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**Effect of Stress in Some Blood Parameters and Heat Shock  
Protein 70 of Awassi Syrian Lambs**

**ABSTRACT**

This study aimed to look into the impact of road transportation in the dry, hot region of Erbil-Kurdistan, Iraq, on blood parameter changes and heat-shock protein 70 (HSP70) expressions in lambs as welfare indicators. A total of thirty Awassi Syrian sheep 1-year-old lambs with an average live weight of  $63.894 \pm 0.48$  kg were subjected to three pre-slaughter handling groups of ten (10) animals each one day before transportation. The first group was kept under normal farm conditions and used as a control (T1). The other two groups were loaded into an open truck and driven for 2 hours before being unloaded and then lairage for 0h (T2) 12h (T3). According to the findings, the response of biochemical parameters and heat-shock protein in kidney and liver tissues rose significantly. Cortisol, adrenaline, noradrenaline, glucose, and lactate levels were considerably higher in T2 than in T1 and T3 lambs. According to heat shock protein 70 expressions, transit without shade was more stressful for the lambs. Therefore, it is necessary to use an appropriate resting time following road transportation pre-slaughter to prevent welfare problems.

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**INTRODUCTION**

Transporting animals to slaughter is an essential step in the meat processing process. It is a traumatic experience with long-term implications for animal welfare and meat quality. High ambient temperature causes physiological stress reactions in animals while transporting them from the farm to the abattoir (Terlouw and Bourguet, 2022). When animals are put through stressful situations, their sympathetic nervous system sets in. When animals are exposed to stressful events or stimuli, stress-metabolites and hormones are released into the circulation. As a result, numerous studies have employed a number of indicators to assess the welfare aspect of pre-slaughter road transportation stress in a variety of animals, including hormones (cortisol, adrenaline, and noradrenaline) and haematological markers (glucose and lactate) (Simonov et al., 2022; Abubakar et al., 2021; Miranda-de la Lama et al., 2011). Furthermore, most protein production is slowed when living organisms are subjected to heat stressors during transit. Heat shock proteins (HSP) is a group of highly conserved proteins that are rapidly synthesised. Heat shock proteins have been shown to protect stressed cells and organisms and prevent or reverse stress-related illnesses. The protein works as a molecular chaperone by interacting with other cellular proteins, aiding intracellular transport, and folding into suitable secondary structures. As a result, preventing protein aggregation during stress is essential (Zulkifli et al., 2010).

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Food animals are carried worldwide because of the strength and specialisation of livestock production in specific areas and the need to sell and kill them in places where they are not cultivated. Animal welfare and carcass quality are negatively affected during transport when animals are exposed to various potential pre-slaughter stresses. However, studies have shown that allowing lambs to rest after transportation allows them to recuperate from the stress of transportation (Xin et al., 2017). Although the little study has been done in this area, there is little information on the effects of pre-slaughter road transportation in dry, hot conditions on biochemical blood parameter and heat shock protein 70 in lambs. As a result, the current study looked into the impact of pre-slaughter road transportation on biochemical blood parameter changes and heat shock protein 70 expressions as welfare indicators in lambs in dry, hot regions.

## **MATERIALS AND METHODS**

### **Animals Transportation and Trials**

The experiment took place over two days (July 25th and 26th, 2021), with an average environmental temperature of 38.14 degrees and relative humidity of 14.9%. Thirty-one-year-old Awassi Syrian sheep lambs with an average live weight of  $63.894 \pm 0.48$  kg were employed. All lambs came from the same farm near Erbil and were treated to the same regular management. Before transportation, the animals were divided into three pre-slaughter groups of ten animals per day. The first group did not travel and was utilised as a control group (T1). The other two groups were put onto an open truck covered in straw and carried by road for 2 hours at a density of 0.25 m<sup>2</sup> per animal (Xin et al., 2017), followed by shade for 0h (T2), and 12h (T3). The route was flat, and the speed limit was 60 kilometres per hour. The animals were carefully loaded and unloaded on a driving board. During shade, the animals had unlimited access to water but were deprived of food.

### **Blood Sampling**

During slaughter, 10 mL of sticky blood was collected through jugular venipuncture and centrifuged in EDTA tubes at 3000 RPM for 15 minutes at 4 °C. The plasma samples were divided into 3 ml and stored at -20 °C until biochemical parameters (glucose and lactate) and stress hormones were determined (cortisol, adrenaline, and noradrenaline).

### **Determination of Biochemical Blood Parameters**

The contents of glucose and lactic acid were determined using a Sigma-Aldrich Diagnostic kit (USA). The analyses were performed according to the manufacturer's instructions for each variable.

### **Determination of Stress-linked Hormones**

Cortisol levels were determined using a competitive enzyme test (EIA, RADIM, Pomezia, Italy). Adrenaline and noradrenaline levels were assessed simultaneously using a non-competitive assay (kit CatCombi ELISA, IBL Laboratories Immuno-biológicos, Hamburg, Germany). Samples were measured in triplicate, as recommended by the manufacturer.

### **Liver and Kidney Samples**

According to Muslim or halal slaughter procedures, the animals were slaughtered in a commercial abattoir (Erbil's slaughterhouse for ruminants - Kurdistan Region, Iraq). The technique involved cutting neck and severing both carotid arteries and jugular veins. Lambs were slain in a lateral position in all treatments (animal upon its left side). The liver and kidney samples were snap-frozen in liquid nitrogen after bleeding and evisceration and stored at -20 °C until HSP 70 analyses.

### **SDS-Quantification and Immunoblot**

Utilising the approach described by Zulkifli et al. (2010), the quantity of HSP 70 was determined. Approximately 0.5 g of heart and kidney tissue was homogenised in 5 ml of cold Tris-HCl solution containing 20 mM Tris pH 7.5, 0.75 mM NaCl, and 2 mM 2-mercaptoethanol test before being centrifuged at 23000 RPM for 30 minutes at 4 °C with a protease inhibitor. The Bicinchoninic Acid Protein Assay Kit (Sigma-Aldrich®, USA) was used to assess the concentration of proteins in the supernatants, with bovine serum albumin serving as the standard. Thirty micrograms of total protein were separated on 1.5 x 80 x 100 mm 12% polyacrylamide gels containing SDS. The gels were then subjected to 150 V electrophoresis until the tracking dye reached the gel's base. The separated proteins were visualised with Coomassie blue staining and

transferred to Thermo Scientific™ PVDF Transfer Membranes (Thermo Scientific, USA). PVDF membranes were stained with 0.5 g/l Ponceau S in TBS buffer (100 mM Tris-HCl; 150 mM NaCl) for 5 minutes with constant shaking on a wave shaker to visualise and mark the locations of proteins used as molecular weight standards. After rinsing the Ponceau S with distilled water, the non-specific binding sites were blocked with 10 ml of cold blocking buffer containing TBST+ 3% bovine serum albumin for 60 minutes. The membranes were blocked with 10 ml of blocking solution containing a 1:1000 dilution of antiserum (mouse anti-chicken HSP 70; Sigma-Aldrich®, USA) at 4 °C overnight. After overnight incubation, the blots were washed three times for five minutes each with 10 ml of cold blocking buffer. For one hour, the blots were then treated with a secondary goat anti-mouse antibody conjugated to alkaline phosphatase (Sigma-Aldrich®, USA). After the bands attained colour, the membranes were air-dried at room temperature on filter paper. Utilising the Quantity One Pro application and a calibrated GS-800 densitometer, the reflective density of HSP 70 was measured (BIO-RAD, USA).

### Statistical Analysis

The experiment was conducted as a completely randomised design (CRD). The GLM procedure of the Statistical Analysis System package (SAS) Version 9.2 software (Statistical Analysis System, SAS Institute Inc., Cary, NC, USA) was utilised for all statistical analyses, and  $p < 0.05$  was chosen as the level of statistical significance. The data were subjected to a one-way analysis of variance (ANOVA) using a model that considered treatment and animal as a possible source of variation. The multiple range tests of Duncan's were utilised to determine which means differed significantly.

### RESULTS AND DISCUSSION

The results of the study showed that transport causes stress in farm animals, which increases their physiological responses to biochemical changes and metabolic products. The mean values of the analysed biochemical variables (Table 1) indicate that transport substantially affected the investigated variables. The lambs that were transported by road for two hours and then shade for zero hours (T2) had significantly greater levels of blood glucose and lactate than those who were not transported (T1) and those who were transported for two hours and then shade for 12 hours (T3). Glucose and lactate levels were also observed to increase after the transporting lambs in other experiments (Liste et al., 2011; Zhong et al., 2011; Cozar et al., 2016; Saribey and Karaca, 2018). Due to glucose's function in the energy metabolism under stress, its quantification has become a practical approach for determining pre-slaughter stress and muscular activity (Stewart et al., 2018). Due to increased glycogenolysis and gluconeogenesis and higher catecholamines and glucocorticoids, glucose levels rise in stressful situations (Hemsworth et al., 2019). By changing the metabolism of carbohydrates, proteins, and lipids, glucocorticoids increase blood glucose levels. On the other hand, stress-induced hyperglycemia involves multiple mechanisms, the most notable being an interplay between glucagon, catecholamines, and glucocorticoid hormones, as well as a reduction in insulin production (Mormède et al., 2007). As a result of anaerobic glycolysis, the significant increase in blood lactate concentration during road travel may be a stress signal in the current experiment. When the circulation system can't longer supply the working muscle with sufficient oxygen, there occurs a shift to anaerobic oxidation, the consequence of which is an increment in the concentration of blood lactate.

**Table (1): Effect of pre-slaughter handling on plasma metabolites in lambs**

| Parameter           | Treatment                  |                             |                            | P-value |
|---------------------|----------------------------|-----------------------------|----------------------------|---------|
|                     | T1                         | T2                          | T3                         |         |
| Glucose (mg/ml)     | 62.200 ± 1.07 <sup>a</sup> | 84.900 ± 24.60 <sup>b</sup> | 69.075 ± 0.60 <sup>a</sup> | 0.5275  |
| Lactic Acid (mg/ml) | 7.982 ± 0.19 <sup>a</sup>  | 14.625 ± 0.39 <sup>b</sup>  | 9.100 ± 0.30 <sup>a</sup>  | <.0001  |

T1: Non-transported group used as control.

T2: Lambs transported by road for 2h with 0h shade.

T3: Lambs transported by road for 2h with 12h shade.

<sup>a,b</sup> Values with different superscripts differ significantly at ( $P < 0.05$ ).

### The concentration of Stress-linked Hormones

The current study showed in Table 2 displays the current investigation's mean values of stress hormone variables. When lambs were carried by road for two hours and then shade for zero hours (T2), plasma cortisol concentrations were considerably higher than when they were not transported (T1) or when they were transported with shade (T3). The results indicate that lambs are stressed after two hours of non-lairage transit in an open vehicle during hot weather (about 38 degrees Celsius). This result aligns with those of Saribey and Karaca (2018) and De la Fuente et al. (2012). They discovered that lambs carried for two hours had considerably higher cortisol levels than lambs not transported. Liste et al. (2011) discovered a substantial increase in cortisol levels in lambs transported without lairage as compared to lambs shade for 12 hours before slaughter. The decreased levels of stress hormones in lambs transported by shade indicate that they have had sufficient time to recover from their stress. According to Grandin (2020), animal shade at the slaughterhouse is a widespread practice in the industry that aids livestock in recovering from transportation stress before slaughter. Lambs transferred without shade for two hours showed higher amounts of adrenaline and noradrenaline than lambs transported with shade and sheep that were not transported (Table 2). These results demonstrated that these hormone levels remained raised for at least two hours following transit, allowing animals to mobilise metabolic resources in response to stress rapidly. This is the explanation offered by Afsal and others (2018). According to the study, large amounts of adrenaline and noradrenaline are released into the bloodstream in stressful conditions to prepare the body for rapid energy expenditure. Similarly, Kadim et al. (2010) found that transporting lambs significantly boosted their adrenaline and noradrenaline levels compared to lambs who were not transferred.

**Table (2): Effect of pre-slaughter handling on plasma stress-linked hormones in lambs**

| Parameter            | Treatment                  |                            |                            | P-value |
|----------------------|----------------------------|----------------------------|----------------------------|---------|
|                      | T1                         | T2                         | T3                         |         |
| Cortisol (ng/ml)     | 93.750 ± 0.63 <sup>b</sup> | 95.750 ± 2.07 <sup>a</sup> | 93.650 ± 1.08 <sup>b</sup> | <.0001  |
| Adrenaline (ng/ml)   | 26.675 ± 0.35 <sup>b</sup> | 53.225 ± 0.40 <sup>a</sup> | 27.350 ± 0.33 <sup>b</sup> | <.0001  |
| Noradrenalin (ng/ml) | 19.665 ± 0.37 <sup>c</sup> | 34.300 ± 0.94 <sup>a</sup> | 22.307 ± 0.38 <sup>b</sup> | <.0001  |

T1: Non-transported group used as control.

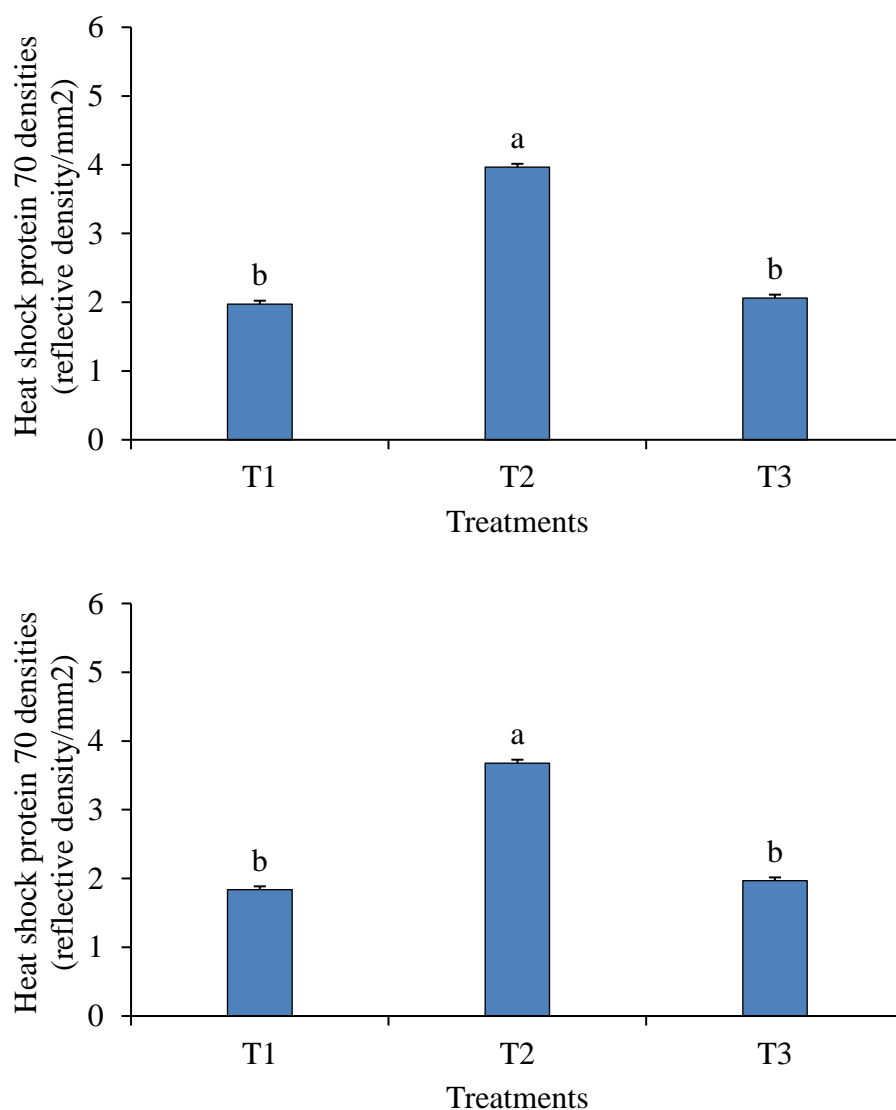
T2: Lambs transported by road for 2h with 0h shade.

T3: Lambs transported by road for 2h with 12h shade.

<sup>a,b</sup> Values with different superscripts differ significantly at (P<0.05).

### Expression of Heat-Shock Protein 70 (HSP70)

Heat shock proteins (HSP) perform an essential function as molecular chaperones during protein formation and disassembly, folding and unfolding, translocation, and interaction with damaged proteins across various environmental and physiological conditions. HSP70, a known sign of stress, is one of the most prominent and widely expressed heat shock proteins. Its expression within cells is a natural reaction to stress (Archana et al., 2017). It has been established that heat shock proteins protect stressed cells and organisms and prevent or reverse diseases caused by transportation stress in animals (Sharma et al., 2013). After transit, HSP 70 densitometry readings revealed organ-specific differences (Figure 1). HSP 70 expression was more significant in the kidney and liver tissues of lambs transported by road without shade compared to lambs that were neither moved nor transported with lairage (p 0.05). The average HSP 70 densities transported by shade lambs did not differ significantly from those conveyed by controls. Transportation by road has been shown to increase HSP 70 expression in goats (Hu et al., 2020) and chickens (Xing et al., 2017). It has been demonstrated that animal transportation increases HSP70 expression in organs such as the kidneys and heart (Rout et al., 2016). The findings of the present study indicate that lamb HSP 70 expression can be induced by transport stress. The high ambient temperature plays a critical role in inducing physiological stress responses during road transport of farm animals.



**Figure (1): Effect of pre-slaughter handling on heat shock protein 70 density of kidney (top) and liver (bottom) tissues in lambs.**

T1: Non-transported group used as control.

T2: Lambs transported by road for 2h with 0h shade.

T3: Lambs transported by road for 2h with 12h shade.

<sup>a,b</sup> Values with different superscripts differ significantly at ( $P < 0.05$ ).

## CONCLUSION

In conclusion, according to biochemical blood parameters and heat shock protein 70, the results of the present study indicated that lambs subjected to pre-slaughter road transit without lairage had a more intense stress response. In order to prevent welfare issues, it is essential to optimise the logistics of vehicle transportation in dry, hot regions.

## Disclosure Statement

The authors have disclosed no competing interests.

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### تأثير الاجهاد في بعض الصفات الدمية وبروتين الصدمة الحرارية 70 للحملان العواسية السوري

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#### الخلاصة

هدفت الدراسة الى معرفة تأثير النقل في ظروف الاجهاد الحراري في بروتين الصدمة الحرارية 70 وبعض الصفات الدمية ورفاهية الحملان اجريت الدراسة على ثلاثين حمل من سلالة الأغنام العواسي السورية البالغة من العمر سنة واحدة وبمتوسط وزن حي  $63.894 \pm 0.48$  كغم قسمت الحملان الى ثلاث مجموعات متساوية قبل الذبح تتكون كل منها من 10 حيوانات، قبل يوم واحد من النقل. تم الاحتفاظ بالمجموعة الأولى في الظروف العادية في المزرعة واستخدامها كمعاملة السيطرة بينما تم تحميل المجموعتين الأخرين في شاحنة مكشوفة وتم نقلهما براً لمدة ساعتين ثم ذبحت مباشرة بدون فترة استراحة (المجموعة الثانية) او ذبحت بعد 12 ساعة من الاستراحة (لمجموعة الثالثة). أظهرت النتائج زيادة معنوية في القيم الدم البيوكيميائية واستجابة بروتين الصدمة الحرارية 70 لنسجي الكلى والكبد. كان لدى الحملان المجموعة الثانية مستويات أعلى من الكورتيزول والأدرينالين والنورادرينالين والجلوكوز واللاكتيك مقارنة بالحملان المجموعة الأولى والثالثة. كان النقل بدون فترة من الاستراحة قبل الذبح أيضًا أكثر إجهادًا للحملان بناءً على تعبير في بروتين الصدمة الحرارية 70. لذلك، من الضروري استخدام وقت الراحة مناسب بعد النقل البري وقبل الذبح الحيوان لمنع مشاكل الرفاهية.

#### الكلمات المفتاحية:

الرفق بالحيوان، المناخ حار وجاف، الاستراحة قبل الذبح، لحملانا، النقل