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Research Paper

Confirmation of Antimicrobial Resistance by Using Resistance Genes of Isolated *Salmonella* spp. in Chicken Houses of North West, South Africa.

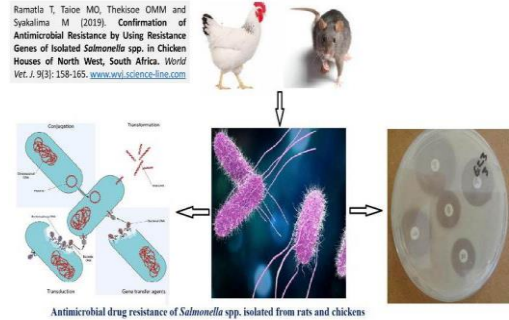
Ramatla T, Taioe MO, Thekiso OMM and Syakalima M.

World Vet. J. 9(3): 158-165, 2019; pii:S232245681900020-9
DOI: <https://dx.doi.org/10.36380/scil.2019.vwj20>

ABSTRACT: The widespread use of antibiotics for treatment of bacterial infections and growth promotion in the poultry industry has effectively increased antibiotic resistance around the world. Antibiotics resistance can be caused by different mechanisms and can be determined through phenotypic and molecular methods. The aim of the present study was to determine the occurrence of antibiotic resistance in *Salmonella* serovars isolated from layer chickens and rats in poultry houses. Phenotypic testing of antimicrobial resistance was performed using the Kirby-Bauer disc diffusion method. Furthermore, molecular evaluations and PCR assay were conducted for detecting resistance genes and class 1 integrons. A total of 144 *Salmonella* isolates (68 from rats and 46 from chickens) serovars were assessed. Evaluation of phenotypic resistance patterns demonstrated that *Salmonella* isolates have the highest antibiotic resistance for rifampicin (100%) followed by tetracycline (68%), ciprofloxacin (48%), sulphonamides (42%), chloramphenicol (39%), nalidixic acid (33%), ampicillin (28%), cephalothin (18%), streptomycin (18%), amoxicillin-clavulanic acid (6%), enrofloxacin (5%), and gentamicin (4%). Some *Salmonella* serovars revealed multi-drug resistance for up to four different antibiotics. According to PCR results, all the tested resistant gene markers (*tet*, *cat*, *bla*TEM, *sul*, *qnrA*, and *aadA*) were detected from the *Salmonella* isolates. The study further confirmed that 68% of *Salmonella* isolates were harboring class 1 integrons and the majority of the isolates (n=52) which were harboring these genes were recovered from the rats. The results of the present study provided that the *Salmonella* spp. isolated from chickens and rats in poultry houses, exhibited significant antibiotic resistance. Moreover, the current research ultimately highlights the importance of rats as carriers of antibiotic-resistant bacteria and the risk of transmission to chickens and humans.

Key words: Antibiotic resistance pattern, Class 1 integrons, Resistance genes, *Salmonella* serovars

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Research Paper

Antibacterial Effect of Aloe Vera Gel Extract on *Escherichia coli* and *Salmonella enterica* Isolated from the Gastrointestinal Tract of Guinea Fowls.

Adzitey F, Agbolosu AA and Udoka UJ.

World Vet. J. 9(3): 166-173, 2019; pii:S232245681900021-9
DOI: <https://dx.doi.org/10.36380/scil.2019.vwj21>

ABSTRACT: *Aloe vera* has a long history as a medicinal plant with diverse therapeutic applications. This study was conducted to assess the antibacterial effect of *Aloe vera* gel extract against *Escherichia coli* and *Salmonella enterica* isolated from the gastrointestinal tract (GIT) of guinea fowls. The conventional method was used for the isolation of *Escherichia coli* and *Salmonella enterica*. The antibacterial activity of *Aloe vera* gel extracts (50, 100 and 200 mg/ml) and standard antibiotics were evaluated using the disk diffusion method. The prevalence of *Escherichia coli* in the GIT of the guinea fowls was 100% (15/15). All the *Escherichia coli* were susceptible to ciprofloxacin. At 48h and 72h of incubation, all the *Escherichia coli* were susceptible to gentamicin but not at 24h. Inhibition zones using the *Aloe vera* gel extract ranged from 7.87-12.23mm (50 mg/ml), 8.53-17.23mm (100 mg/ml) and 7.43-10.67mm (200 mg/ml) for *Escherichia coli*. Also, antibacterial test for *Escherichia coli* using the *Aloe vera* gel extract revealed an inhibition zone of 9.10-12.23mm for *Escherichia coli* isolate GIT1, 7.8-8.57mm for *Escherichia coli* isolate GIT2 and 7.43-17.23mm for *Escherichia coli* isolate GIT7. The prevalence of *Salmonella enterica* in the GIT of the guinea fowls was 40% (6/15). All *Salmonella enterica* were susceptible to suphamethoxazole/trimethoprim and tetracycline but not at 24h. Inhibition zones using *Aloe vera* gel extract ranged from 7.13-12.57mm (50 mg/ml), 4.2-6.7mm (100 mg/ml) and 0-9.23mm (200 mg/ml). Furthermore, antibacterial test for *Salmonella enterica* using the *Aloe vera* gel extract revealed an inhibition zone of 5.3-12.57mm for *Salmonella enterica* isolate GIT9, 0-7.8mm for *Salmonella enterica* isolate GIT10 and 4.2-9.0mm for *Salmonella enterica* isolate GIT15. The study revealed that *Aloe vera* gel extract possessed antibacterial properties. Therefore, it can be added to the feed of guinea fowls as a prophylactic to reduce bacterial infections.

Key words: *Aloe vera*, Antibiotics, *Escherichia coli*, Gut, *Salmonella enterica*



Adzitey F, Agbolosu AA and Udoka UJ (2019). Antibacterial Effect of Aloe Vera Gel Extract on *Escherichia coli* and *Salmonella enterica* Isolated from the Gastrointestinal Tract of Guinea Fowls. *World Vet. J.* 9(3): 166-173. www.wjv.science-line.com

Review

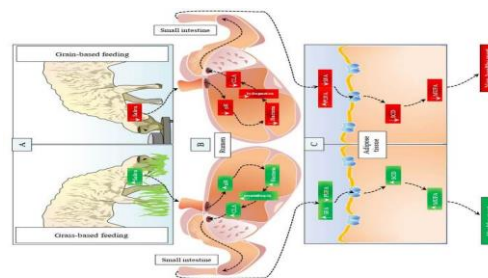
The Effects of Grass-Based versus Grain-Based Feeding of Ruminants on the Human Hygienic Status, a Review.

Al-Thuwaini TM and Al-Shuhaib MBS.

World Vet. J. 9(3): 174-180, 2019; pii:S232245681900022-9
DOI: <https://dx.doi.org/10.36380/scil.2019.wvj22>

ABSTRACT: Ruminant meat quality is one of the important factors contributing to the recent spreading of several diseases, such as obesity, cancer, and cardiovascular problems, which have increased predominately. Feeding regiment plays an important role in the determination of the composition of fatty acids and meat quality in ruminants. This review aims to highlight the main factors that lie behind the variability of ruminant meat quality and its effect on human being's health. The reduction in grass-feeding decreases saliva levels in the ruminants, which has several consequences on the rumen, including a reduction in pH level, along with a reduction in the microorganism activities and conjugated linoleic acid levels. In adipose tissues, the expression of the stearyl-CoA desaturases gene is negatively affected by the decreased conjugated linoleic acid levels in the rumen, which leads to a decreased transformation of saturated fatty acids to monounsaturated fatty acids. Therefore, the lower monounsaturated fatty acids and the parallel increase in the proportion of saturated fatty acids in the consumed meat can be associated with some human diseases. Thus, the present study provided a molecular explanation for the superiority of grass-based feeding in ruminants raised at pasture in term of production of meat with a healthier quality for consumers than those raised on grains.

Key words: Grain; Grass, Human disease, Ruminant meat, SCD enzyme



Al-Thuwaini TM and Al-Shuhaib MBS (2019). The Effects of Grass-Based versus Grain-Based Feeding of Ruminants on the Human Hygienic Status, a Review. *World Vet. J.* 9(3): 174-180. www.wvj.science-line.com

Research Paper

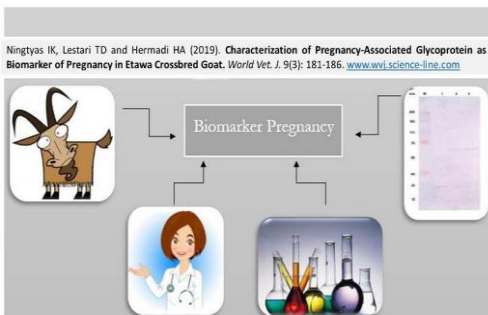
Characterization of Pregnancy-Associated Glycoprotein as a Biomarker of Pregnancy in Etawa Crossbred Goat.

Ningtyas IK, Lestari TD and Hermadi HA.

World Vet. J. 9(3): 181-186, 2019; pii:S232245681900023-9
DOI: <https://dx.doi.org/10.36380/scil.2019.wvj23>

ABSTRACT: Pregnancy-Associated Glycoprotein (PAG) is secreted by the placenta, which is produced in mononucleate and binucleate trophoblast cells. The current research was conducted to find out a substance for diagnosing early pregnancy in Etawa crossbred goats. Six Etawa crossbred goats (not pregnant, three months pregnant and four months pregnant) were subjected in the present study from Livestock Government Institution Breeding in Singosari, Malang. The research methods consisted of sample collection, identification PAG with sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the determination of concentration with Biuret method and specificity test with Western Blot assay. The obtained results showed that the molecular weight of PAG from pregnant Etawa crossbred goats was 55.85 kDa. The average concentrations of PAG in the goats of non-pregnant, three months pregnant, and four months pregnant were 1.83 ± 2.98 , 3.79 ± 2.72 and 4.36 ± 2.63 , respectively. The results of the specificity test with the Western Blot molecular revealed a molecular mass of PAG was 55 kDa. The findings of the present study demonstrated PAG in Etawa crossbred goats can be used as an indicator of pregnancy.

Key words: Biomarkers, Etawa crossbred, PAG, Pregnancy



Ningtyas IK, Lestari TD and Hermadi HA (2019). Characterization of Pregnancy-Associated Glycoprotein as a Biomarker of Pregnancy in Etawa Crossbred Goat. *World Vet. J.* 9(3): 181-186. www.wvj.science-line.com

Research Paper

Incretin Mimetics Vildagliptin and Exenatide Improve Pedicle Skin Flap Survival in Rats.

Danilenko LM, Tarasova AP, Pokrovskiy MV, Trunov KS, Stepenko YV, Artyushkova EB and Gudyrev OS.

World Vet. J. 9(3): 187-191, 2019; pii:S232245681900024-9
DOI: <https://dx.doi.org/10.36380/scil.2019.wvj24>

ABSTRACT: Hypoxia and tissue ischemia are the leading factors in the alteration of tissues in many pathological conditions. Prevention and reversion of the effects of local ischemia, which develops during various surgical interventions, is an actual problem of modern medicine. The aim of the present study was to investigate the effect of exenatide and vildagliptin on the survival rate of an isolated pedicle skin flap in sixty adults Wistar rats. Simulation of a pedicle skin graft was performed on the second day of the experiment. After anesthesia under aseptic conditions, a skin graft was cut out:



isolated in a plastic bag, the edges of the skin were stitched with interrupted sutures (nylon 3/0). Rats were divided into six groups: control group, exenatide group (10 µg/kg/day subcutaneously for nine days after surgery), vildagliptin group (0.2 mg/kg/day intraperitoneally for nine days after surgery) and pentoxifylline group (100 mg/kg/day intravenously, two hours before the surgical intervention). In the other two groups, glibenclamide (5 mg/kg) were administered before injection of incretin mimetics. On the third, seventh and tenth day, area of the surviving tissue was measured. Subsequently, the survival rate of the skin graft was calculated. The area of the surviving tissue in exenatide and vildagliptin group was 1.5 and 1.7 times more compared to the control group, respectively. Preliminary blockade of ATP-dependent potassium channels by glibenclamide eliminated the protective effect of exenatide and vildagliptin. The increase in the survival of ischemic tissues using exenatide and vildagliptin has been experimentally proved. The current study confirmed the important role of ATP-dependent potassium channels in dermatoprotective properties of incretin mimetics.

Key words: Dermatoprotective properties, Exenatide, Ischemia, Pedicle skin graft, Vildagliptin.

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Research Paper

Use of Untreated and Autoclave-Treated Wheat Germ Meal in Growing Rabbit Diets.

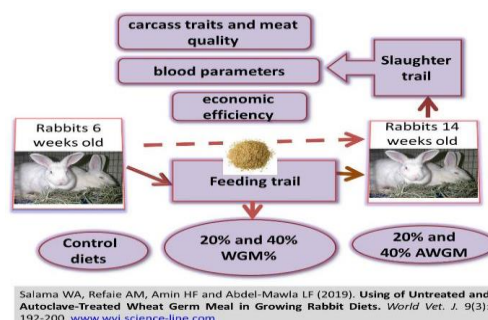
Salama WA, Refaie AM, Amin HF and Abdel-Mawla LF.

World Vet. J. 9(3): 192-200, 2019; pii:S232245681900025-9

DOI: <https://dx.doi.org/10.36380/scil.2019.wvj25>

ABSTRACT: The present study was intended to investigate the influence of using 20% and 40% treated or untreated wheat germ meal in growing New Zealand rabbit diets. A total of 75 weaned New Zealand White rabbits aged six weeks old, with an average initial weight of 659.60±18.84g were divided into five groups with five replicates in each group (three rabbits per replicate). The first group was fed on a basal diet (T₁), second and third groups received diets containing Wheat Germ Meal (WGM), as replacement of soybean meal protein, at levels of 20% and 40% and were labeled as T₂, T₃, respectively. Fourth and fifth groups were fed with 20% and 40% autoclave-treated autoclaved WGM (T₄ and T₅, respectively). The trial was continued until 14 weeks of age. The present study was evaluated growth performance, blood parameters, carcass traits, meat quality in different groups and also economic efficiency was calculated. There were insignificant differences in terms of live weight, daily weight gain, carcass weight and dressing percentages among rabbits in groups of T₁, T₂, and T₄. Rabbits in the group of T₄ achieved the best feed conversion ratio. Digestion coefficients of crude protein, crude fiber, ether extract, nitrogen-free extract, and nutritive value in terms of digestible crude protein, total digestible nutrition, and digestible energy did not significantly differ between T₁ and T₄. However, these factors significantly decreased in T₃ and T₅ compared to T₁. Plasma total protein and globulin significantly increased in rabbits of T₂ and T₄ compared to those fed in T₁ group. A significant decrease in total cholesterol and total lipid for rabbits in groups of T₄, T₅, and T₂ was observed. Moreover, rabbits fed on T₄ or T₂ diets had the highest economic efficiency. Conclusively, the untreated or autoclaved WGM can be used in growing rabbit diets up to 20% for replacing the soybean meal protein, which caused low feed costs without adverse effects on the growth performance of rabbits.

Key words: Rabbits, Soybean meal, Wheat germ meal



Salama WA, Refaie AM, Amin HF and Abdel-Mawla LF (2019). Use of Untreated and Autoclave-Treated Wheat Germ Meal in Growing Rabbit Diets. *World Vet. J.* 9(3): 192-200. www.wvj.science-line.com

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Research Paper

Productive Characteristics and Reproductive Responses to Estrus Synchronization and Flushing in Abou-Delik Ewes Grazing in Arid Rangelands in Halaieb - Shalateen - Abouramad Triangle of Egypt.

Farrag B.

World Vet. J. 9(3): 201-210, 2019; pii:S232245681900026-9

DOI: <https://dx.doi.org/10.36380/scil.2019.wvj26>

ABSTRACT: There are a few reports about the reproductive aspects or uses of both of flushing and estrus synchronization in Abou-Delik ewes grazing in the South Eastern zone of Egypt. Thirty-three Abou-Delik ewes were allocated to three experimental groups (n = 11 in each) to study the effects of estrus synchronization and flushing on reproductive responses and productive characteristics under arid conditions of South Eastern zone of Egypt. Group one served as control represent the system dominant in the area (without estrus synchronization and flushing ration). Ewes in group two were estrus synchronized with two doses of PGF_{2α}, 10 days apart without flushing ration. Ewes in group three were estrus synchronized just like the second group and received 300g of barley grain/head/day as flushing meal for three weeks before the start of breeding season. All ewes were grazed *Panicum turgidum* (natural vegetation dominant in the area) for eight hours daily. Results showed that, the percentage of estrus exhibition in group three reached 100%, while the lowest percentage was observed in group one (81.82 %). Estrus activity signs in synchronized groups, occurred in 70 and 81.81% during the first 48 h after the second dose of PGF_{2α}, for groups two and three respectively, compared to control group (22.22 %). The onset of estrus was earlier in synchronized groups than control group. Duration of estrus did not differ significantly. Estrus intensity in group three was higher (P < 0.05) compared to the other groups. Conception and lambing rates were 100% in group three. Third group showed the highest insignificant litter size that was 18% higher than the other groups.



The overall mean of birth weight, weaning weight and average daily gain of Abou-Delik lambs were 2.91, 16.89 and 0.116 kg, respectively. There is no significantly effect on concentrations of plasma progesterone among groups. While there were significant differences between sampling periods. In conclusion, under grazing on arid rangelands conditions in the South Eastern zone of Egypt, using flushing and/or estrus synchronization can be useful to improve reproductive and productive characteristics of Abou-Delik sheep.

Key words: Abou-Delik sheep, Estrus synchronization, Flushing, Productive performance, Rangelands, Reproduction

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Research Paper

Histopathological Alterations of Ceca in Broiler Chickens (*Gallus gallus*) Exposed to Chronic Heat Stress.

Adji AV, Plumeriastuti H, Ma'ruf A and Legowo D.

World Vet. J. 9(3): 211-217, 2019; pii:S232245681900027-9
DOI: <https://dx.doi.org/10.36380/scil.2019.wvj27>

ABSTRACT: Heat stress has been found to cause adverse effects on small intestinal microstructure, but little is known about its impact on chicken's cecum. In this research, the histopathological alterations of broiler chicken's cecum following chronic heat stress were evaluated. 20 broiler chickens were randomly divided into control group and treatment group containing 10 replicates, respectively. Both groups were reared under standard conditions until 21 days of age. From day 22 to day 42, the control group was kept at 24-28°C as well as relative humidity of 40-55%, while the treatment group was exposed to high temperature of 36-40°C and relative humidity of 45-65% for eight hours per day. At the end of the period, proximal part of each chicken's cecum was collected and made into histopathological slides with Hematoxylin and Eosin staining. Villus height, villus width, crypt depth, villus surface area, and villus height to crypt depth ratio were examined from 10 villi per replicate. Results analysis revealed that chronic heat stress profoundly ($P < 0.05$) reduced the crypt depth. Insignificant ($P > 0.05$) changes of the villus despite the long-term heat exposure might imply that the damage is at its early phase. In conclusion, chronic heat stress can produce morphological alterations in the ceca of broiler chickens, though requiring longer duration due to cecum's durability.

Key words: Broiler chicken, Cecum, Heat stress, Intestinal morphology



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Research Paper

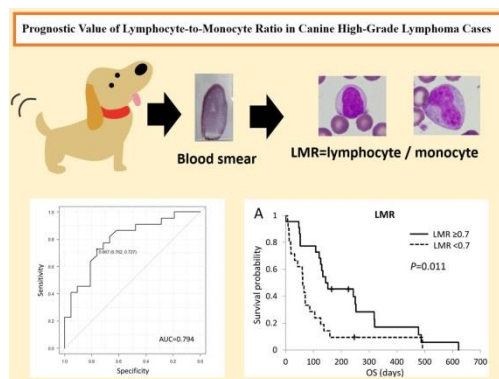
Prognostic Value of Lymphocyte-to-Monocyte Ratio in Canine High-Grade Lymphoma Cases.

Tagawa M, Shimbo G, Matsumoto K and Miyahara K.

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ABSTRACT: The Lymphocyte-to-Monocyte Ratio (LMR) has been described as a useful prognostic marker for human patients with various cancers and dogs with diffuse large B-cell lymphoma. The objective of this study was to determine whether the LMR could predict disease outcome as measured by the Time To Progression (TTP) and Overall Survival (OS) of dogs with different types of high-grade lymphoma. The medical records of 43 dogs diagnosed with high-grade lymphoma at the Veterinary Medical Center of Obihiro University of Agriculture and Veterinary Medicine between 2013 and 2018, were retrospectively analyzed. Receiver Operating Characteristic (ROC) curve analysis was used to determine the optimal LMR cutoff values. The prognostic influence of the LMR and other clinicopathological data on TTP and OS was studied by Kaplan-Meier curves. To identify the independent prognostic factors, univariate and multivariate Cox proportional analyses were used. The optimal cutoff value of the LMR was 0.7, which corresponded to the maximum sensitivity (0.727) and specificity (0.762) of the LMR for predicting the median days of OS with ROC analysis (area under the curve, 0.794). Log-rank tests showed that dogs with a high LMR had significantly longer TTP and OS than dogs with a low LMR. Moreover, immunophenotype, body weight, treatment regimen and response to treatment were significantly associated with TTP and OS. In multivariate analysis, treatment and response to treatment were independent risk factors for TTP. Moreover, the LMR, treatment regimen and response to treatment were independent predictors of OS.

Key words: Dog, Lymphocyte to monocyte ratio, Lymphoma, Prognosis



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Research Paper

Computed Tomography Scan and Polyester resin 40 Plastination Technique: Teaching Aids to Illustrate Anatomical Structure of Donkey Brain.

Mohamed ShKhA, El-Behery EI and Mahdy EAA.

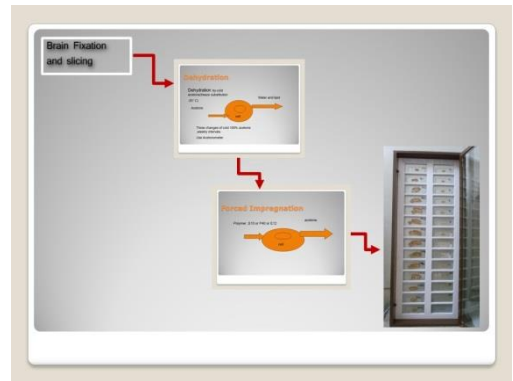
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ABSTRACT: The present study investigated the collaboration between the plastinated sagittal sections and computed tomography (CT) images of the donkey brain. Four adult healthy donkeys of both sexes from native breeds were analyzed. The animals were sedated with a combination of xylazine and ketamine. The donkeys were positioned in sternal recumbency during CT brain examination and contiguous sagittal 3 mm thick slices of the head were obtained. Polyester resin 40 (P40) technique for the brains were done through five main steps including fixation, slicing, dehydration, forced impregnation and light-curing of brain glass chambers by UVA light source. Furthermore, a survey was conducted using questionnaires involving second-year veterinary medical students of anatomy department, Zagazig University to compare the educational capacity of CT brain images and P40 plastination of brain slices. 52% of students voted that using of brain sheeted slices are much better, 38% prefer to learn both parallel to each other. The obtained results revealed that both the plastinated brain sections and the CT images portrayed a combination of many complex neuroanatomical structures that provide an excellent aid for researchers in educational and diagnostic purposes. In addition, the plastinated brain serves as a good tool for the interpretation of CT images.

Key words: Brain, CT, Donkey, Plastination, Polyester resin 40

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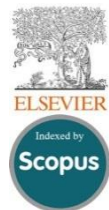
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Histopathological Alterations of Ceca in Broiler Chickens (*Gallus gallus*) Exposed to Chronic Heat Stress

Antonia Vania Adji¹, Hani Plumeriastuti², Anwar Ma'ruf^{3*} and Djoko Legowo²

¹Faculty of Veterinary Medicine, Airlangga University, Surabaya 60115, Indonesia

²Department of Veterinary Pathology, Faculty of Veterinary Medicine, Airlangga University, Surabaya 60115, Indonesia

³Department of Basic Veterinary Medicine Science, Faculty of Veterinary Medicine, Airlangga University, Surabaya 60115, Indonesia

*Corresponding author's E-mail: anwarunair@gmail.com; ORCID: 0000-0001-7158-9156

ABSTRACT

Heat stress has been found to cause adverse effects on small intestinal microstructure, but little is known about its impact on chicken's cecum. In this research, the histopathological alterations of broiler chicken's cecum following chronic heat stress were evaluated. 20 broiler chickens were randomly divided into control group and treatment group containing 10 replicates, respectively. Both groups were reared under standard conditions until 21 days of age. From day 22 to day 42, the control group was kept at 24-28°C as well as relative humidity of 40-55%, while the treatment group was exposed to high temperature of 36-40°C and relative humidity of 45-65% for eight hours per day. At the end of the period, proximal part of each chicken's cecum was collected and made into histopathological slides with Hematoxylin and Eosin staining. Villus height, villus width, crypt depth, villus surface area, and villus height to crypt depth ratio were examined from 10 villi per replicate. Results analysis revealed that chronic heat stress profoundly ($P < 0.05$) reduced the crypt depth. Insignificant ($P > 0.05$) changes of the villus despite the long-term heat exposure might imply that the damage is at its early phase. In conclusion, chronic heat stress can produce morphological alterations in the ceca of broiler chickens, though requiring longer duration due to cecum's durability.

Key words: Broiler chicken, Cecum, Heat stress, Intestinal morphology

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INTRODUCTION

Heat Stress (HS) is defined as biological response to high ambient temperature which disrupts the heat exchange equilibrium (Lara and Rostagno, 2013). Over the past decades, the Earth's surface temperature has gone beyond the former baseline, going as far as nominating 2016 as the warmest year since the recording was initiated in 1880. Consequently, the risk of HS' emergence in livestock projects to a higher level (Thornton et al., 2009). Broiler chickens are particularly more prone to contract heat stress because their cardiovascular and respiratory systems, which are vital for heat loss mechanisms, are unable to cope with their heavier body weight and high metabolism rate resulting from genetic selection (Sozcu, 2019).

HS has generally been found to be detrimental to small intestine's morphology in broiler chickens (Al-Fataftah and Abdelqader, 2014; Yi et al., 2016). The underlying mechanisms involve hypothalamic-pituitary-adrenal (HPA) axis activation, ischemia, oxidative stress, or perturbation of intestinal microbiota (Bolek, 2013; Song et al., 2014; Yi et al., 2016). The activated HPA axis subsequently elevates corticosterone level which in turn undermines immunity (Mishra and Jha, 2019). It has also been discovered that HS can suppress the number of goblet cells and the mRNA expression of tight junctions and adherence junctions in broiler chickens subjected to 33°C, 10 hours/day for 21 consecutive days (Zhang et al., 2017). These occurrences along with the impaired immune system facilitate the penetration of both toxins and pathogenic bacteria into the circulation afflicting various organs. Meanwhile, cecum is a part of chicken's intestine with the most abundant and diverse microorganisms colonizing its lumen (Ijaz et al., 2018). Furthermore, cecum regularly receives the backflow of urine rendering potentially more severe damage by the pathogens trespassing (Kum et al., 2015).

The available research on the influence of HS on cecal morphology produced conflicting results. Reduction in villus length and crypt size in the ceca of Japanese quails (*Coturnix coturnix japonica*) exposed to chronic HS was demonstrated by El-Daly et al. (2014). In contrast, negative results were obtained in Jaafar (2013)'s research. Other experiments, for the most part, are concerned with the microbiome in cecum, for instance HS was proven to be able to restrain the growth of normal flora colonies, like *Lactobacillus* sp., while enhancing the colonies of *Escherichia* sp., *Salmonella* sp., and aerobic bacteria in the cecum of broiler chickens (Park et al., 2013).

It was proposed that HS influenced the intestine variably in compliance with susceptibility level of each segment and the duration of HS occurrence (acute or chronic) (Loyau et al., 2015; Varasteh et al., 2015). This was borne out by analogous findings in Cherry-Valley ducks exhibiting no substantial crypt depth discrepancy in the cecum despite pronounced changes in the jejunum and ileum (He et al., 2019). Taking into account the lack of consistent and adequate number of results, the current research had an objective to evaluate the impact of chronic HS for 21 days on the microstructure of broiler chicken's cecum.

MATERIALS AND METHODS

Ethical approval

This experiment was performed on the basis of approval by the laboratory animals use research ethics committee of faculty of veterinary medicine, Airlangga University, Indonesia.

Experimental animals

In this research, 20 one day old Cobb broiler chicks were housed at the cage of laboratory animals, Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia. The chicks marketed as Wonchick originated from Wonokoyo Jaya Corporindo Ltd. (Limited Company, Surabaya, Indonesia). The rearing temperature followed the guidelines provided by the referred company, namely the leaflet containing Wonchick broiler chicken rearing guide. The chicks were allowed *ad libitum* access to feed and water. This went on up to 21 days.

Experimental design

The chickens were randomly allocated to two groups (n= 10, respectively), the control (C) group and the treatment (T) group, from day 22 onwards. This starting point was chosen because broilers in the later stage of life have greater sensitivity to high surrounding temperature than the younger birds (He et al., 2018). In that, true experimental design, specifically the post-test-only control group design, was applied. The C group was maintained on thermoneutral conditions (24-28°C and relative humidity (RH) 40-55%), while the T group was exposed to high ambient temperature and humidity (36-40°C and RH 45-65%) for eight hours/d then returned to 24-28°C and RH 40-55% for the rest of the day. On day 42, all the chickens were humanely slaughtered as affirmed by the research ethics committee and organ collection was carried out.

Cecal histopathology

The proximal part of cecum was dissected from 23.59% - 23.65% proximal of its length for all chickens. About one cm of the segment was fixed in neutral buffered formalin 10% and then routinely processed into slides with Hematoxylin and Eosin (H&E) stain. The sections were analyzed under light microscope with computer-assisted digital image analyzer (NIS Elements) for Villus Height (VH), Villus Width (VW), and Crypt Depth (CD) (Figure 1). Measurements of VH, VW, and CD were based on the methods used by Abdelqader and Al-Fataftah (2016); Godwin et al. (2016) and Shokryazdan et al. (2017). The Villus Surface Area (VSA) was calculated from the formula $= \pi \times VW \times VH$. The Villus Height to Crypt Depth (VH:CD) ratio is a comparison of VH to CD (Santos et al., 2015). Collection of all the variables was done on 10 intact and the longest villi with their associated crypts in one cecal cross section per chicken. These values were averaged to obtain mean value of each variable for each chicken.

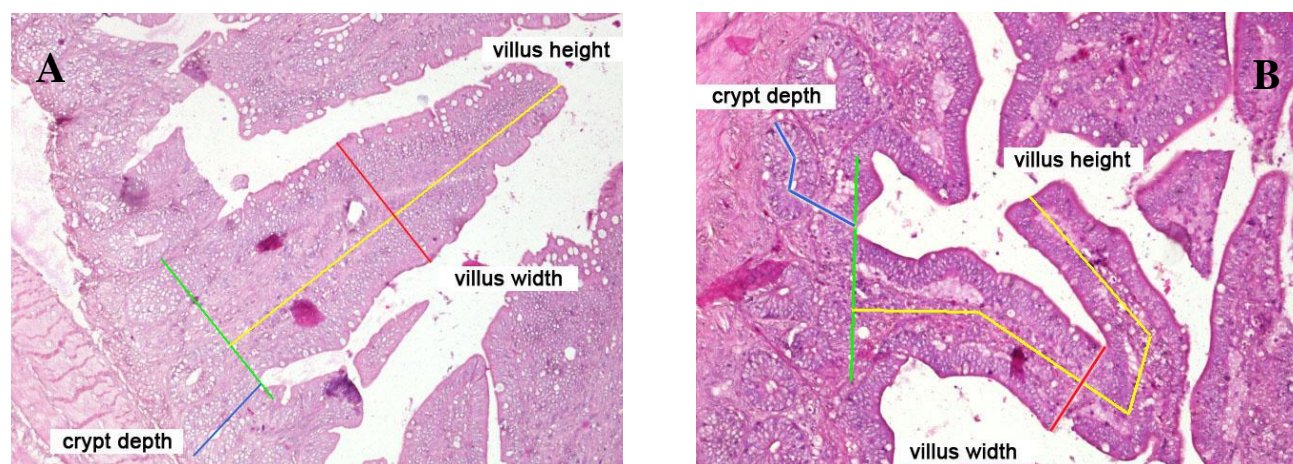


Figure 1. Methodology for morphometric assessment of villus-crypt unit of cecum in broiler chickens, **A:** in straight unit and **B:** in irregularly-oriented unit (H&E, 200×), Villus height (yellow line) was measured from the tip of the villi to the villus-crypt junction (green line), Villus width (red line) was measured from side to side of the villi at its half height, Crypt depth (blue line) measurement was based on the depth of invagination between adjacent villi.

Statistical analysis

Data analysis used the two-tailed T Test for independent samples, and $p < 0.05$ was the accepted significant value. It was conducted with SPSS (Version, 24) for Windows OS (SPSS, Chicago, IL, USA). The results are presented in mean \pm standard error.

RESULTS AND DISCUSSION

From the five dependent variables examined, profound decline of CD ($p < 0.05$) was observed in the chickens of T group compared to C group (Figure 2 and figure 3). The remaining variables did not express significant difference between the two groups ($p > 0.05$) (Table 1).

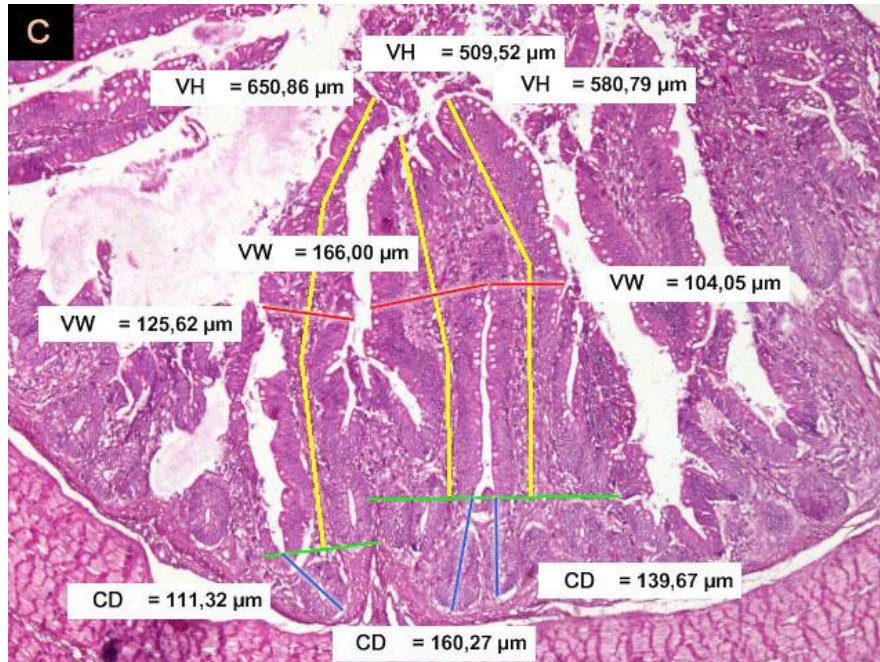


Figure 2. Cecal morphology measurements of broiler chickens of control group from the study in Surabaya, Indonesia between August and September 2017. The yellow lines, red lines, and blue lines represent villus height, villus width, and crypt depth respectively (H&E, $\times 100$). VH: villus height; VW: villus width; CD: crypt depth

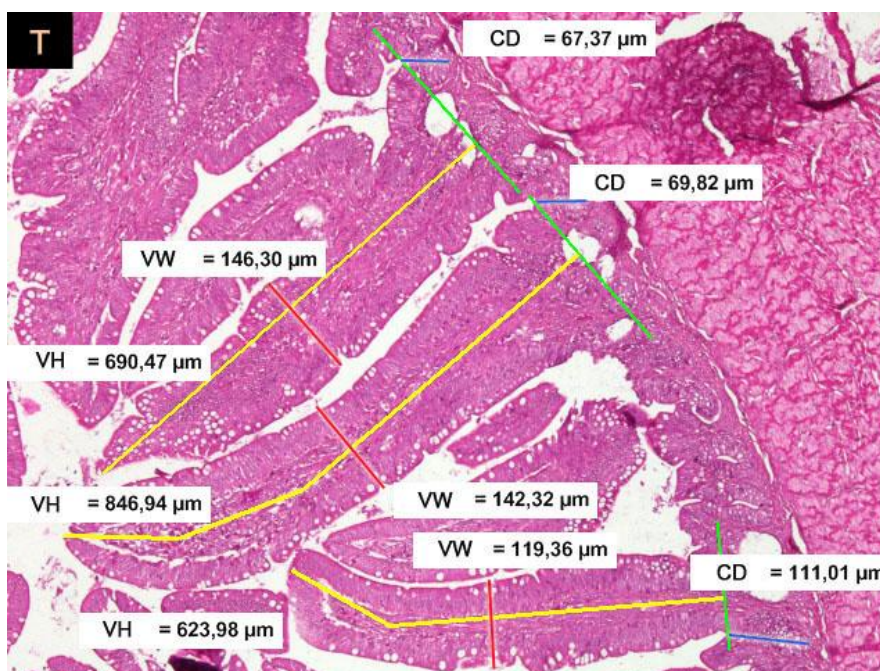


Figure 3. Cecal morphology measurements of broiler chickens from treatment group which were exposed to high ambient temperature and humidity from 22 to 42 days of age during the study in Surabaya, Indonesia between August and September 2017. The yellow lines, red lines, and blue lines represent villus height, villus width, and crypt depth respectively (H&E, $\times 100$). VH: villus height; VW: villus width; CD: crypt depth.

Table 1. The effect of heat stress on histopathology of ceca in broiler chickens in Surabaya, Indonesia between August and September 2017

Variable	Group C	Group T
VH (μm)	518.66 \pm 31.44	493.22 \pm 45.01
VW (μm)	115.23 \pm 2.86	122.92 \pm 5.25
CD (μm)	116.67 \pm 4.65 ^a	98.84 \pm 5.83 ^b
VSA (μm^2)	189,980.84 \pm 14,538.90	195,195.07 \pm 22,117.80
VH:CD	4.75 \pm 0.28	5.38 \pm 0.56

C: control; T: chickens were exposed to high ambient temperature and humidity from 22 to 42 days of age; VH: villus height; VW: villus width; CD: crypt depth; VSA: villus surface area; Values are provided with mean \pm standard error; ^{a,b} Different superscripts within the same row mark significantly different means ($p < 0.05$).

HS is able to delay epithelial turnover by means of decreasing feed intake as a result of HPA axis activation (Hu and Guo, 2008). Elevated plasma corticosterone in broiler chickens is indirectly held responsible for the decrease in VH and CD in duodenum and jejunum, but this only occurs in the acute phase instead of chronic (Aggarwal and Upadhyay, 2013). Another proposed mechanism is ischemia in the event of blood diversion to heat-releasing organs, such as the turbinates and the respiratory muscles (Song et al., 2014). Ischemia will lead to vacuolation, rapid sloughing, and loss of microvilli in the intestinal epithelium (Al-Fataftah and Abdelqader, 2014). Moreover, ischemia is also reported to cause oxidative and nitrosative stress which implicated damaged cell membrane, disrupted cellular ion homeostasis, opened tight junctions, and reduced integrity of intestinal mucosa (Al-Fataftah and Abdelqader, 2014). This may proceed to shorter villi but deeper crypts to compensate (Song et al., 2014). The injured mucosa also disables the attachment of commensal bacteria. In addition, the expression of Heat Shock Protein (HSP) may act as receptor for pathogens, while lack of goblet cells which equals falling mucin production promotes the adhesion of pathogens to mucosa (Burkholder et al., 2008; Shah et al., 2019).

The VH, VW, VSA, and VH:CD ratio in present research were barely affected by the heat exposure (36-40°C and RH 45-65%) for eight hours/day along 21 days. Similar findings were attributed by Quinteiro-Filho et al. (2010) to the rapid periodical re-epithelization of intestinal mucosa in the absence of structural adaptation. This explanation is however inappropriate for this case given that it normally occurs in acute or shorter period of cyclic chronic HS, i.e. (seven days). Chronic HS for over one week has been recorded to consistently diminish VH in the absence of adaptation or compensation, although temperature exceeding 36 \pm 1°C could accelerate this process into less than a week (Santos et al., 2015; Abdelqader and Al-Fataftah, 2016; Yi et al., 2016). Meanwhile, CD showed variable responses to HS (decreased, increased, or no difference) regardless of the HS duration. This denoted that there are different sequences of alterations, and their progression is dependent on the level of temperature and internal variation of the organisms subjected to HS. Within the earlier period of HS when the CD of cecum and small intestine have recovered, their VH often did not reach to a similar state, in other words changes in villi are initiated by the crypts (Deng et al., 2012; Abdelqader and Al-Fataftah, 2016).

Crypt of Lieberkühn, being the location of stem cells, is the center for proliferation of intestinal epithelium. The newly generated enterocytes then migrate up and are ejected at the villus tip. Because crypts merely grow in length, the activity level of crypts is proportional to CD (Cummins et al., 2011). Significantly ($p < 0.05$) shallower CD in the T group thus indicated less proliferation rate. The crypts due to proliferative property, were the first part of intestine observed to suffer from stress as remarked by Burkholder et al. (2008). By combining this fact with current results, it can be interpreted that the cecum of chickens in T group is on early phase of damage despite the prolonged HS. The possibility that injury on the cecum of T group was a start can also explain the insignificant ($p > 0.05$) alterations of other variables besides CD. This idea is reinforced by the inference that colon (including cecum) is one of the segments more resistant to HS characterized by lower expression of HSP70 and HSP90 mRNA (Varasteh et al., 2015). If the order of segment susceptibility is to be associated with epithelial migration time, they may lay the basics for cecum's resistance against HS. The proximal cecum of Leghorns had demonstrated longer turnover time than duodenum (four days compared with 72 hours) in the research performed by Takeuchi et al. (1998). Similarly, epithelial migration time in the ceca of Japanese quails was much higher than in their small intestine, and it was second only to rectum, as noted by Starck (1998).

The development of adaptations by the chickens subjected to present study can be considered as another reason. It takes longer period to impair the intestinal compensatory mechanisms in adaptive birds according to Santos et al. (2015). This was reflected by the zero mortality of the Cobb broiler chickens taking part in comparison to 12.5% death of Hubbard chickens following a shorter period of HS with slightly lower temperature applied in Al-Fataftah and Abdelqader (2014)'s research. The adaptive trait might have originated from the Cobb strain itself or from inherited

adaptation subsequent to breeding attempts in the warm climate of Indonesia. Warm ambience could unknowingly perform an act termed as thermal manipulation on chickens prior to hatching which is capable of assisting thermotolerance acquisition (Al-Zghoul et al., 2019). Likewise, it is known that poultry are very likely to develop acclimatization when kept under tropical and subtropical climates with over two months of high temperature (He et al., 2018). Therefore, it can be deduced that the chickens in this study did not need deep crypts in their ceca to preserve their villi from potential casualties because they had created such an effective mechanism enabling them to withstand the lengthy HS (Biasato et al., 2018).

These results contradict those obtained by Deng et al. (2012) which found that the CD of layer hens' ceca was initially reduced by chronic HS on day six but returned to normal on day 12 (last day) while VH remained low throughout the experiment. This difference may be linked to breed, implying that broilers are more resistant than layers, or age of the chicken at the time of HS.

The retarded crypt activity might bring about decreased VH later as the HS progresses (Al-Fataftah and Abdelqader, 2014). CD, VW, and VSA can be enhanced in order to compensate the increased destruction, but still providing sufficient absorption area for nutrients (Santos et al., 2015). The VH:CD ratio inversely portrays the rate of epithelial cell turnover in the intestine, its value is smaller in heat stressed chickens due to induced inflammatory reaction (Laudadio et al., 2012; Shah et al., 2019). When the value is constant, the number of villi per unit area is required to be taken into account. It is recommended to eliminate the possibility that HS has reduced the number of villi instead of the villus morphometry (Marchini et al., 2016).

CONCLUSION

The resilience of broiler chickens' ceca to chronic HS was confirmed in this research. This feature can be credited to intestinal segment-specific resistance which may be explained by the slower epithelial turnover rate in cecum than in small intestine. Additionally, the current findings also suggest that broiler chickens in Indonesia have undergone an adaptation process to the high daily temperature. Nevertheless, it did not remove the possibility of damage progression if the heat exposure is extended.

DECLARATIONS

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Competing interests

The authors have declared that no competing interests exist.

Consent to publish

All the authors approved and agreed to publish the manuscript and declared that this work has not been previously published elsewhere.

Authors' contributions

Hani Plumeriastuti drafted and revised the manuscript while monitoring the course of the study. Anwar Ma'ruf was involved in revising the manuscript, also data analysis, presentation, and interpretation. Djoko Legowo devised the study and participated in its design and coordination, as well as guided the examination and analysis of the histopathological slides. Antonia Vania Adji participated in the execution of the study, data analysis, composition and revision of the manuscript. All authors read and approved the final manuscript.

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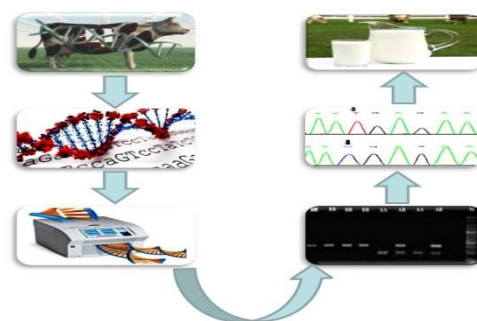
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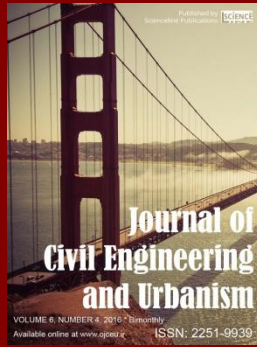
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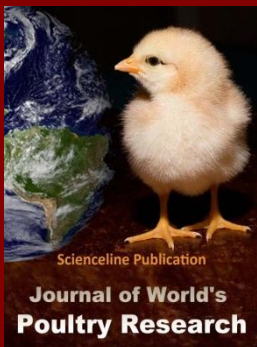
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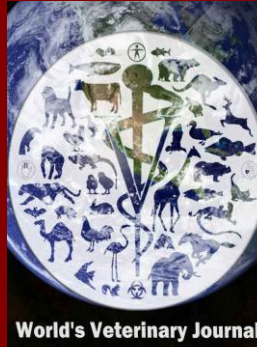
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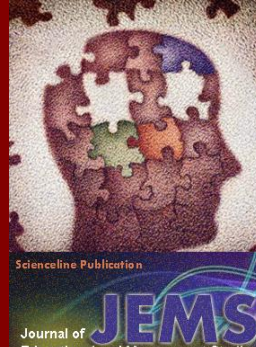
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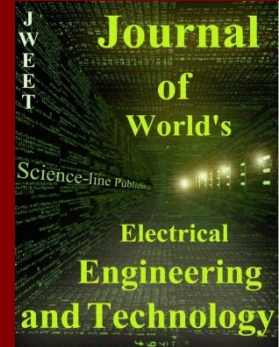
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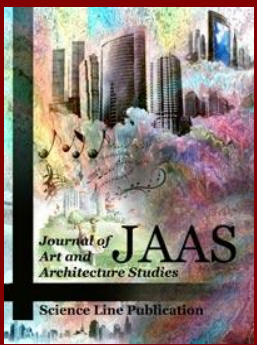
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