were measured [9, 10]. The statistical analysis of the results evidenced that in terms of stroke volume group 1 demonstrated a significant difference in comparison to groups 4 and 5 (p<0.05). Likewise, ejection fraction in group 6 appeared to be meaningfully different from groups 2 and 3 (p<0.05) (Table 1).

### DISCUSSION

In examining the impact of nanocid disinfectants on some cardiovascular parameters, in this study, the effective noninvasive technique of echocardiography was exploited. Analysis of the results seemed to substantiate a tangible parameter variation in group 6, which consumed the highest nanocid doses among the groups. Therefore, one can safely infer that the diminution of heart functioning in group 6 in association with its fractional shortening percentage decrease as an important parameter in the left ventricle functioning as well as its direct influence on the heart contraction force has been illustrated in this study.

Ascites was a clinical examination in some experimental chickens. Therefore, in this study, heart function indices such as LVIDd, LVIDs, IVSd, IVSs and SV were investigated. Previously, Deng *et al.* [11] in a study in China utilized echocardiography in two modes of bright (B mode) and movement (M mode) to inspect the ascitic broilers' heart functioning. They found that RVDd and RVDs in ascitic broilers in all age ranges were higher and that the left ventricular shortening after normalization of ascetic chickens aged 4, 5 and 6 weeks was lower. As a whole, the results indicated heart disfunctioning in both ventricles, especially the right one [11].

In one research, in echocardiography procedure, illustrated that measurements of identical variables between broiler chickens and Laghoren chickens manifested that broilers had smaller hearts structurally and functionally in comparison to Laghoren chickens [9]. Shahabzadeh *et al.* [12] scrutinized the effect of nanosilver on Rainbow Trout survival percentage. They found lethal concentrations of 2.3, 3 and 3.5 mg.lit 48, 72 and 96 hours after the experiment, respectively. Which in turn showed the average quantity of nanosilver lethality in Rainbow Trout [10].

Akrami *et al.* [13] studied Nanosilver effect on Bursa fabrisius. They showed that nanosilver can induce edema on bursa in broiler chickens.

In 2009 Muhling embarked on a research on nanosilver effects in order to study the antibiotic resistance and exhibited that silver ion antimicrobial activity seemed to be due to the connection of silver cations to electron giver groups, the generation of free radicals from the silver particle surfaces and eventually the flaw in bacteria functioning [14].

Nano products and upshots seem to be dominating the disinfectant industry that in turn has a growing importance in the poultry production line. With due consideration to the already unknown high dosage effects of such disinfectant products and the resistance emanated from them, the present study focused its attention on the possible effects of various doses of nanosilver disinfectants (nanocids) on the cardiovascular system of broiler chickens, which is considered by experts as the most vigorously energetic organ in their body.

In this research, significant variation in the most critical functional parameters of the broilers' cardiovascular system in the experimental group with the highest consumption dose evidenced the diminution of heart functioning in the whole course of the experiment, the predisposition of the birds to cardiovascular disorders and the increase in the group fatality rate.

Moreover, the decline of Fractional Shortening (FS), the most important left-ventricular functional parameter, occurred in the group which consumed the highest nanocid dose, illustrating the myocardial power drop that leads to cardiovascular ailments and ascites syndrome.

Likewise, the increase in the left-ventricular internal diameter at the end of systole and diastole and the significant difference of this parameter in group 6 in comparison to groups 2, 3 and 4 is highly likely to be indicative of the devastating impact of the administered dose on the bird's hearts.

The group 6 ejection fraction reached the lowest value of all groups and portrayed a significant difference from that of group 2, 3 and 4 ( $p < 0.05$ ).

The inter-ventricular septum thickness at the end of diastole in group 6 attained the lowest amount of all groups which enables one to observe the statistically significant distinction between this group and groups 1, 4 and 5 ( $p < 0.05$ ).

The stroke volume approached its highest value in group 1, elucidating that this group gained a statistically significant difference from groups 4 and 5.

Pericardial Effusion that has an effective qualitative role in the diagnosis of heart maladies has also been taken into consideration. Although the results in this respect showed an increase in the amount of Pericardial Effusion with the increase in the nanocid dosages of the groups, they did not point to a significant statistical difference among the groups.

Some qualitative parameters such as pericardial effusion associated with group 6 achieved its highest value. It seems that congestion and vascular congestion provokes the heart to enlarge in size to compensate for its inefficiency. Considering the important role of the left ventricle in the heart total activity, this can justify the left-ventricular internal diameter increase in group 6. Until now, there have been no scientific research about the effect of different doses of nanosilver on the heart functioning and structure.

In conclusion, recent study revealed that Nanosilver impact on the heart muscle can cause diminished Fractional shortening as a myocardial contraction power index. The present study showed that Nanosilver consumption in high dose leads to a change in cardiovascular function and forming different degree of Ascites in broiler chickens.

## REFERENCES

1. Devereux, R.B., D.R. Alonso, E.M. Lutas, G.L. Gottlieb, E. Campo, I. Sachs and N. Reichek, 1986. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Animal Journal of Cardiology*, 57: 450-458.
2. Devereux, R.B. and N. Reichek, 1977. Echocardiographic determination of left ventricular mass in man: anatomic validation of the method. *Circulation*, 55: 613-618.
3. Kitabatake, A., M. Inoue, M. Asao, T. Masuyama and J. Tanouchi, 1983. Noninvasive evaluation of pulmonary hypertension by a pulsed doppler technique. *Circulation*, 68: 302-309.
4. Masuyama, T., K. Kodama, A. Kitabatake, H. Sato, S. Nanto and M. Inoue, 1986. Continuous-wave doppler echocardiographic detection of pulmonary regurgitation and its application to noninvasive estimation of pulmonary artery pressure. *Circulation*, 74: 484-492.
5. Einzing, S., N.A. Staley, E. Mettler, D.M. Nicoloff and G.R. Noren, 1980. Regional myocardial blood flow and cardiac function in naturally occurring congestive cardiomyopathy of turkeys. *Cardiovascular Research*, 14: 396-407.
6. Balwin, C.I. and D.A. Denham, 2008. Isolation and characterization of the three subpopulation of IgG. *Poultry Immunology*, 81: 157-160.
7. Ban, P.W., Y. Anurag-Mittal and W. Cao-Starr, 2008. Practical nanodrug is daescoles agromomias. *Veterinary Gona*, 18: 98-104.
8. Nyland, T.G. and J.S. Mattoon, 2002. *Small Animal Diagnostic Ultrasound*. 2<sup>nd</sup> Edn., Elsevier Health Sciences, Philadelphia, ISBN: 0721677886, pp: 461.
9. Martinez-Lemus, L.A., M.W. Miller, J.S. Jeffrey and T.W. Odom, 1998. Echocardiographic evaluation of cardiac structure and function in Broiler and leghorn chickens. *Poultry Science*, 77: 1045-1050.
10. Nautrup, C.P. and R. Tobias, 2000. *Dignostic Ultrasonography of the Dog and Cat*. 2<sup>nd</sup> Edn., Manson publishing Ltd., London, ISBN: 1874545103, pp: 400.
11. Deng, G., Y. Zhang, X. Peng, D. Guo and C. Li, 2006. Echocardiographic characteristics of chickens with ascites syndrome. *British Poultry Science Journal*, 47: 756-762.

12. Shahbazzadeh, D., H. Ahar, N.M. Rahimi, F. Dastmalchi, M. Soltani *et al.*, 2009. The effect of Nanosilver (Nanosid) on survival percentage of Rainbow Trout (*Oncorhynchus mykiss*). Pakistan Journal of Nutrition, 8: 1178-1179.
13. Akrami, M.M.A., A.N.S. Jeedi and F. Ahmadi, 2005. Evaluation of the effectiveness of different levels of nanosilver on bursa of fabricius development and on its histopathological lesions in broiler chickens. Poultry Science, 11: 718-723.
14. Muhling, M., A. Bradford, J.W. Readman, P.J. Somerfield and R.D. Handy, 2009. An investigation into the effects of silver nanoparticles on antibiotic resistance of naturally occurring bacteria in an estuarine sediment. Environmental Research, 68: 278-283.