SERUM BIOCHEMISTRY AND HORMONAL PROFILE OF FINISHER BROILERS FED DIETARY SUPPLEMENTATION OF ACETYLSALICYLIC ACID DURING DRY SEASON

AWONEYE, Olusola Olufisayo

Department of Animal Health and Production Technology, Rufus Giwa Polytechnic, PMB 1019, Owo, Ondo State, Nigeria. **Email**: olusolaawoneye@gmail.com **Phone:** +234 706 834 7056

***Received** January 15, 2024; **Revised** March 05, 2024; **Accepted** March 07, 2024*

ABSTRACT

Acute heat stress contributes greatly to sudden death in animals and induces serious intra-corporal damage even if the animal survives. This trial was therefore conducted to unravel the effect of dietary supplementation of acetylsalicylic acid (ASA) on the serum biochemical composition and hormonal status of finisher broiler chickens during the transition from dry to rainy season (January – March). One hundred and ninety-two (192) day-old broiler chicks (ANAK) were randomly allotted to four treatment diets supplemented with 0, 0.025, 0.050, and 0.075% of ASA at 48 birds/treatment in four replicates of 12 birds each. The result revealed that broilers fed 0.075% ASA had the highest ($p<0.05$) aspartate aminotransferase and alkaline phosphatase with the values of 69.12 ± 6.52 IU/L, 58.02 ± 11.75 IU/L respectively, while broilers fed 0.050% ASA had the lowest ($p<0.05$) cholesterol value of 57.27 ± 2.61 mg/dL. The highest ($p<0.05$) total protein value of 4.20 ± 0.11 g/dL was observed in broilers fed the control diet. There were significant treatment effects ($p<0.05$) in all the hormonal parameters investigated. Estradiol was observed to increase as the level of ASA supplementation increased. Similarly, broilers fed 0.075% ASA had the highest ($p<0.05$) progesterone value. It could be concluded that ASA supplementation improves serum biochemical profile, as well as reduces the cholesterol level of experimental birds.

Keywords: Serum, Biochemistry, Hormone, Broiler, Acetylsalicylic acid

INTRODUCTION

Animal cells synthesize a small number of heat-shock proteins (HSPs) under heat stress conditions, and low production of heat-shock proteins (HSP) expression causes serious intra-corporal damage in poultry species (Rylander *et al*., 2006; Kamboh *et al*., 2013). It increases the membrane permeability of myocardial cells, which enhances the release of enzymes from myocardial cells into the serum (Saravanan *et al.,* 2013). These enzymes, including iso-enzyme creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH), are widely regarded as indicators of acute myocardial injury (Wu *et al*., 2013; Zeren *et al*., 2013). High environmental temperatures alter the activity of the neuroendocrine system of poultry, resulting in activation of the hypothalamic-pituitary-adrenal (HPA) axis, along with the sympathetic-adrenal-medullar axis (SAM), which promotes the release of glucocorticoids. Furthermore, glucocorticoids initiate the hydrolysis of circulating triglycerides, intensifying the activity of lipoprotein lipase that leads to an increase in lipolysis. Insulin growth factor (IGF-1) is negatively regulated by glucocorticoids to worsen skeletal muscle damage (Quinteiro-Filho *et al*., 2012; Kuo *et al.,* 2013). Glucocorticoids enhance glucose synthesis to confirm the survival of animals under heat stress. Long-term secretion of corticosterone during chronic heat stress is linked to many deleterious consequences in broiler chickens; these include reduced immunity, muscle breakdown, cardiac issues, and depression. Heat stress also induces infertility by altering the activities of reproductive hormones, it affects poultry gut health (leaky gut) (Romero *et al*., 2015). Several researches on the adverse effects of environmental stress on domestic birds showed that the plasma level of corticosteroids increases, while that of plasma proteins decreases with a marked increase in blood glucose concentration and calcium levels (Oladele *et al.,* 2001; Sahin *et al.,* 2003). It was reported by McDaniel *et al.* (1995; 1996) that serum calcium and phosphorus levels, calcium and potassium ion exchange, and spermatogenesis were significantly lowered in heat-stressed birds. Ambient temperatures influence reproduction by reducing the feed intake of chickens, this leads to decreased body weight, feed efficiency, egg production, and quality, reduced dietary digestibility, and decreased plasma protein (Ardon *et al*., 2008).

Aspirin (acetylsalicylic acid) is one of the most widely prescribed non-steroidal anti-inflammatories, antipyretic, and analgesic drugs. Aspirin has been reported to play a key role in lowering cholesterol and triglycerides in blood, meat, and eggs, and is used in the cure and anticipation of normal cardiovascular disorders (van Diepen *et al*., 2011). It acts to prevent prostaglandin synthesis; and therefore, it alters the hypothalamic thermostat capacity (Wu *et al*., 2015; Truong *et al*., 2016). Aspirin decreases blood clotting, and inhibition of blood clotting is due to the disruption of prostaglandin synthesis in thrombocytes (Balog *et al.,* 2000). The attributes of ASA have thus necessitated this experiment to investigate the effects of feeding ASA supplemented diet on the serum biochemistry and hormonal profile of finisher broiler chicken during the dry season.

MATERIALS AND METHODS

Experimental Site: The experiment was carried out at the Teaching and Research Farm (Poultry Unit) of the Rufus Giwa Polytechnic, Owo, Nigeria. It is located on latitude 7°18′N and longitude 5°10′E. The site is within the hot, wet equatorial climate with high rainfall (1,500 mm) and relative humidity (well above 75 %) all year round with an annual temperature of about 27°C.

Procurement of Dietary Supplement: Aspirin (Acetylsalicylic acid) was purchased from was purchased from Bayer Pharmaceutical Company, Berlin, Germany.

Experimental Animals and Feeding: One hundred and ninety-two (192) day-old broiler chicks (ANAK) were purchased from Chi Farm Limited, Ibadan. The birds were initially fed a standard broiler starter diet containing 23.10% crude protein and 11.78 MJ/Kg metabolizable energy (Table 1) from the 1st to 4th weeks of age after which they were switched to a standard broiler finisher's diet with 19.60% crude protein and 12.21 MJ/Kg metabolizable energy (Table 2) from the 5th week to the 8th week of the feeding trial. Both the starter and finisher diets were supplemented with 0, 0.025, 0.050, and 0.075% of acetylsalicylic acid (ASA) respectively. Diet 1 (T1) which is the control had no inclusion of acetylsalicylic acid (ASA), Diet 2 (T2) contained 0.025% inclusion of ASA, Diet 3 (T3) contained 0.050% inclusion of ASA, and Diet 4 (T4) had 0.075% of ASA. The experimental diets were formulated to meet the nutrient requirements of the bird.

Experimental Layout and Feeding Trial: The birds were randomly divided into four dietary treatments, each treatment comprising 48 birds, which was replicated 4 times containing 12 birds per replicate. At the end of the 8-week feeding trial, the final weight was taken; three birds per replicate were randomly selected, and humanely slaughtered.

Data Collection: Blood samples were collected from birds into a sterile glass tube without anti-coagulant for serum biochemical analyses. From the clotted blood inside the test tube, sera were separated following centrifugation at 3000 rpm for about 10 minutes. The sera samples of 12 birds (3 birds per treatment group) were then taken to the Chemical Pathology Department of University College Hospital (UCH) Ibadan in Oyo State for laboratory assay. The following serum biochemical parameters were determined: serum protein, serum albumin, serum globulin, serum cholesterol, serum creatinine, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT).

Table 1: Composition (g/100g) of broiler starter diets (0-4 Weeks) during the transition from dry to rainy season (January - March)

Ingredients	Percentage Composition			
	T_1 (0.00%)	T_2 (0.025%)	T_3 (0.050%)	T_4 (0.075)
Maize	52.00	52.00	52.00	52.00
Wheat Offal	5.00	5.00	5.00	5.00
Groundnut Cake	16.00	16.00	16.00	16.00
Soya Bean Meal	21.40	21.40	21.40	21.40
Fish Meal	1.50	1.50	1.50	1.50
Bone meal	1.50	1.50	1.50	1.50
Limestone	1.00	1.00	1.00	1.00
Lysine	0.10	0.10	0.10	0.10
Methionine	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50
Premix	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Acetylsalicylic acid (ASA)	0.00	0.025	0.050	0.075
Calculated Analysis				
Crude Protein (%)	23.10	23.10	23.10	23.10
Metabolizable Energy (MJ/Kg)	11.78	11.78	11.78	11.78
Ether Extract (%)	4.67	4.67	4.67	4.67
Crude Fibre (%)	3.92	3.92	3.92	3.92
Phosphorus (%)	0.72	0.72	0.72	0.72
Calcium (%)	1.03	1.03	1.03	1.03
Lysine (%)	1.19	1.19	1.19	1.19
Methionine (%)	0.85	0.85	0.85	0.85

T1 = Diet with 0.00%ASA; T2 = Diet with 0.025% ASA; T3 = Diet with 0.050%ASA; T4 = Diet with 0.075% ASA

Alkaline phosphatase (ALP) was determined using the procedure provided by Randox. ALP activity was measured by monitoring the concentration of p- p-nitro phenol formed when ALP reacts with p-nitro phenyl phosphate. ALT and AST were determined using the principle described by Reitman and Frankel (1957) with the procedure provided by Randox. The serum was analyzed for cholesterol content using the Randox cholesterol kit. The serum samples were analyzed for creatinine content using a Randox R creatinine kit (32 Mol/l picric acid and 0.32 Mol NaOH). Also, an enzyme-based immunoassay procedure was employed to determine oestradiol, progesterone, testosterone, cortisol, and luteinizing hormone in the serum samples.

Statistical Analysis: All data were subjected to a one-way analysis of variance (ANOVA) using the SAS (2008) statistical package. Duncan's multiple range test of the same statistical package was used to separate significant means at $p<0.05$.

RESULT AND DISCUSSION

The serum biochemical profile of broiler finisher chickens fed dietary supplementation of acetylsalicylic acid during the transition from dry to rainy season (January- March) indicated that the total protein, albumin, globulin, aspartate aminotransferase, alkaline phosphatase, and cholesterol were significantly influenced ($p<0.05$) by the dietary treatments (Table 3). The highest ($p<0.05$) total protein value of 4.20 ± 0.11 g/dL was observed in broilers fed the control diet. Broilers fed 0.050% ASA had the highest albumin value of 1.62 ± 0.18 g/dL, which was not statistically different ($p>0.05$) from the value obtained in broilers fed the control diet and those fed 0.075% ASA. Globulin was highest ($p<0.05$) in broilers fed 0.025% ASA. The result also showed that broilers fed 0.075% ASA had the highest ($p<0.05$) in aspartate aminotransferase and alkaline phosphatase with the values of 69.12 ± 6.52 IU/L, 58.02 ± 11.75 IU/L, while broilers fed 0.050% ASA had the lowest ($p<0.05$) cholesterol value of 57.27 ± 2.61 m g/dL.

Table 2: Composition (g/100g) for broiler finisher's diets during the transition from dry to rainy season (January – March)

Ingredients	Percentage Composition			
	T_1 (0.00%)	T_2 (.025%)	T_3 (0.050%)	T_4 (0.075%)
Maize	62.00	62.00	62.00	62.00
Wheat Offal	4.70	4.70	4.70	4.70
Groundnut Cake	17.20	17.20	17.20	17.20
Soya Bean Meal	11.00	11.00	11.00	11.00
Fish Meal	1.00	1.00	1.00	1.00
Bone meal	1.50	1.50	1.50	1.50
Limestone	1.00	1.00	1.00	1.00
Lysine	0.20	0.20	0.20	0.20
Methionine	0.40	0.40	0.40	0.40
Salt	0.50	0.50	0.50	0.50
Premix	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Acetylsalicylic acid (ASA)	0/00	0.025	0.050	0.075
Calculated Analysis				
Crude Protein (%)	19.60	19.60	19.60	19.60
Metabolizable Energy (MJ/Kg)	12.21	12.21	12.21	12.21
Ether Extract (%)	4.76	4.76	4.76	4.76
Crude Fibre (%)	3.53	3.53	3.53	3.53
Phosphorus (%)	0.68	0.68	0.68	0.68
Calcium (%)	1.00	1.00	1.00	1.00
Lysine (%)	1.03	1.03	1.03	1.03
Methionine (%)	0.70	0.70	0.70	0.70

T1 = Diet with 0.00%ASA; T2 = Diet with 0.025% ASA; T3 = Diet with 0.050%ASA; T4 = Diet with 0.075% ASA

Table 3: Serum biochemistry of broiler finisher chickens fed dietary supplementation of acetylsalicylic acid at the transition from dry to rainy season (January – March)

Parameters	T1	T2	T3	T4
Total Protein (g/dL)	4.20 ± 0.11^{b}	3.88 ± 0.08^{a}	3.92 ± 0.26^{a}	3.70 ± 0.22^{a}
Globulin (g/dL)	2.48 ± 0.08^{b}	2.55 ± 0.02^{c}	2.28 ± 0.20^{a}	2.14 ± 0.17^{a}
Albumin (g/dL)	1.52 ± 0.16^{b}	1.33 ± 0.09^{a}	1.62 ± 0.18^{b}	1.56 ± 0.25^{b}
AST (IU/L)	58.21 ± 7.74^{a}	60.47 ± 4.40^{a}	60.90 ± 5.13^{a}	69.12 ± 6.52^{b}
ALP(IU/L)	38.14 ± 5.83^{ab}	42.74 ± 2.71^{ab}	31.38 ± 3.97^{a}	58.02 ± 11.75^{b}
ALT (IU/L)	8.41 ± 0.49	9.11 ± 0.72	8.66 ± 0.29	8.82 ± 0.89
Cholesterol (m g/dL)	65.57 ± 3.64^{b}	80.27 ± 14.10^{c}	57.27 ± 2.61^{a}	68.08 ± 9.15^{bc}
Creatinine (m g/dL)	0.77 ± 0.04	0.83 ± 0.04	0.80 ± 0.05	0.77 ± 0.09

a, ab, b, c = Means on the same rows but with different superscripts are statistically ($p<0.05$) significant. T1 = Diet with 0.00% ASA; T2 = Diet with 0.025% ASA; T3 = Diet with 0.050% ASA; T4 = Diet with 0.075% ASA; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; ALP = Alkaline Phosphatase

The result obtained in this study was in agreement with the findings of Kutlu and Forbes (2000) and Sahin *et al.* (2003) who reported a significant increase in serum protein, and Sahin *et al*. (2003) who reported a decrease ($p<0.05$) in cholesterol when ASA supplement was fed to broilers. On the contrary, McKee *et al.* (1997) and Konca *et al*. (2009) reported that dietary ASA supplementation did not have any significant effect on serum total protein and cholesterol.

The variation in the results could be a result of differences in the level of supplementation, breed, and environmental condition at the time of the experiment. High levels of alanine aminotransferase values and alkaline phosphatase reported in broilers fed 0.075% ASA is a pointer of the toxicity of ASA, which indicates liver damage. The hormonal profile of broiler finisher chickens fed dietary supplementation of acetylsalicylic acid during the transition from dry

Table 4: **Hormonal profile of broiler finisher chickens fed dietary supplementation of acetylsalicylic acid at the transition from dry to rainy season (January - March)**

Parameters	T1	T2	T3	T4
Estradiol (nmol/L)	0.07 ± 0.00^{a}	0.22 ± 0.01^{b}	0.24 ± 0.01^{b}	0.24 ± 0.05^{b}
Progesterone (ng/ml)	0.82 ± 0.01^{a}	1.25 ± 0.39^{ab}	1.01 ± 0.07^{ab}	1.63 ± 0.18^{b}
Testosterone (nmol/L)	0.93 ± 0.09^{a}	1.15 ± 0.03^{b}	0.76 ± 0.03^{a}	0.93 ± 0.05^{a}
LH(IU/L)	0.20 ± 0.04^{b}	0.15 ± 0.03^{ab}	0.10 ± 0.00^{a}	0.15 ± 0.02^{ab}
Cortisol (**IU/L**)	1.55 ± 0.06^{b}	1.30 ± 0.00^{a}	1.35 ± 0.03^{a}	1.40 ± 0.00^{a}

a, ab, b = Means on the same rows but with different superscripts are statistically (p<0.05) significant. T1 = Diet with 0.00% ASA; T2 = Diet with 0.025% ASA; T3 = Diet with 0.050% ASA; T4 = Diet with 0.075% ASA; LH = Luteinizing hormone

to the rainy season (January - March) indicated that there were significant treatment effects ($p<0.05$) in all the hormonal parameters investigated (Table 4). Estradiol was observed to increase as the level of ASA supplementation increased. Similarly, broilers fed 0.075% ASA had the highest ($p<0.05$) progesterone value of 1.63 ± 0.18 ng/mL which varies significantly from values obtained in other treatments. Broilers fed the control diet had the highest ($p<0.05$) luteinizing hormone and cortisol value of 0.20 ± 0.04 IU/L and 1.55 ± 0.06IU/L respectively, while the lowest ($p<0.05$) cortisol value of 1.30 ± 0.00 IU/L was recorded in broilers fed 0.025% ASA, which was statistically similar ($p>0.05$) with the values obtained in broilers fed 0.050% and 0.075% ASA. The significantly higher values of progesterone and estradiol with the lowest ($p<0.05$) cortisol recorded in ASA-supplemented diets reflects the potentiality of ASA to suppress prostaglandins, which invariably stimulates the release of circulatory pituitary, steroid hormones, and also suppressed cortisol, which is the stress indicator hormone (Abou El-Soud *et al.*, 2006; Abdel-Fattah, 2006).

Conclusion: It can be concluded that ASA supplementation improved the serum protein, and albumin globulin hormonal status, as well as reduced the cholesterol levels in the blood. However, the inclusion of ASA above 50% level was toxic and may have detrimental effects on the health and general well-being of broiler chickens.

ACKNOWLEDGEMENTS

I am thankful to the Tertiary Education Trust Fund for the research grant provided for this study. I also especially thank Prof. S. O. Aro of the Federal University of Technology, Akure for supervising the research work.

REFERENCES

ABDEL-FATTAH, S. A. (2006). Physiological and immunological adjustments of dietary ascorbic acid and acetyl salicylic acid in heat stressed Japanese quail. *Egyptian Poultry Science*, 26(IV): 1395 – 1418.

ABOU EL-SOUD, S. B., EBEID, T. A. and EID, Y. Z. (2006). Physiological and antioxidative effects of dietary acetyl salicylic acid in laying Japanese quail (*Coturnix japonica*) under high ambient temperature. *Journal of Poultry Science*, 43(3): 255 – 265.

ARDON, F., HELMS, D., SAHIN, E., BOLLWEIN, H., TOPFER-PETERSEN, E. and WABERSKI, D. (2008). Chromatin-unstable boar spermatozoa have little chance of reaching oocytes in vivo. *Reproduction*, 135(4): 461 – 470.

BALOG, J. M., HUFF, G. R., RATH, N. C. and HUFF, W. E. (2000). Effect of dietary aspirin on ascites in broilers raised in a hypobaric chamber. *Poultry Science*, 79(8): 1101 – 1105.

KAMBOH, A. A., HANG, S. Q., BAKHETGUL, M. and ZHU, W. Y. (2013). Effects of genistein and hesperidin on biomarkers of heat stress in broilers under persistent summer stress. *Poultry Science*, 92(9): 2411 – 2418.

KONCA, Y., KIRKPINAR, F., MERT, S. and YURTSEVEN, S. (2009). Effects of dietary ascorbic acid supplementation on growth performance, carcass, bone quality and blood parameters in broilers during natural summer temperature. *Asian*

Journal of Animal and Veterinary Advances, 4(3): 139 – 147.

KUO, T., HARRIS, C. A. and WANG, J. C. (2013). Metabolic functions of glucocorticoid receptor in skeletal muscle. *Molecular and Cellular Endocrinology*, 380(1-2): 79 – 88.

KUTLU, H. R. and FORBES, J. M. (2000). Effects of environmental temperature and dietary ascorbic acid on the diurnal feeding pattern of broilers. *Turkish Journal of Veterinary and Animal Sciences*, 24(5): 479 – 491.

MCDANIEL, C. D., BRAMWELL, R. K. and HOWARTH JR, B. (1996). The male contribution to broiler breeder heat-induced infertility as determined by sperm-egg penetration and sperm storage within the hen's oviduct. *Poultry Science*, 75(12): 1546 – 1554.

MCDANIEL, C. D., BRAMWELL, R. K., WILSON, J. L. and HOWARTH JR, B. (1995). Fertility of male and female broiler breeders following exposure to elevated ambient temperatures. *Poultry Science*, 74(6): 1029 – 1038.

MCKEE, J. S., HARRISON, P. C. and RISKOWSKI, G. L. (1997). Effects of supplemental ascorbic acid on the energy conversion of broiler chicks during heat stress and feed withdrawal. *Poultry Science*, 76(9): 1278 – 1286.

OLADELE, S. B., OGUNDIPC, S., AYO, J. O. and ESIEVO, K. A. N. (2001). Effects of season and sex on packed cell volume, haemoglobin and total proteins of indigenous pigeons in Zaria, Northern Nigeria. *Veterinarski Arhiv*, 71(5): 277 – 286.

QUINTEIRO-FILHO, W. M., GOMES, A. V. S., PINHEIRO, M. L., RIBEIRO, A., FERRAZ-DE-PAULA, V., ASTOLFI-FERREIRA, C. S., FERREIRA, A. J. P. and PALERMO-NETO, J. (2012). Heat stress impairs performance and induces intestinal inflammation in broiler chickens infected with *Salmonella enteritidis*. *Avian Pathology*, 41(5): 421 – 427.

REITMAN, S. and FRANKEL, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, 28(1): 56 – 63.

ROMERO, L. M., PLATTS, S. H., SCHOECH, S. J., WADA, H., CRESPI, E., MARTIN, L. B. and BUCK, C. L. (2015). Understanding stress in the healthy animal – potential paths for progress. *Stress*, 18(5): 491 – 497.

RYLANDER, M. N., FENG, Y., BASS, J. O. N. and DILLER, K. R. (2006). Thermally induced injury and heat-shock protein expression in cells and tissues. *Annals of the New York Academy of Sciences*, 1066(1): 222 – 242.

SAHIN, K., SAHIN, N. and KUCUK, O. (2003). Effects of chromium, and ascorbic acid supplementation on growth, carcass traits, serum metabolites, and antioxidant status of broiler chickens reared at a high ambient temperature (32 °C). *Nutrition Research*, 23(2): 225 – 238.

SARAVANAN, G., PONMURUGAN, P., SATHIYAVATHI, M., VADIVUKKARASI, S. and SENGOTTUVELU, S. (2013). Cardioprotective activity of *Amaranthus viridis* Linn: effect on serum marker enzymes, cardiac troponin and antioxidant system in experimental myocardial infarcted rats. *International Journal of Cardiology*, 165(3): 494 – 498.

SAS (2008). *Statistical Analysis System (SAS/STAT Program, Version 9.1).* SAS Institute Incorporated, Cary, North Carolina, USA.

TRUONG, A., SAYAGO, M. M., KUTTEH, W. H. and KE, R. W. (2016). Subchorionic hematomas are increased in early pregnancy in women taking low-dose aspirin. *Fertility and Sterility*, 105(5): 1241 – 1246.

VAN DIEPEN, J. A., VROEGRIJK, I. O., BERBÉE, J. F., SHOELSON, S. E., ROMIJN, J. A., HAVEKES, L. M., RENSEN, P. C. and VOSHOL, P. J. (2011). Aspirin reduces hypertriglyceridemia by lowering VLDL-triglyceride production in mice fed a high-fat diet. *American Journal of Physiology, Endocrinology and Metabolism*, 301(6): E1099 – E1107.

WU, D., XU, J., SONG, E., TANG, S., ZHANG, X., KEMPER, N., HARTUNG, J. and BAO, E. (2015). Acetyl salicylic acid protected against heat stress damage in chicken myocardial cells and may associate with induced Hsp27 expression. *Cell Stress and Chaperones*, 20: 687 – 696.

ZEREN, G., ERER, H. B., KIRIŞ, T., SAHIN, O., AKSU, H., KÖPRÜLÜ, D., GÜVENÇ, T. S., ERDOĞAN, G., SAYAR, N., GÜNAYDIN, Z. Y. and EREN, M. (2013). Relation of heart-type fatty acid-binding protein with the degree and extent of atherosclerosis in patients with non-ST elevation acute coronary syndrome. *Turk Kardiyoloji Dernegi Arsivi: Turk Kardiyoloji Derneginin Yayin Organidir*, 41(7): 610 – 616.