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DETERMINATION THE EFFECTS OF ADMINISTRATION OF COMBINATION OF NSAIDs ON BROILERS PERFORMANCE AND HEALTH INDICATORS WITH THE ASSOCIATION OF HEAT SHOCK PROTEIN-70

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Abstract

Introduction: The most challenging environmental situation in commercial poultry production is stress including vaccination, heat stress (summer), cold stress (winter) and drug intake stress. The use of NSAIDs may help to minimize the disease outbreaks in broilers. The gene expression of HSP-70 is an endogenous mechanism by which living cells conform to stress. It is reported that, NSAIDs have ability to produce different HSPs in several species. The prophylactic measures should be implemented to avoid the excessive misuse of antibiotics. **Objective:** This study was planned to determine the effects of NSAIDs including acetylsalicylic acid (ASA), acetaminophen (APAP) and ibuprofen by short term and prolonged use with different combinations on performance and health indicators in broilers and their association with heat shock protein 70, i.e., to conclude the protected level of NSAID's use in poultry. **Materials and methods:** In present experiment, by using completely randomized design (CRD) three subgroups in different combinations of non-steroidal anti-inflammatory drugs (NSAIDs) were given orally via drinking water (DW) from 10^{th} to 32^{nd} day of age of birds. The birds were slaughtered on the 14^{th} and 42^{nd} day of age. In general parameters, including weekly live weight, slaughter weight, carcass weight, clinical signs and behavioral alterations were also accessed in slaughtered birds The blood samples were collected without anti-coagulant to study the serum-biochemistry and tissue samples were collected at time of sampling to determine the level of HSP-70 at these stages. **Results:** The weekly live weight and carcass weight were also showed significant results at higher doses of NSAIDs. The higher doses of NSAIDs were showing severe clinical signs and behavioral alterations including dull feathers, low attraction towards feed and water and loose to watery droppings. The serum biochemical parameters and mRNA gene expression of HSP-70 showed significant results according to the dose manner of NSAIDs. **Conclusion:** Administration of low and medium doses of NSAIDs results in improved body weights. The toxicity due to excess dose of acetaminophen was observed in this research.

Index Terms: Acetaminophen, Acetylsalicylic acid, Broilers, HSP-70, Ibuprofen, NSAIDs, Serum biochemistry.

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1. INTRODUCTION

The poultry industry is the second largest business in Pakistan after the textile industry, providing employment for over 1.5 million people directly or indirectly (Khan et al., 2022). The NSAIDS have been shown to enhance the heat shock response (HSR) and a reaction to hyperthermia and other toxic situations (Batulan et al., 2005), in addition to their anti-inflammatory response which is characterized by the induction of heat shock proteins (HSPs).

The history of NSAIDs dates as far back as thousands of years with Hippocrates and other physicians prescribing willow bark for a wide range of conditions (Rao and Knaus, 2008). Nonsteroidal anti-inflammatory drugs are used throughout the world, with or without prescription (Bozimowski, 2015). NSAIDs are effective, particularly in both acute and chronic orthopedic pain such as rheumatoid arthritis and osteoarthritis, as well as in post-surgical pain (KuKanich et al., 2012). The NSAIDs are also effective for somatic pain (headache, pain in muscle and joints), while they are not effective in reducing discomfort from visceral organs (lung, liver and heart) (Bindu et al., 2020). In veterinary practice, NSAIDs are normally used for the treatment of different musculoskeletal inflammatory disorders. They are used in certain clinical situations such as endotoxic shock, mastitis, laminitis and as well as in colic in horses. They are also used for the control of pain which is associated with surgery or trauma (Boothe, 2001).

Acetylsalicylic acid (ASA) is commonly used for its broadly therapeutic antipyretic and anti-inflammatory properties. It has heart protective functions, and this was initially considered to act through the platelet interactions (Patrono et al., 2005). Although, non-acetylsalicylic acid NSAIDs have anti-platelet and anti-inflammatory properties, few of them have the same myocardial protective effects against acute myocardial infarction (Solomon et al., 2002). It is probable that acetylsalicylic acid confers protection of heart by another mechanism, e.g., upregulation of the expression of different HSP protein family members including HSP27 (Ebert et al., 2005), in different species (Sandoval-Montiel et al., 2013).

Acetaminophen (APAP) is normally used as an antipyretic and analgesic drug. Usually, it is safe at therapeutic doses; however, at high doses it is converted to a highly toxic metabolite Nacetyl-p- benzoquinone imine (NAPQI) through the cytochrome P-450 pathway, that has potential to cause nephrotoxicity and hepatic necrosis in both animals and humans (Yaman et al., 2011).

Ibuprofen was used to treat fever, pain and inflammation. After 1-2 h of oral administration, ibuprofen serum concentrations peaked, with up to 99% bound to plasma proteins. However, within 24 h, 99% of ibuprofen was metabolized and eliminated in the urine, with the remaining unchanged 1% removed by biliary excretion (Bushra and Aslam, 2010). High environmental temperatures produce heat waves, which ultimately induces heat stress in animals.

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Heat stress is a condition in which an animal cannot dissipate their body temperature into the surrounding due to the relatively higher temperature in the environment. Within the poultry industry there are global concerns over heat stress, given its adverse effects on the functions of organs (Wasti et al., 2020) as well as on the growth performance of broiler chickens (Lara and Rostagno, 2013; Abd El-Hack et al., 2019).

Heat stress induces poor digestion and absorption, losses of intestinal integrity, as well as reduced body weight (Siddique et al., 2020). The most challenging environmental situation in commercial poultry production is stress. The gene expression of HSP-70 is an endogenous mechanism by which living cells conform to stress (Gu et al., 2012). It was reported that NSAIDs, and in particular acetylsalicylic acid, have the ability to produce different HSPs in several species, including HSP-B1 (Ebert et al., 2005).

Heat shock proteins play an important role in repair and protection of tissues and cells. They are well-known as a family of endogenous, protective proteins. These proteins are situated in the mitochondria (HSP-60), cytoplasm and nucleus (HSP-70 and HSP-90) to maintain cellular function. In poultry, the expression of HSPs is induced by number of stressors including hyperthermia, handling, hypertension, crating, transport and oxidative stress (Al-Aqil et al., 2013; Awad et al., 2021).

Signals, such as reactive oxygen species (ROS), cytoskeletal alterations and cytotoxic lysosomal markers can accelerate expression of HSP in the cell. Cells initiate a cascade of events that helps to involve HSPs as molecular chaperones (Hassanpour et al., 2013). In cell lines, the NSAIDs propagates the heat shock factor 1 (HSF-1) which help in binding to heat shock element such in response to heat shock, that helps in transactivation of HSP genes, which was increased relative to the cells, that had not been treated with NSAIDs (Housby et al., 1999).

Poultry production practices in the future are to minimize the use of antibiotics. The use of NSAIDs in stress conditions such as heat stress of summer, cold stress of winter, and vaccine stress may prevent or minimize disease outbreaks in poultry. To the best of our knowledge, the use of NSAIDs has not been investigated in poultry. The aim of this research was to determine the effect by short term and prolonged use of NSAIDs at different combinations on production performance and health indicators in poultry and check their association with HSP -70.

2. MATERIALS AND METHODS

The total of 80 broiler chicks were procured from a local hatchery. After 10 d of acclimatization, these birds were randomly distributed into different groups. The birds were provided water and feed *ad-libitum*. The Group-A was acted control (-ve) group. The treatment groups (Group B) consisted of combinations of acetylsalicylic acid, ibuprofen and acetaminophen as presented in Table 1. The birds from each group were slaughtered at either 14^{th} and 42^{nd} day of the experiment. The proposed study was approved by ethical committee of University of Agriculture, Faisalabad with ref number (D.No.3676/ORIC).

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Table 1: Experimental framework

Group/ Subgroups		No. of Birds	Treatment
A		20	Control negative group
B	B1	20	300 mg/L acetylsalicylic acid (tablet) + 20 mg/L ibuprofen (syrup) + 30 mg/L acetaminophen (syrup)
	B2	20	600 mg/L acetylsalicylic acid (tablet) + 60 mg/L ibuprofen (syrup) + 90 mg/L acetaminophen (syrup)
	B3	20	1200 mg/L acetylsalicylic acid (tablet) + 120 mg/L Ibuprofen (syrup) + 160 mg/L acetaminophen (syrup)

a. General parameters:

Weekly live weight, Slaughter and carcass weights

In the general parameters assessed included weekly live weight, slaughter and carcass weight in slaughtered birds.

Clinical signs and behavioral alterations

The clinical signs and behavioral alterations were observed twice a day. These included attraction towards water, feed and consistency of droppings which were categorized as either mild, moderate or severe.

b. Serum biochemistry:

The blood was collected without anti-coagulant at Day 14 and Day 42 of each experiment for determination of serum biochemistry. The serum was collected after 2-3 h of blood collection and stored at -20^{o} C for further use. The following serum parameters were studied.

Alanine aminotransferase

The ALT was determined using a commercially available kit (Quimica Clinica Aplicada, S.A, REF # 99.95.00) and according to the manufacturer's instructions. The sample was diluted (0.1 mL serum + 0.9 mL physiological saline) where required (with the dilution factor included in the calculation of enzyme activity).

Aspartate aminotransferase

The level of AST was also measured using a commercially kit of (Quimica Clinica Aplicada, S.A., number REF # 99.95.00) and according to the manufacturer's instructions. The sample was diluted (0.1 mL serum + 0.9 mL physiological saline) where required (with the dilution factor included in the calculation of enzyme activity).

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Lactate dehydrogenase

Lactate dehydrogenase consists of five different isoenzymes which catalyze the interconversion of L-lactate and pyruvate. It is present in the cytoplasm of all tissues with higher concentrations found in the liver, muscles, and heart (Drent et al., 1996). Increased LDH activities are found in a variety of pathological conditions such as myocardial infarction, liver diseases, blood diseases, and cancer or muscle diseases (Lagana et al., 2019). However, because of the lack of organ specificity, determination of its isoenzymes or other enzymes such as ALP is necessary for differential diagnosis by Sharma at al. (2013). Lactate dehydrogenase concentration was measured using a commercially available kit (Diagnostic Systems GmbH, Germany, REF # 844 4211 10 02 00) and according to the manufacturer's instructions.

Total serum proteins

Total serum protein comprises of albumin and globulin. A colorimetric method was used to determine total serum protein using a commercially available kit (Biorays diagnostic kit) and Erba serum analyzer. The samples, including standards were prepared and analysed as per the manufacturer's instructions. In brief, 10 µL of thawed serum sample (or standard) was combined with 10 µL of deionized water and 500 µL of biuret reagent in an appropriately labeled Eppendorf tube. The tubes were then incubated at room temperature (25-30^{o}C) for 25 min, transferred into a 1 cm light path cuvette and absorbance measured at 540 nm wavelength.

Serum albumin

Albumin is a carbohydrate free protein, which constitutes 55-65% of total plasma protein. The measurement of serum albumin is based on its quantitative binding to the indicator bromocresol green (BCG). The albumin-BCG-complex absorbs maximally at 578 nm. Serum albumin concentration was determined using a commercially available kit (Biorays diagnostic kit), Erba serum analyzer and according to the manufacturer's instructions. In brief, 5µL of thawed serum (or standard) was combined with 5 µL of deionized water and 1000 µL BCG in an appropriately labelled Eppendorf tube. The tubes were then incubated at room temperature (25-30^{o}C) for 15 min, transferred into a 1 cm light path cuvette and absorbance measured at 630 nm wavelength in an automated Erba biochemical analyzer.

Serum globulins

Serum globulin concentration was determined measured by subtracting the total albumin concentration from the total serum proteins concentration.

Serum urea

The kinetic method was used for determination of serum urea concentration. A commercially available kit (Diagnostic Systems GmbH, Germany, REF # 1.1711.99.10.021) was used. In brief, the serum sample (or standard) were combined with reagent 1 and reagent 2 in an Eppendorf tube. The reagents and sample were thoroughly mixed using a vortex. After mixing, the tubes were incubated at room

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temperature for 5 min, and then transferred into a cuvette and absorbance measured at 340 nm wavelength in an automated Erba biochemical analyzer.

Creatinine

The serum creatinine was measured by the use of commercially available kit (Diagnostic Systems GmbH, Germany), having the REF # 1.1711.99.10.021). Briefly, either 50 µL of sample or the standard was combined with 250 µL of reagent 1 in an appropriately labelled Eppendorf tube and then mixed using a vortex.

The tubes were incubated at room temperature for 5 min after which 250 µL of reagent 2 was added then again mixed using the vortex mixer before transferring into a cuvette and reading the absorbance at 340 nm wavelength in an automated Erba biochemical analyzer.

c. Gene expression of HSP-70 by real-time PCR:

RNA isolation: Tissue samples were kept at -70°C in liquid nitrogen. Sample was triturated in liquid nitrogen. 100µl trizol reagent was added in sample. 200µl chloroform was added after homogenization. 3 layers were formed after 15 min. Took supernatant and added 200µl ISO-propanol and mixed it.

Sample was preserved at -20°C for 24 hrs. The pellet was observed after centrifugation. Supernatant was discarded and centrifuged it by adding 70% ethanol for 2 min. The previous step was repeated 3 time. RNA pellet was preserved in RNA's free water.

Primers: The following sequence of oligo primers were used check the gene expression of HSP-70.

5'-AGCGTAACACCACCATTCC-3' (forward primer)

5'-TGGCTCCCACCCTATCTC-3' (reverse primer) (Yu and Bao, 2008).

cDNA synthesis: After RNA extraction, by the kit method synthesis of cDNA was done. The thermos scientific RevertAid First Strand cDNA Synthesis kit was used having catalog # K1622.

Relative quantification by real-time* PCR*: SYBER Green I-based one-step real-time quantitative amplification was done by using (iCycler iQ) Real-Time PCR detection system. In RT-PCR conditions, the denaturation temperature was 94°C for 30 secs. The annealing and extension temperature was, 58°C and 72°C for 30 secs, respectively.

Statistical analysis:

The experimental collected data was analyzed by using the analysis of variance technique ($p \leq 0.05$). The means were also analyzed by Tukey, s test by using SAS statistical software (SAS, 2007).

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3. RESULTS

Weekly live weight

One week following treatment, there were no differences (P > 0.05) in live weight of broilers in Group B1 and Group B2 compared with the control negative birds; however, the broilers in Group B3 weighed less (P< 0.05) than the control birds. Two weeks after treatment, there were no differences (P > 0.05) in the live weight of NSAIDs-treated birds (Groups B1, B2 and B3) compared with the control birds. Three weeks after treatment, there was no difference (P > 0.05) in the average live weight of birds in Group B1, while the live weights of the Groups B2 and B3 birds were lower (P≤0.05) lower than control birds. After 4 weeks after treatment, the average live weight of birds in Groups B1 and Group B2 did not differ (P > 0.05) to those of the control negative (Group A) birds, while the live weight of the Group B3 was lower (P ≤ 0.05) than the control negative birds.

Table 2: Mean (± SD) weekly live weight of broilers administered varying doses of combinations of NSAIDs and percentage comparison with control negative (Group-A)

Treatment group / Subgroups		Weekly Live weight (g)			
		Week-1	**Week-2**	**Week-3**	**Week-4**
A		812.00± 90.05	1104.40 ±143.71	1735.40 ±83.69	2029.20 ±237.20
B	B1	891.60 ±78.56 (9.80%)	1276.20 ±111.62 (15.55%)	1786.20 ±112.67 (2.92%)	1938.80 ±89.72 (-4.45%)
	B2	711.00 ±48.85 (-12.43%)	1160.00 ±173.97 (5.03%)	**1533.40* ±46.66 (-11.64%)**	1850.60 ±130.31 (-8.80%)
	B3	**592.80* ±33.02 (-26.99%)**	905.20 ±115.18 (-18.03%)	**1395.40* ±66.34 (-19.59%)**	**1254.60* ±254.37 (-38.17%)**

A: Negative control; B1: ASA, 300 mg/L, Ibuprofen, 20 mg/L and APAP, 30 mg/L; B2: ASA, 600 mg/L, Ibuprofen, 60 mg/L and APAP, 90 mg/L; B3: ASA, 1200 mg/L, Ibuprofen, 120 mg/L and APAP, 160 mg/L

Bolded and * values are significantly different (P ≤ 0.05) from control negative

Slaughter and carcass weights

At Day 14, there were no differences (P > 0.05) in the average live weights and carcass weights of the birds in Groups B1 and B2 of NSAID-treated groups: however, the average live weight and carcass weight was less (P < 0.05) for the Group B3 birds. At Day 42, the average live weight of birds in Group B1 was higher (P < 0.05) than control negative birds. However, there was no difference (P > 0.05) in the average carcass weight of the birds in Group B1 compared to the control birds. The average live weight and carcass weight of the broilers in Group B2 did not vary (P > 0.05) to that of control birds. The average live weight of the Group B3 birds was lower (P < 0.05) than the control negative birds,

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while there was no difference (P > 0.05) in the average carcass weight of the birds in Group B3 compared to control negative (Group A) birds.

Table 3: Mean (± SD) live weight prior to slaughter and carcass weight of broilers administered varying doses of combinations of NSAIDs and percentage comparison with control negative (Group-A)

Treatment group / Subgroups		Live weight (g)		Carcass weight (g)	
		S1	S2	S1	S2
A		742.26 ±52.01	1925.67 ±200.52	426.66 ±71.18	1404.67 ±64.14
B	B1	641.00 ±131.73 (-13.64%)	**2539.00* ±177.99 (31.85%)**	387.60 ±44.64 (-9.15%)	1399.60 ±451.10 (-0.36%)
	B2	605.33 ±53.90 (-18.44%)	1959.67 ±116.84 (1.76%)	373.25 ±84.29 (-12.51%)	1250.40 ±216.64 (-10.98%)
	B3	**534.66* ±21.50 (-27.96%)**	**1458.67* ±27.15 (-24.25%)**	**284.66* ±56.94 (-33.28%)**	884.40 ±285.54 (-37.03%)

A: Negative control; B1: ASA, 300 mg/L, Ibuprofen, 20 mg/L and APAP, 30 mg/L; B2: ASA, 600 mg/L, Ibuprofen, 60 mg/L and APAP, 90 mg/L; B3: ASA, 1200 mg/L, Ibuprofen, 120 mg/L and APAP, 160 mg/L

S1: slaughtered at 14 d; S2: slaughtered at 42 d

Bolded and * values are significantly different (P ≤ 0.05) from control negative

Clinical signs and behavioral alterations

The control negative (Group A) birds behaved “normally”. When the cage wall was tapped, the birds became alert and rushed towards feed and water. Their feathers were shiny and droppings were of “normal” consistency.

By 1 and 2 weeks post-treatment, the behavior of the Group B1 birds did not differ to that of control negative (Group A) birds. However, the birds in Groups B2 and B3 showed slightly dull, depressed behavior and loose droppings compared to control negative birds. By 3 weeks post-treatment, the behavior of birds in Group B1 showed depressed behavior, ruffled feather and loose droppings in comparison to the control negative group, while those in Groups B2 and B3 showed lower interest towards feed and loose to watery droppings. By 4 weeks post-treatment, behavioral alterations and changes in fecal consistency were evident in the Group B2 birds, while Group B2 and Group B3 birds were showing severe clinical signs and behavioral alterations including dull feathers, low attraction towards feed and water and loose to watery droppings.

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Table 4: Clinical signs and behavioral alterations scores of broilers administered varying doses of combinations of NSAIDs compared to control negative (Group-A)

Weeks	Treatment groups	Clinical signs and alterations in behavior (score range 0-4)				
		Alertness	Attraction towards feed	Attraction towards water	Fecal consistency	Total score
	A	4	4	4	4	16
1	B1	4	4	4	4	16
	B2	4	3	4	4	15
	B3	4	4	3	3	14
2	B1	4	4	3	4	15
	B2	4	4	3	3	14
	B3	3	4	4	3	14
3	B1	4	4	4	3	15
	B2	3	4	3	3	13
	B3	3	3	4	3	13
4	B1	4	4	4	3	15
	B2	3	3	4	3	13
	B3	3	3	3	3	12

A: Negative control; B1: ASA, 300 mg/L, Ibuprofen, 20 mg/L and APAP, 30 mg/L; B2: ASA, 600 mg/L, Ibuprofen, 60 mg/L and APAP, 90 mg/L; B3: ASA, 1200 mg/L, Ibuprofen, 120 mg/L and APAP, 160 mg/L

Serum biochemistry

Alanine-aminotransferase

At 1st sampling (Day 14), treatments with the varying dosages of the combined NSAIDS had no effect ($P > 0.05$) on serum ALT concentrations. By Day 42, serum ALT of the Group B1 and B2 birds did not vary ($P > 0.05$), while those of Group B3 birds were higher ($P \leq 0.05$) higher than that of the control negative (Group A) birds.

Aspartate-aminotransferase

At Day 14, mean serum AST of the Group B1 and Group B2 birds did not vary ($P > 0.05$), while those of Group B3 birds were lower ($P \leq 0.05$) than that of the control negative birds. By Day 42, mean serum AST of the Group B1 and Group B2 birds did not vary ($P > 0.05$), while those of Group B3 birds were higher ($P \leq 0.05$) higher than that of the control negative birds.

Creatinine

At both Day 14 and Day 42, treatment with the combined NSAIDs had no effect ($P > 0.05$) on serum creatinine concentrations.

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Urea

At Day 14, treatment with the combined NSAIDs had no effect (P > 0.05) on serum urea concentrations. By Day 42, serum urea concentrations of Group B1 and Group B2 birds did not vary (P > 0.05), while those of Group B3 birds were lower (P≤0.05) lower than the control negative (Group A) birds.

Table 5: Mean (± SD) ALT, AST, creatinine and urea concentrations of broilers administered varying doses of combinations of NSAIDs and percentage comparison with control negative (Group-A)

Treatment group / Subgroups		ALT (IU/L)		AST (IU/L)		Creatinine (mg/dL)		Urea (mg/dL)	
		S1	S2	S1	S2	S1	S2	S1	S2
A		14.59 ±4.91	11.91 ±1.47	263.33 ±79.31	142.00 ±7.48	2.07 ±0.90	2.20 ±0.59	28.91 ±7.13	34.18 ±5.66
B	B1	15.54 ±1.70 (10.66%)	12.58 ±1.14 (6.55%)	191.33 ±9.23 (-27.77%)	166.00 ±17.66 (17.24%)	2.31 ±0.21 (35.29%)	1.63 ±0.31 (-27.27%)	22.63 ±0.60 (-29.68%)	31.38 ±6.05 (-8.82%)
	B2	12.86 ±2.78 (-12%)	13.04 ±0.19 (9.83%)	198.00 ±10.19 (-25.92%)	160.00 ±22.44 (13.79%)	1.48 ±0.38 (-17.64%)	2.29 ±1.06 (0%)	25.73 ±4.06 (-20.31%)	33.00 ±3.63 (-2.94%)
	B3	13.71 ±1.03 (-5.33%)	**14.65* ±0.88 (24.59%)**	**152.66* ±21.96 (-42.59%)**	**200.00* ±10.09 (40.84%)**	1.46 ±0.27 (-17.64%)	2.28 ±0.56 (0%)	29.71 ±1.69 (-13.23%)	**15.70* ±6.64 (-51.56%)**

A: Negative control; B1: Acetylsalicylic acid, 300 mg/L, Ibuprofen, 20 mg/L and Acetaminophen, 30 mg/L; B2: Acetylsalicylic acid, 600 mg/L, Ibuprofen, 60 mg/L and Acetaminophen, 90 mg/L; B3: Acetylsalicylic acid, 1200 mg/L, Ibuprofen, 120 mg/L and Acetaminophen, 160 mg/L

S1: slaughtered at 14 d; S2: slaughtered at 42 d

Bolded and * values are significantly different (P ≤ 0.05) from the control negative group

Total proteins

At both Day 14 and Day 42, serum total proteins concentrations in combination of NSAIDs-treated birds did not vary (P > 0.05) to that of the control negative birds.

Albumin

At Day 14, serum albumin concentration of the birds in Group B1 and Group B2 did not vary (P > 0.05), while that in Group B3 birds was higher (P≤0.05) compared to the control negative birds. By the 2nd sampling (Day 42), serum albumin concentration of the birds in Group B1 and Group B2 did not vary (P > 0.05), while those in Group B3 were lower (P≤0.05) than the control negative birds.

Globulins

At Day 14 and Day 42, treatment with the NSAIDs combinations had no effect (P > 0.05) on serum globulins concentrations with the control negative (Group A) birds.

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Lactate dehydrogenase

At Day 14, LDH in the Group B1 birds did not vary (P > 0.05), while that of the Group B2 and B3 birds was lower (P≤0.05) than that of the control negative birds. By Day 42, treatment with the varying doses of the combined NSAIDs had not effect (P > 0.05) on mean LDH.

Table 5: Mean (± SD) Total proteins, albumin, globulins and LDH concentrations of broilers administered varying doses of combinations of NSAIDs and percentage comparison with control negative (Group-A)

Treatment group / Subgroups		Total proteins (g/dL)		Albumin (g/dL)		Globulins (g/dL)		LDH (IU/L)	
		S1	S2	S1	S2	S1	S2	S1	S2
A		5.73 ±0.87	5.69 ±1.07	2.46 ±0.25	2.53 ±0.23	3.27 ±1.09	3.15 ±1.05	3882.90 ±713.12	2830.55 ±258.61
B	B1	6.39 ±1.71 (11.51%)	4.73 ±0.89 (-16.87%)	2.58 ±0.24 (4.87%)	2.39 ±0.30 (-5.53%)	3.34 ±1.88 (2.14%)	2.33 ±1.14 (-26.03%)	3445.77 ±356.21 (-11.25%)	2935.79 ±307.25 (3.71%)
	B2	6.63 ±0.85 (15.70%)	5.75 ±2.00 (1.05%)	2.50 ±0.35 (1.62%)	2.67 ±0.40 (5.53%)	4.12 ±0.73 (25.99%)	3.90 ±1.83 (23.80%)	**2903.41* ±238.31 (-25.22%)**	2674.05 ±73.60 (-5.52%)
	B3	5.80 ±1.31 (1.22%)	4.22 ±0.19 (-25.83%)	**3.04* ±0.24 (23.57%)**	**1.84* ±0.28 (-27.27%)**	3.21 ±1.10 (-1.83%)	1.54 ±0.60 (-51.11%)	**2760.40* ±132.02 (-28.90%)**	2895.31 ±53.89 (2.28%)

A: Negative control; B1: Acetylsalicylic acid, 300 mg/L, Ibuprofen, 20 mg/L and Acetaminophen, 30 mg/L; B2: Acetylsalicylic acid, 600 mg/L, Ibuprofen, 60 mg/L and Acetaminophen, 90 mg/L; B3: Acetylsalicylic acid, 1200 mg/L, Ibuprofen, 120 mg/L and Acetaminophen, 160 mg/L

S1: slaughtered at 14 d; S2: slaughtered at 42 d

Bolded and * values are significantly different (P ≤ 0.05) from the control negative group

Gene expression of HSP-70

The mRNA expression level of HSP-70 did not vary (P > 0.05) in Group B1 birds, but was increased (P ≤ 0.05) in Group B2 and Group B3 birds compared to the control negative birds.

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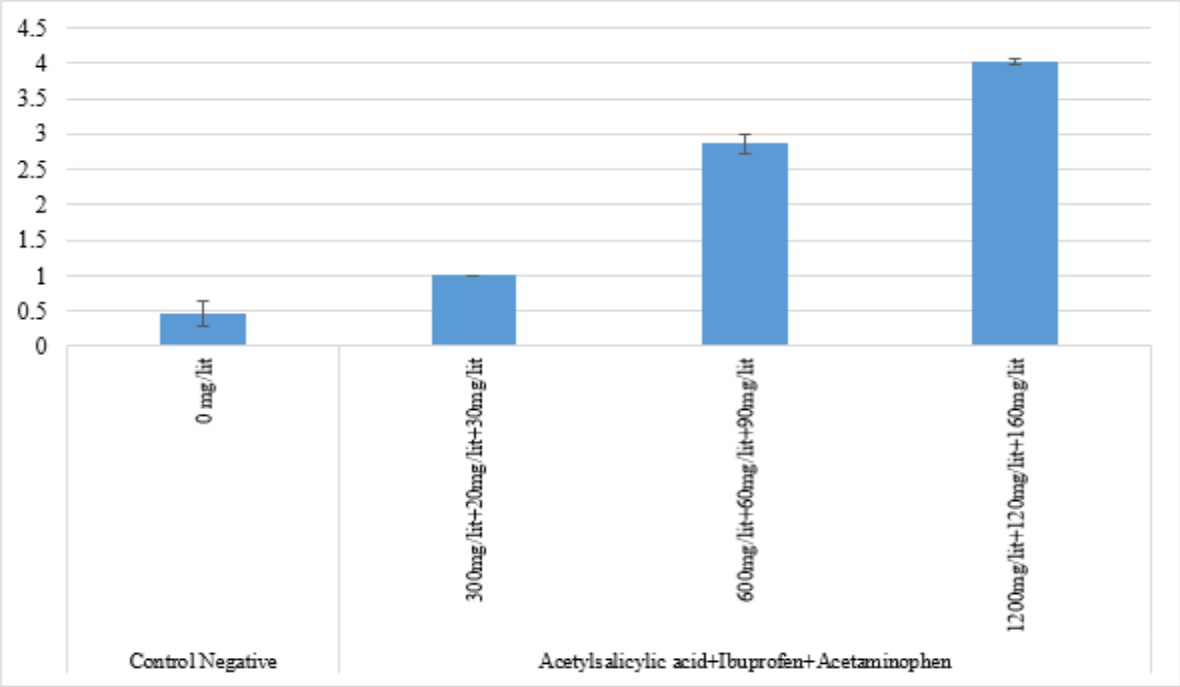

Fig 1: mRNA expression level of hsp-70 of broilers administered varying doses of combinations of NSAIDs compared with control negative (Group-A)

4. DISCUSSION

The major objective of current study was to examine the effects of varying dosages of combined NSAIDS either (acetylsalicylic acid, acetaminophen and ibuprofen) in broilers. The results showed the effects of NSAIDs with combinations of weekly live weight, slaughter and carcass weights at 1st slaughtering (Day 14) and 2nd slaughtering (Day 42).

Administration of 600 mg/L ASA + 60 mg/L APAP + 90 mg/L ibuprofen (Group B2) resulted in a 11.64% reduction in weekly liveweight gain by week 3, while administration of 1200 mg/L ASA + 120 mg/L APAP + 160 mg/L ibuprofen (Group B3) resulted a 26.99%, 19.59% and 38.17% reduction in weekly liveweight at 1, 3 and 4 weeks, respectively, compared to the control negative (Group A) birds (Shafi et al., 2015). The Group B3 birds also had lower slaughter weights (Day 14). In contrast, administration of 300 mg/L ASA + 20 mg/L APAP + 30 mg/L ibuprofen (Group B1) resulted in a 31.85% increase in slaughter weight (Day 42), which was similar to the results reported by Alagawany et al. (2017). The decrease in growth with the high dose of the combined NSAIDS could be attributed to decreased protein synthesis and also due to decreased production of pancreatic digestive enzymes (Fathi et al., 2016).

Adverse effects were noted in both the Group B2 and Group B3 broilers, indicating that these doses of the combined NSAIDs were inappropriate for poultry by (Israr et al., 2021).

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Muneeb et al. (2022) also described that, both the medium (Group B2) and high (Group B3) doses of the combined NSAIDs affected serum biochemistry, including ALT, creatinine and AST. Hori (2010) and Gomaa (2018) also reported similar results of NSAIDs on serum biochemistry. Serum albumin was lower in Group B3 broilers at Day 42, while Group B3 broilers had higher serum albumin at Day 14, similar to the findings of Godhasara et al. (2014). The mean LDH of both Group B2 and Group B3 broilers was lower in comparison with the control negative (Group A) birds at 1st slaughtering (Day 14). The NSAIDs block the prostaglandins and may lead to decreased blood flow towards the kidneys, which means a lack of oxygen to keep the kidneys alive and ultimate cause kidney damage (Zoubek et al., 2020).

The mRNA expression level of hsp-70 was increased in the Group B2 and Group B3 broilers. The increased expression of Hsp-70 may be a response to the repairing of damaged proteins (Hartl, 1996; Gouda et al., 2024) in tissues experienced by the birds.

5. CONCLUSION

Only a low dose of the combined NSAIDs could be safely recommended for use in broilers, as both the medium and high doses of the combined NDAIDs produced various adverse effects in the broilers. Thus, while the NSAIDs combinations could be used as a possible substitute for antipyretic, anti-inflammatory and analgesic drugs for poultry.

Conflict of Interest:

According to authors, there is no conflict of interest.

Authors contribution:

IH, MTJ and MKS contributed in planning of research, data analysis and manuscript writing. IH, MTJ, MKS and MMG executed research plan, laboratory analysis. MTJ, MMG and MKS helped in proof reading, manuscript preparation and data interpretation.

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Abbreviations:

APAP	N-acetyl-para-aminophenol / acetaminophen
ASA	Acetylsalicylic acid
ALT	Alanine transaminase
AST	Aspartate transaminase
BCG	Bromocresol green
BW	Body weight
cDNA	complementary DNA
CRD	Completely randomized design
dL	Deciliter
h	Hour
HSF	Heat shock (transcription) factor
HSP-60	Heat shock protein-60
HSP-70	Heat shock protein-70
HSP-90	Heat shock protein-90
g	Gram
kg	Kilogram
L	Liter
LDH	Lactate dehydrogenase
min	Minute
ml	Milliliter
mRNA	Messenger ribonucleic acid
nm	Nanometer
NSAIDs	Non-steroidal anti-inflammatory drugs
NAPQI	N-acetyl-p- benzoquinone imine
PGE	Prostaglandin
RNA	Ribonucleic acid
rpm	Revolutions per minute
sec	Second
tab	Tablet
µl	Microliter