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Effect of Acetylsalicylic Acid Treatment on Gait Score and Femur Osteometry in Broiler Chickens

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Abstract: The current research examined the effects of adding 0.03%, 0.06%, 0.1%, 0.3%, and 0.6% acetylsalicylic acid (ASA) to drinking water between 24 and 45 days on gait score and femur osteometry. The gait score average of the birds slaughtered on the 49th day was above 3, and no difference was found between the groups. It was determined that ASA treatment did not affect the osteometric and index values of the femur bones taken and examined after slaughter. In conclusion, it was concluded that ASA was not effective in preventing skeletal deformations caused by rapid growth in broilers.

Keywords: *Acetylsalicylic acid, Femur, Rapid growth, Welfare.*

Etlik Piliçlerde Asetilsalisilik Asit Uygulamasının Yürüme Skoru ve Femur Osteometrisi Üzerine Etkisi

Özet: Yapılan araştırmada 24 ve 45. günler arasında içme suyuna %0.03, %0.06, %0.1, %0.3 ve %0.6 asetilsalisilik asit (ASA) eklenmesinin yürüyüş skoru ve femur osteometrisi üzerine etkileri incelenmiştir. 49. günde kesilen tavukların yürüyüş skoru ortalaması 3'ün üzerinde olup gruplar arasında fark bulunmamıştır. Kesim sonrası alınarak incelenen femur kemiklerinin osteometrik değerlerini ve indeks değerlerini etkilemediği belirlenmiştir. Sonuç olarak ASA'nın etlik piliçlerde hızlı büyümeye bağlı iskelet deformasyonlarını önlemede etkili olmadığı sonucuna varılmıştır.

Anahtar Kelimeler: *Asetilsalisilik asit, Femur, Hızlı büyüme, Refah.*

Introduction

Orthopaedic problems are considered an important concern for broiler production and welfare. Foot and leg problems cause lameness, which causes pain, decreased mobility, and difficulty acting on basic behaviours, such as accessing water resources in chickens. In conclusion, this results in reduced yield and economic losses (Bessei, 2006; de Jong et al., 2016; Gocsik et al., 2017; Yang et al., 2023). Fast growth is a critical aspect impacting musculoskeletal health in commercial broilers. Physiologically, skeletal development should be consistent with the organism's growth rate. The growth rate of broilers increases dramatically in the early stage, but the skeletal development rate is slower than weight gain. Furthermore, these phenomena make weaker skeletons relative to huge body weight, quickly leading to skeletal disorders in broilers, especially in the legs and feet (Duggan et al., 2015; Shim et al., 2012; Xu et al., 2022; Yan et al., 2019). Some reports demonstrate that between 14% and 50% of broilers exhibit moderate to severe foot problems in commercial flocks during the final stages of the growing period (de Jong et al., 2016; Yang et al., 2023). In addition, studies indicate that fast-growing broilers have decreased bone mineral density and a lower bone ash percentage than slow-growing broilers (Shim et al., 2012).

Acetylsalicylic acid (ASA) is a non-steroidal anti-inflammatory drug that affects numerous biological pathways. As it is known, the ASA's mechanism of action acts by inhibiting cyclo-oxygenase-1 (COX-1), cyclo-oxygenase-2 (COX-2), and anti-platelet aggregation by reducing cytokine production (Hida et al., 2023). ASA additionally supports osteogenesis and prevents osteoclastogenesis, which means it can potentially improve bone health. (Cao et al., 2015; Liu et al., 2015; Wada et al., 2013). Moreover, some studies suggest that ASA could regulate bone metabolism (Fang et al., 2018; Shi et al., 2008; Yamaza et al., 2008).

This study aimed to benefit from ASA's osteogenesis effects to help reduce orthopedic problems in fast-growing broiler chickens. This research examined the impact of different ASA dosages on the gait score and femur osteometry of chickens during their growth phase.

Materials and Methods

Housing, feeding protocol, and experimental design: 300 one-day-old Ross 308 male broiler chickens (Aviagen, Newbridge, UK) were obtained from a commercial farm and participated in a 49-day study. The chicks were selected at random into six groups: (C) Control (untreated); (ASA1) added 0.03% acetylsalicylic acid to drinking water; (ASA2) added 0.06% acetylsalicylic acid to drinking water; (ASA3) added 0.1% acetylsalicylic acid to drinking water; (ASA4) added 0.3% acetylsalicylic acid to drinking water; (ASA5) added 0.6% ASA to drinking water. Each group included five repetitions and ten birds over the study period. The diet was uniform for all six treatment groups. The broilers have full access to both feed and water. They followed a feeding protocol, starting with a starter diet for the first 24 days of their lives, then transitioning to a grower diet from day 24 to day 35, and then switching to a finisher diet from day 36 to day 49 (Table 1). Additionally, water-soluble ASA treatment was administered to the groups throughout the growth phase until the end of the finisher phase (24-45 days). The nutritional content and requirements of the subject were assessed according to NRC guidelines. The chicks were housed in a 1 m×1 m pen; the floor was covered with wood shavings in a mechanical ventilation room. Throughout the initial stage (first five days), the temperature within the feeding chamber was maintained at a uniform range of 32-34°C. Afterward, the temperature was systematically decreased by two °C weekly until it reached the final range of 23±1°C. The relative humidity was consistently maintained at 40 to 60 percent. For the initial three days, the broilers were exposed to nonstop illumination, which was then replaced by a routine of 23 hours of light and 1 hour of night-time. At the end of the experiment, 90 animals, three from each replication and 15 from each group reflecting the replication average, were selected and ethically slaughtered.

Gait score: Gait score (GS) assessment was performed before the chickens were slaughtered. The gait scores were determined by consensus between the two trained observers. Trained assessors have classified all broilers into six score levels (0–5) following the Welfare Quality Assessment Protocol (Welfare Quality®, 2009), shown in Table 2.

Femur osteometry: The surrounding tissues were removed after grouping the right femur bone samples obtained from slaughtered chickens. Then, samples were kept in hydrogen peroxide for 2 hours. Measurements were made after the bones were cleaned of fat and dried. Measurements were conducted following the data-collecting protocol established by A. von den Driesch (von den Driesch, 1976). Figure 1 presents the metrics, including abbreviations and definitions used in the dataset.

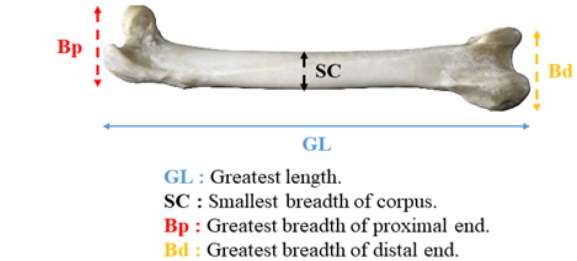

Figure 1. Abbreviations and definitions used in right femur osteometric measurements.

Statistical analysis: The ANOVA test was performed to compare the groups. For significant parameters, the Tukey HSD test was used for intra-group comparisons. For data with scoring, the Kruskall-Wallis H test and Mann-Whitney U

Table 1. Ingredients, chemical composition, and energy of the diets used during the grower period (24 to 35 d of age) and finisher period (36 to 49d of age).

Basal diet ingredients	Grower period (24-35)	Finisher period (36-49)
Corn	60.29	63.53
Soybean meal (44% crude protein)	28.34	24.20
wheat bran (Razmol)	4.00	5.00
Vegetable oil	4.21	4.15
Dicalcium Phosphate	1.33	1.33
Ground Llimestone	0.97	0.89
DL- Methionine	0.25	0.25
L- Lysine hydrochloride	0.16	0.20
L- Threonine	0.08	0.08
Salt	0.35	0.35
Vitamin and mineral supplements*	0.20	0.20
Nutritional Composition, (%)**		
Dry matter	90.00	89.90
Crude protein	19.50	18.00
Ether extract	6.07	6.08
Ash	5.51	5.25
Crude fiber	2.73	2.72
Starch	41.61	43.65
Calcium	0.78	0.74
Available phosphorous	0.39	0.38
Sodium	0.17	0.17
Chlorine	0.25	0.25
Methionine+Cystine	0.90	0.85
Lysine	1.15	1.07
Threonine	0.80	0.73
Tryptophan	0.26	0.23
Linoleic acid	3.45	3.48
ME, kcal/kg**	3200	3225

*: Vitamin-mineral premix supplied per kg: Vitamin A, 12000 IU; Vitamin D3, 3000 IU; Vitamin E, 30 mg; Manganese, 80 mg; Iron, 60 mg; Zinc, 60 mg; Copper, 5 mg; Iodine, 1.5 mg; Cobalt, 0.3 mg; Selenium 0.15 mg.
**: Calculated.

Table 2. Practicing a gait score for broiler chickens

Gait score	Measurement for gait score assessment
0	Typical, smooth, and agile. The toes are flexed while lifted
1	There is a slight anomaly, but it cannot be identified.
2	It is an actual and identifiable abnormality, but it has limited effects on walking ability.
3	Apparent abnormality affects the ability to walk. The bird has imbalanced steps and squats within 15 seconds.
4	Severe abnormality, but still capable of stepping. The chicken takes over five seconds to rise when nudged and squats after a few steps.
5	Incapable of stepping

test were utilized. The SPSS 21 package tool was used for statistical analysis. The difference between group means was statistically significant (P<0.05).

Results

Gait score: The gait score is an indicator commonly utilized for the measuring lameness in broilers. In current research, broiler gait scores were not affected by whether ASA was applied or not (P>0.05). As stated in Figure 2, the average gait score of the broilers was between 3-4 in all groups.

Femur osteometry: Table 3 shows the impact of adding acetylsalicylic acid to drinking water at different doses on the osteometric properties of the right femur in broiler chickens. Both osteometric measurements and index values showed no difference between the groups (P>0.05).

Discussion and Conclusion

Previous research has suggested that a gait score of 3 or more is a painful situation linked to poor welfare and inactivity (Caplen et al., 2013; Nääs et al., 2009). Broiler gait score is highly correlated with body weight. The rapid growth of broilers can lead to the placement of abnormally high weights on relatively infancy bones, which could impact their gait score (Kittelsen et al., 2017; Yang et al., 2023). Researchers have reported that ASA treatment positively affects broilers' carcass weight and yield (Fathi et al., 2016; Tavakoli et al., 2022). Also, researchers have found that administering one-time painkillers may increase the walking ability of heavier and older chickens. Studies point to a slight improvement in gait score, particularly for birds severely affected by lameness (Almeida Paz et al., 2019; Caplen et al., 2013; Nääs et al., 2009). However, this study showed that long-time ASA treatment did not affect the gait score in birds. The absorption of ASA following oral administration is fast and complete. The absorption is directly proportional to the dose and, thus, follows first-order pharmacokinetics

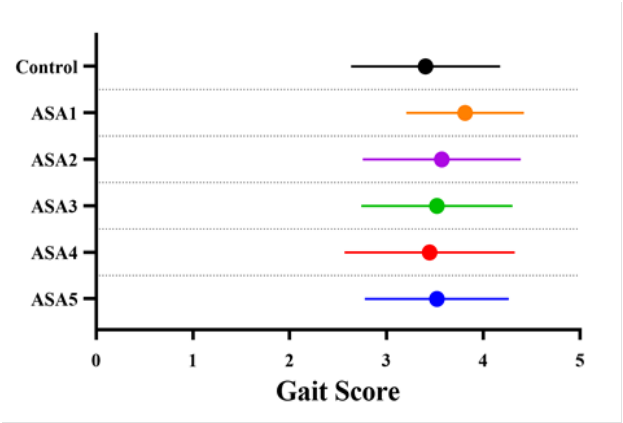

Figure 2. Effect of ASA treatment in drinking water on broiler Gait score. Control (without treatment); (ASA1) added 0.03% acetylsalicylic acid to water; (ASA2) added 0.06% acetylsalicylic acid to water; (ASA3) added 0.1% acetylsalicylic acid to water; (ASA4) added 0.3% acetylsalicylic acid to water; and (ASA5) added 0.6% ASA to water. The mean and standard error represent the values. P>0.05.

Table 3. The effect of acetylsalicylic acid added to drinking water at different doses on broiler chickens' osteometric properties of the right femur.

Osteometric Properties (mm)	Control	ASA Groups					P
		ASA1	ASA2	ASA3	ASA4	ASA5	
GL	114.89±1.40	114.16±1.39	114.77±0.78	115.60±0.55	115.37±0.53	115.48±0.81	NS
Bp	20.43±0.37	20.89±0.26	20.87±0.35	20.33±0.35	20.64±0.19	20.51±0.32	NS
SC	8.76±0.21	9.14±0.17	8.72±0.18	8.88±0.24	9.20±0.21	8.89±0.23	NS
Bd	24.08±0.43	24.21±0.47	23.94±0.33	23.81±0.44	24.30±0.31	23.36±0.36	NS
Bp*100/GL	17.78±0.28	18.13±0.23	18.19±0.26	17.60±0.32	17.90±0.17	17.78±0.30	NS
SC*100/GL	7.63±0.19	8.03±0.24	7.60±0.14	7.68±0.20	7.97±0.17	7.71±0.22	NS
Bd*100/GL	20.80±0.36	21.22±0.37	20.88±0.34	20.60±0.39	21.07±0.31	20.26±0.39	NS

Control (without treatment); (ASA1) added 0.03% acetylsalicylic acid to water; (ASA2) added 0.06% acetylsalicylic acid to water; (ASA3) added 0.1% acetylsalicylic acid to water; (ASA4) added 0.3% acetylsalicylic acid to water; and (ASA5) added 0.6% ASA to water. Data are shown as means ± standard error of the mean. Not Significant (NS): P>0.05, Greatest length (GL), Greatest breadth of the proximal end (Bp), Smallest breadth of the corpus (SC), Greatest breadth of the distal end (Bd).

(Stevens et al., 2019). The pharmaceutical effect of the drug can explain why long-term use of ASA did not affect lameness in fast-growing broilers in this study.

Over the last 60 years, the genetic selection of broilers has concentrated primarily on production qualities, including growth rate and feed efficiency. This has resulted in severe

welfare troubles for broilers, such as cardiovascular issues (sudden death syndrome, ascites, etc.) or musculoskeletal disorders (Contact dermatitis, Bacterial chondronecrosis with osteomyelitis, leg weakness, and lameness) (Hartcher and Lum, 2020). Restricting feeding due to chronic hunger and stress could not be an option (Decuypere et al., 2010). Genetic lines of broilers with slower growth rates exhibit better cardiovascular function, reduced mortality, and a decreased prevalence of musculoskeletal problems and bone deformities. Numerous studies argue for slower-growing strains, which do not present the same welfare problems as fast-growing commercial strains (Bessei, 2006; Hartcher and Lum, 2020; Wilhelmsson et al., 2019).

Rapidly growing broilers may want to avoid walking or exercising. Broilers spend about 76% of their time lying down, which increases with age. As they get closer to market age, broilers spend only 3.3% of their time walking (Weeks et al., 2000). Fast-growth broilers with limited circumstances for movement and exercises are particularly weak regarding osteoporosis, lower tibia and femur mineral density, bone mass, and bone-breaking strength. Also, rapid growth is associated with skeletal deformities such as tibial dyschondroplasia, which results in lameness. One study showed that (Derakhshanfar et al., 2013) dietary ASA administration at a dose of 400 mg/L for three weeks induced histopathological changes in the development of tibiotarsal bones in chickens. However, the authors put forward the thesis that the ASA application may be beneficial based on the indirect evidence they found. Despite using much higher doses of ASA in this study, the gait score remained above 3, and the femur index was similar among groups. Acetylsalicylic acid (ASA) is primarily effective as a non-steroidal anti-inflammatory drug. Many studies suggest that ASA possesses bone protective effects (Dadwal et al., 2020; de Souza Rendohl et al., 2021). ASA can promote osteoblast precursor stem cell survival and osteoblast differentiation (Cao et al., 2015). However, these effects provide limited benefits and still need to be proved. Our study showed no difference between the groups regarding osteometric measurements, index values, or gait scores.

While breeding is a competitive industry influenced by economic factors, it is also a sensitive domain due to its importance for animal welfare. Ensuring animal welfare is essential for a sustainable chicken meat industry. More research is required to avoid musculoskeletal welfare problems related to genetic selection. As a result, adding ASA to drinking water may not be a helpful solution for musculoskeletal problems. Therefore, using ASA to improve bone condition and walking ability may not be suitable.

Conflict of Interest

The authors stated that they did not have anyreal, potential or perceived conflict of interest.

Ethical Approval

This study was approved by the Harran University Animal Experiments Local Ethics Committee (05.08.2021, 2021/01-05 Number Ethics Committee Decision). In addition, the authors declared that Research and Publication Ethical rules were followed.

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Control/Supervision: ÜGŞ
Data Collection and / or Processing: GG, İD, YK, SA, BK
Analysis and / or Interpretation: GG, İD, YK, SA, BK
Literature Review: GG, İD
Writing the Article: GG, İD, ÜGŞ
Critical Review: ÜGŞ

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