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Impact of Long-distance Ship Transport on Physiological Parameters of Imported Sheep to Saudi Arabia

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ABSTRACT

Background: Transporting of live animals by ship raises serious animal welfare concerns. Studying and evaluating the physiological impact is essential because no studies cover all the transport effects. This study aims to compare the potential impact of long-distance sheep transportation duration within the lower and upper decks of the ships from Romania and Spain.

Methods: The study included 96 healthy 12-month-old male sheep of the CONJ MESTIZO breed who arrived at Jeddah Islamic Port following a 10-day sea voyage from Romania and 12 days in Spain. The sheep were categorised based on the distance journeyed, weight (40-49 and 50-59 kg) and deck location (upper and lower). A comparative analysis between groups was utilised to assessthe influence of long-distance maritime excursions on changes in physiological parameters and liver enzyme activity, as well as cortisol and cytokine levels.

Result: Significant differences in temperature, pulse and respiration between Romanian and Spanish sheep. Heavier sheepe xhibited higher blood pressure, indicating greater changes. Liver enzyme activity (ALT, AST, γ-GT, AST/ALT) also varied significantly. Cortisol levels were higher in lower deck sheep (14.49±0.84, 12.12±0.77, 35.50±2.09 and 35.47±2.10 ng/mL) compared to upper deck sheep (6.614±0.39, 5.71±0.86, 27.69±1.97 and 24.81±0.98 ng/mL). Smaller sheep had higher cortisol, indicating increased vulnerability. Lower deck sheep from both Romania and Spain had higher IL-17 (37.47±1.503, 36.30±2.076 and 41.31±1.846, 44.37±1.662 pg/mL) and lowerIL-10 (50.14±0.82, 50.86±2.344 and 62.83, 61.25±1.445 pg/mL) compared to upper deck sheep. In conclusion, long-distance sea transport induces significant physiological changes in sheep, with the lower deck exhibiting the most pronounced effects.

Key words: Animal health, Immunity, Physiology, Sea transport, Sheep.

INTRODUCTION

Transportation is unavoidable in sheep farming owing to the necessity for breeding, fattening and marketing. The importance of animal welfare during transportation is a major issue that, if handled, has the potential to reduce mortality and avert economic losses (Carnovale, 2022). Transporting animals in ships poses serious threats to their welfare, particularly when the distance is great (Phillips and Santurtun, 2013). Sheep are prone to a variety of illnesses and health difficulties, which cause numerous physiological changes and disturb the functional activities of several critical organs. Because disease outbreaks can occur during long-distance live animal transportation, there are inherent and significant dangers to both animal and public health (Boada-Sana *et al.*, 2021). Physical and psychological strains can be combined during transportation; loading and unloading, mixing with strange animals, loud noises, lack of food and water, extremely high temperatures and the novelty of the transporter or new housing environment can all be stressful on their own, let alone when combined (Earley and Murray, 2010). An animal's responding to stressful events is impacted by its species, breed and prior exposure to the elements, as well as its health, performance level, bodily condition, psychological state and age (Kuraz *et al.*, 2021).

Sheep transport across the sea requires special procedures for safety and health, including space, ventilation, medical care and nutrition. Challenges include famine, waiting periods at ports and handling upon arrival, making livestock vessels more risky than road transport (Nielsen *et al.*, 2022). There was a link between the increasing signs of physiological stress and temperature stress during the transportation; The sheep that carried long distances showed higher levels of stress at the bottom site, as well as those in the front of the transporter (Miranda-de *et al.*, 2018). Stressors affecting animals in transit pose a serious

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threat to their wellbeing and economy (Minka and Ayo, 2010). Although transporting the animal raised its body temperature, it did not appear to change its heart rate or body weight (Miller *et al.*, 2021). An effective method for researching ruminants' acute and long-term stress reactions during sea transport is heart rate variability (Grisel, 2019). Livestock transportation affects the animals' body temperature, heart rate, respiration rate and reduced body weight (Trisiana *et al.*, 2021). A significant percentage of hunger, thirst and exposure to severe weather are among the serious effects on animal welfare. Ship technical faults or infrastructure concerns directly affected animal wellbeing and conditions at discharge jeopardized the welfare of certain animals (Hing *et al.*, 2021). Stress and an increase in respiration and heart rate might be brought on transportation (Baihaqi *et al.*, 2022). Stress is a huge economic issue and its consequences extend to health and well-being *via* physiological and behavioural changes (Kumar *et al.,* 2018). Body temperature, pulse and respiratory frequency all rose considerably after transportation when compared to pre-transportation values (Li *et al.*, 2024). Transport of livestock causes an increase in temperature, respiratory rate and pulse rate immediately after unloading (Gupta *et al.*, 2019). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were increased during the sea transport (Zulkifli *et al.*, 2019). When animals are transferred over a long distance, their ALT concentration is noticeably greater (Avila-Jaime *et al.*, 2021). After both acute and chronic stress, cortisol levels had an impact on interleukin 10 (IL-10) production; compared to acute stress, chronic stress resulted in a greater generation of IL-10 (Ciliberti *et al.*, 2017). Animal immune systems are impacted by hormones that trigger stress, *via* immune cell multiplication or inhibition, hormonal variations in reaction to stress, which include elevated cortisol levels, are known to have immunomodulatory actions (Jain and Shakkarpude, 2024). Neuroendocrine reactions are triggered by certain ongoing physiological alterations that take place to cope with a variety of environmental circumstances (Sahib *et al.*, 2024). According to Li *et al.* (2019), transportation stress increased the levels of the pro-inflammatory cytokine TNF-α in all livestock breeds; to maintain endocrine balance and adapt to the shifting environment, the anti-inflammatory cytokine IL-10 rose in response. Animals with low body weights that were transported had far greater levels of interleukin 17 (IL-17a) than those of transported, normal-weight animals (Masmeijer *et al.*, 2019). Increased levels of the anti-inflammatory cytokine IL-10 are present, the produced stress elicited no reaction from the cytokines IL-17α (Naylor *et al.*, 2020). There were no differences found between IL-17a stress response groups and there was no change in IL-17a concentrations between 0 and 4 hours after stress induction (Naylor *et al.*, 2021). Certain breeds of livestock showed a rise in IL-10 and IL-17A expression in response to heat stress (Park *et al.*, 2021). Animal transport boosted IL-10 but did not appear to influence IL-17α (Miller *et al.*, 2021). Sheep with varying stress responses, especially those with high and moderate stress reactivity, produce more IL-10 (Avila-Jaime *et al.*, 2021). Therefore, the aim of the study is to identify the extent to which there are effects resulting from the sea transport of sheep over long distances within the lower and upper decks of the ship that may affect the physiological health status of sheep by comparative analysis of the results.

MATERIALS AND METHODS

Experiment design

This study involved 96 healthy male CONJ MESTIZO sheep, aged 12 months, randomly selected from two vessels arriving at Jeddah port from long-distances during the period from October to December 2024. forty-eight sheep were from Romania (G1), traveling 2183 nautical miles for 10 days with loading time of 8 hours, The temperature before departure was 5°C and the humidity was 7% and upon arrival it was 19°C and the humidity was 21%. and 48 were from Spain (G2), traveling 2743 nautical miles for 12 days with loading time of 24 hours. The temperature before departure was 16°C and the humidity was 50% and upon arrival it was 22°C and the humidity was 25%. Both groups were divided by weight (40-49 kg and 50-59 kg) and deck location (upper and lower). G1 (Romania) consisted of BR1 (40-49 kg, lower deck, 12 sheep), UR1 (40-49 kg, upper deck, 12 sheep), BR2 (50-59 kg, lower deck, 12 sheep) and UR2 (50-59 kg, upper deck, 12 sheep). G2 (Spain) consisted of BS1 (40-49 kg, lower deck, 12 sheep), US1 (40-49 kg, upper deck, 12 sheep), BS2 (50-59 kg, lower deck, 12 sheep) and US2 (50-59 kg, upper deck, 12 sheep).

Blood analysis

Blood samples were collected on the vessels before unloading. From the Romania ship, 24 samples were collected from each deck (upper and lower). From the Spain ship, 24 samples were collected from the upper section of the upper deck and 24 from the lower section of the basement. Nine ml needles were used for jugular venipuncture and blood was placed into two sterile tubes per animal. An amount of 5 ml of blood was transferred to a single tube without anticoagulant (Hebei Xinle Sciand Tech Co. Ltd, Chaina) to collect serum for biochemical measurements. The remaining 4 ml blood was placed in Another tube containing anticoagulant (EDTA 3 ml tube K3, Hebei Xinle Sciand Tech Co. Ltd, Chaina) to determine hematological parameters. Plasma was extracted by centrifuge model: Lsc-3a Product Code: D0642206151.

Measurements

Systolic (Syst-Bp), diastolic (Diast-Bp) blood pressure and pulse rate (PR/min) were measured for each animal *via* the animal's right leg, using the appropriate cuff according to the circumference of the leg, placing the foot at the level of the heart and reassuring the animal before taking the

measurement using an electronic sphygmomanometer device (B0BQ6WTFJQ pet sphygmomanometer, Jiadofo, China). The reading was recorded direct. The body temperature of each sheep was measured using an electronic thermometer, model Tuweiuzswtc164y, Asin: B0ckx6p312, TUWEI, China, using infrared radiation through the forehead of each animal, at a distance of 5 cm. The breathing movement, strength and regularity were monitored, observations were made of chest and abdomen movements while they were in a resting state. Each rise and fall cycle of the chest or abdomen was counted as one breath. Then the respiratory rate (RR) of each animal was measured with a stethoscope for 30 seconds, multiplied by 2 to obtain the RR/min. The following chemical tests were conducted: (ALT, AST, AST/ALT) using a device veterinary chemistry analyzer model: vetube 30 - Shenzhen Mindray Animal Medical Technology Cp, Ltd- China. Sheep cortisol levels were measured using sheep cortisol ELISA kit (Catalog No.SL00014Sp). Sheep IL- 17 and IL-10 levels were measured using commercial sheep IL-17 and IL-10 ELISA kit (Biorbyt LTD., Catalog No: orb781937 and Sun Long Biotech Co., LTD- Catalog No: SL00128Sp) respectively. The CV for inter-assay accuracy was less than 12% for cortisol and IL-10 and less than10% for IL-17. The CV for intra-assay precision was less than 10% for cortisol and IL-10 and less than 8% for IL-17, demonstrating significant consistency across testing protocols. The assay ranges (IL-10: 3 pg/ml- 200 pg/ml, IL-17: 15.63-1000 pg/mL and cortisol: 3.6ng/ml-100ng/ml) and sensitivity (0.5 pg/ml, 5.6 pg/mL and 0.5 ng/ml for IL-10, IL-17 and cortisol, respectively). The standard log-dose response curves represented by XY equation for IL-10, IL-17 and cortisol were (Y) OD = 0.1005 + 0.008910(X) pg/m, (Y) OD = 0.5489 + 0.008601 (X) pg/mL and(Y) OD = 1.405 - 0.02269(X) ng/mL respectively.

All laboratory testing for the research was carried out at Qassim University's College of Veterinary Medicine laboratories.

Statistical analysis

Excel was initially utilized to organize and preprocess the data, followed by the GraphPad prism 9 was used to make statistics and calculate the probability value, using the one-way ANOVA system. The Tukey's HSD test was used in a post hoc analysis to compare between groups. The statistical outcomes were represented as Mean±SE, considering $p<0.05$ to indicate a notable difference.

RESULTS AND DISCUSSION

Vital signs

Table 1 shows data on the effect of sea transport on vital signs of two weights of sheep on the lower and upper decks of a ship coming from Romania and Spain. The results showed that in G1 and G2, there was no difference in body temperature (°C) between the decks for both (40-49, 50-59 kg). When comparing G1 and G2, the upper deck body temperature of sheep weighing 40-49 kg showed a significant difference between UR1 and US1 ($p<0.05$). Specifically, G2 had a lower body temperature than G1. UR2 vs US2 also showed a significant difference between the upper deck °C of sheep weighing 50-59 kg at $p<0.05$. Again, G2 had a lower °C than G1 at $p<0.05$. In G1, there was no significant difference in respiratory rate (RR/min) between the lower (BR) and upper (UR) decks for either weight category: 40-49 kg (BR1: 42.93±3.28 RR/min; UR1: 41.00±2.42 RR/min) or 50-59 kg (BR2: 42.36±2.59 RR/min; UR2: 47.00±3.75 RR/min). In G2 at 40-49 kg weight BS 1 and US 1 show a significant difference at $p<0.05$. In weight 50-59 kg there is no significant difference between BS 2 and US 2. When comparing G1 and G2, the

Table 1: Effect of sea transport on vital signs of two weights of sheep on the lower and upper decks of a ship coming from Romania and Spain.

Country	Sheep weight (kg)	Ship deck	°C	RR/min	PR/min	Syst-Bp	Diast-Bp	MAP
Romania (G1)	40-49	BR 1	38.71±0.21	42.93±3.28^{u}	99.57±4.58	159.29±6.68	109.43±4.63	125.71±5.01
		UR 1	38.78±0.22^{v}	41.00±2.42	95.60±3.85	161.93±5.45	109.27±5.99	126.27±5.41
	50-59	BR 2	38.69±0.117	42.36±2.59^{x}	99.73±4.51	160.82±4.33	108.73±4.50	125.82±4.08
		UR 2	39.29±0.45^{y}	47.00±3.75	95.10±5.11	164.70±8.59	123.10±9.60	136.70±9.12
Spain (G2)	40-49	BS 1	38.76±0.22	69.21±7.77^{cu}	99.21±4.55	160.00±12.30	133.40±13.70	141.70±13.20
		US 1	38.35±0.16^{v}	49.15±4.56^{c}	95.31±5.82	149.08±9.57	117.80±10.90	127.80±10.20
	50-59	BS 2	38.61±0.16	68.09±8.76^{x}	100.18±5.05^{d}	138.40±12.20	106.70±13.90	117.00±13.20
		US 2	38.47±0.11^{y}	56.17±6.84	84.17±6.84^{d}	151.60±10.00	122.10±10.90	131.60±10.60
	P-values		<0.0001	<0.0001	<0.0365	<0.0905	<0.0606	<0.2438

Mean±Standard error. °C: Body temperature; RR/min: Respiratory rate per minute, PR/min: Pulse rate per minute; Syst-Bp: Systolic blood pressure; Diast-Bp: Diastolic blood pressure; MAP: Mean arterial pressure; BR1: Basement of Romania ship; UR1: Upper deck of Romania ship; BR2: Basement of Romania ship; UR2: Upper deck of Romania ship; BS1: Basement of Spain ship; US1: Upper deck of spain ship; BS2: Basement of Spain ship; US2: Upper deck of Spain ship, values with the same letters mean significant difference between them at P<0.05.

results showed that BR 1 and BS 1 show a significant difference at $p<0.05$. Also, BR 2 and BS 2 (show a significant difference at ($p<0.05$). In G1, there was no significant difference in pulse rate (PR/min) between the lower (BR) and upper (UR) decks for sheep weighing 40-49 kg (BR1: 99.57±4.58 PR/min; UR1: 95.60±3.85 PR/min) or 50-59 kg (BR2: 99.73±4.51 PR/min; UR2: 95.10±5.11 PR/min) ($p>0.05$). In G2, there is no difference in PR/min of 40-49 kg, BS 1 and US 1. But on the contrary in the weight 50-59 kg, BS 2 and US 2 there was a significant difference between them at $p<0.05$.

Liver function enzymes activity

Table (2) shows data on the effect of sea transport on liver function enzymes activity of two weights of sheep on the basement and upper decks of a ship coming from Romania and Spain. In (G1), there was no significant difference in ALT activity between the two weight groups. In contrast, G2 sheep, weighing 40-49 kg only, showed a significant difference between BS1 and US1 at $p<0.05$. When comparing G1 and G2, there was no significant difference in ALT activity between the groups. AST activity in G1 in the 40-49 kg and 50-59 kg groups did not show significant differences between (BR) and (UR). This is consistent with the following values: 98.7±15.1 U/L (BR1), 146.7±19.9 U/L (UR1), 230.0±71.6 U/L (BR2) and 128.5±14.1 U/L (UR2). In (G2) group 40-49 kg, the results indicated a significant difference between (BS1) and (US1) and the AST activity was (179.33±4.90 and 229.00±2.31 U/L). Also, in the 50-59 kg group, a significant difference was shown between (BS2) and (US2). When comparing G1 and G2 in the 40-49 kg group, a significant difference was found between BR1 and BS1 at ($p<0.05$). Similarly, the difference between UR1 and US1 was significant. However, the 50-59 kg group did not show a significant difference. γ-GT activity in G1, within the 40-49 kg group, showed no significant difference between BR1 and UR1. In the 50-59 kg group, similarly, there was no significant difference between BR2 and UR2. In G2 sheep weighing 40-49 kg, there was a significant difference between BS1 and US1 at $p<0.05$. However, in the 50-59 kg group, there was no significant difference between BS2 and US2 at ($p<0.05$). Regarding AST/ALT, In G1 sheep weighing 40-49 and 50-59 kg, there was no significant difference between BR and UR in AST/ALT. However, in G2 sheep weighing 40-49 kg, a significant difference was found between BS1 and US1 at $P<0.05$. There was nosignificant difference between 50-59 kg sheep. When comparing G1 and G2, a significant difference was found ($p<0.05$) between BR1 and BS1 only.

Cortisol, pro and anti-inflammatory cytokines

Table 3 shows data on the effect of sea transport on cortisol, IL-10 and IL-17 of two weights of sheep on the upper and lower decks of a ship coming from Romania and Spain. First, regarding cortisol, the results clearly indicated that sheep (G1) weighing 40-49 kg showed a highly significant difference between BR 1 compared to UR 1 at $p<0.05$. Sheep weighing 50-59 kg from the same group followed a similar trend to the lighter group, showing a significant difference between BR 2 compared to UR 2 at $p<0.05$. The results for sheep (G2) also indicated that sheep weighing 40-49 kg showed a significant difference between BS 1 compared to US 1 at $p<0.05$. The same trend was observed in heavier Spanish sheep (50-59 kg), with BS 2 compared to US 2 at $p<0.05$. When comparing G1 with G2 sheep, a significant difference was observed between them, with G2 showing significantly higher cortisol levels than G1 in all weight categories and decks ($p<0.05$). Secondly, IL-10 levels in G1 sheep weighing 40-49 kg showed a significant difference between BR1 and UR1 decks at $p<0.05$. In sheep weighing 50-59 kg, no significant difference was observed in IL-10 levels between BR 2 and UR 2 decks. In G2 sheep weighing 40-49 and 50-59 kg, no significant difference was observed in IL-10 levels between the ship decks (BS 1 vs. US 1, BS 2 vs. US 2). When comparing G1 and G2, (40-49 kg),

Table 2: Effect of sea transport on liver function enzymes activity of two weights of sheep on the basement and upper decks of a ship coming from Romania and Spain.

Country	Sheep weight (kg)	Ship deck	ALT (U/L)	AST (U/L)	γ-GT (U/L)	AST/ALT
Romania (G1)	40-49	BR 1	42.0±12.3	98.7±15.1^{u}	65.3±14.9	3.83±1.01^{u}
		UR 1	24.67±0.56	146.7±19.9^{v}	79.2±11.4	7.66±1.25
	50-59	BR 2	28.50±2.60	230.0±71.6	126.9±31.6	7.58±1.82
		UR 2	28.00±4.62	128.5±14.1	80.30±9.01	4.73±0.27
Spain (G2)	40-49	BS 1	20.33±1.52^{c}	179.33±4.90^{cu}	82.27±3.09^{c}	9.02±0.62^{cu}
		US 1	45.50±0.87^{c}	229.00±2.31^{cv}	133.3±28.4^{c}	5.04±0.044^{c}
	50-59	BS 2	22.00±3.46	121.00±4.62^{d}	79.30±7.97	5.84±0.71
		US 2	29.00±1.67	172.3±11.0^{d}	107.23±5.80	5.94±0.212
	P-values		<0.0370	<0.0032	<0.0309	<0.0066

Mean±Standard error, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase; γ-GT: γ-Glutamyl transferase; AST/ALT: Aspartate aminotransferase /alanine aminotransferase ratio; BR1: Basement of Romania ship; UR1: Upper deck of Romania ship; BR2: Basement of Romania ship; UR2: Upper deck of Romania ship; BS1: Basement of Spain ship; US1: Upper deck of Spain ship; BS2: Basement of Spain ship; US2: Upper deck of Spain ship. Values with the same letters mean significant difference between them at $P<0.05$.

IL-10 levels were significantly different between BR1 and BS1 at $p<0.05$. There was also a significant difference in IL-10 levels in sheep weighing 50-59 kg between BR2, BS2 and UR2, US2 andat $p<0.05$. Third, in G1 sheep, weight group 40-49 kg, IL-17 levels in BR1 and in UR1 showed significant differences between the two groups ($p<0.05$). Conversely, in the 50-59 kg weight group, IL-17 levels in BR 2 and in UR 2 showed no significant differences between the two groups. In G2 sheep, weight group 40-49 kg, there was no significant difference in IL-17 levels in BS 1 and in US1. Weight group 50-59 kg, IL-17 levels in BS 2 were (44.37±1.662) and in US2 (35.73±1.579 pg/mL), a significant difference between these two groups at $p<0.05$. When comparing G1 and G2, there was no significant difference in the 40-49 kg weight category, in contrast to the 50-59 kg weight category, which showed a significant difference between BR 2 and BS 2 at $p<0.05$.

Maritime transport is a very important aspect of the global livestock trade. However, this process can be extremely stressful for these animals, leading to a number of welfare issues and potential economic losses (Minka and Ayo, 2010). Understanding sheep's physiological parameters during maritime transport is crucial for developing welfare strategies and ethical treatment. Estimating blood parameters helps assess adaptation and living capacity of animals during long journeys (Sahib *et al.*, 2024). In this study, we monitored the alterations in the levels of physiological parameters (vital signs, cortisol, liver enzymes and cytokines) and conducted a comparative analysis between sheep transported from Romania and sheep transported from Spain (same breed) to their final destination.

Vital signs

For sheep (G1) on the lower and upper deck, differences were observed in RR, PR and BP. While the rest of the vital parameters were relatively stable, this is in line with what was reported by (Trisiana *et al.*, 2021) who showed that livestock transportation affects the animals' body temperature, heart rate, PR. In detail in our results, a slight decrease in the BW of sheep in the lighter weight range (sheep with a body weight of 40-49 kg) occurred compared to those in the heavier weight range, both on the lower and upper deck, which is in agreement with (Earley and Murray, 2010) who reported that the animals who were first carried by ferry and then by land weighed less both after reaching their location and staying on the carrier as well as after being transported to the barn. The study noted that °C was relatively stable in all groups, suggesting that the sheep were able to maintain their temperature regulation during sea transport. This is inconsistent with what was reported by Miller *et al.* (2021) who concluded that although transporting the animal raised its body temperature, it did not appear to change its heart rate. As for the RR, a slight increase was found in the sheep on the upper deck, especially in the heavy weight range, thus this could be attributed to the warmer environment and increased ventilation on the upper deck. The PR remained relatively stable in all groups and was in agreement with what was reported by (Miller *et al.*, 2021) indicating that the cardiovascular systems of the sheep were functioning adequately and the results of this study are in agreement with the RR result while differing with the PR result that was reached with what was reported by (Wojtas *et al.*, 2014) mentioned that it was discovered that when exposed to heat stress, the heart rate, white blood cell count and respiration rate all rose. Syst-BP and Diast-BP were slightly higher in the heavy weight range compared to the lightweight range on upper decks, but were not statistically significant at $P<0.05$. The study found that sea transport had little effect on the vital signs of Romanian sheep. However, sheep on the upper deck showed a slight decrease in °C, lower PR and a slightly higher PR, possibly due to wind or cold air currents. The rates of rise and fall varied depending on the animal's location on the ship. Li *et al.* (2024) reported that body temperature, pulse and respiratory frequency all rose considerably after

Table 3: Effect of sea transport on cortisol, pro and anti-inflammatory cytokines of two weights of sheep on the upper and lower decks of a ship coming from Romania and Spain.

Country	Sheep weight (kg)	Ship deck	Cortisol (ng/mL)	IL-10 (pg/mL)	IL-17 (pg/mL)
Romania (G1)	40-49	BR 1	14.49±0.84^{a}	50.14±0.82^{au}	37.47±1.503^{a}
		UR 1	6.614±0.39^{a}	58.05±2.144^{a}	31.34±1.523^{a}
	50-59	BR 2	12.12±0.77^{b}	50.86±2.344^{x}	36.30±2.076^{x}
		UR 2	5.71±0.86^{b}	55.35±1.352^{y}	34.19±1.881
Spain (G2)	40-49	BS 1	35.50±2.09^{c}	62.83±2.254^{u}	41.31±1.846
		US 1	27.69±1.97^{c}	63.08±1.441	36.50±1.586
	50-59	BS 2	35.47±2.10^{d}	61.25±1.445^{x}	44.37±1.662^{dx}
		US 2	24.81±0.98^{d}	66.00±1.067^{y}	35.73±1.579^{d}
	P-values		<0.0001	<0.0001	<0.0001

Mean±Standard error. IL-10: Interleukin 10; IL-17: Interleukin 17; BR1: Basement of Romania ship; UR1: Upper deck of Romania ship; BR2: Basement of Romania ship; UR2: Upper deck of Romania ship; BS1: Basement of Spain ship; US1: Upper deck of Spain ship; BS2: Basement of Spain ship; US2: Upper deck of Spain ship. Values with the same letters mean significant difference between them at $P<0.05$.

transportation when compared to pre-transportation values. Our results are in complete agreement with Baihaqi *et al.* (2022) who mentioned that an increase in respiration and heart rate might be brought on transportation. The study found that G1 and G2 sheep had slightly different body temperatures on both ships, possibly due to environmental conditions, ship design, or distance. G1 sheep had higher respiratory rate (RR) on the upper deck, while G2 had higher RR on the lower deck. Weight also played a role in physiological responses during transport.

Liver function enzymes activity

The results did not observe any significant difference between the two weight groups in sheep (G1) for ALT activity on the lower and upper decks. However, the decrease was more pronounced in the 40-49 kg group on the upper deck. AST activity appeared significantly higher in sheep in the heavier group compared to those in the lighter group and these levels appeared significantly higher on the upper deck, which is inconsistent with what was reported by (Dalmau *et al.*, 2013) who said that AST activity did not change substantially during transport. Also, contrary to what was reported by Kassab and Mohammed (2014) stated that AST and ALT activity in sheep decrease after transportation compared to before transportation. While the results of this study agree with him regarding the decrease in ALT. Also, it is also consistent with the findings of (Zulkifli *et al.*, 2019) regarding AST and differs from it in ALT, who reported that liver enzymes were increased during the sea transfer following road transfer. The study found that sea transport may affect liver function in sheep, particularly in heavier animals and those on the upper deck, despite no significant difference in γ-GT levels. The higher AST and AST/ALT ratios in heavier weight sheep may indicate increased hepatocellular damage due to higher metabolic demands, physiological changes, or body composition differences and may be influenced by temperature and ventilation. As stated by (Soni *et al.*, 2022), animals exposed to temperature stress caused by ambient temperatures showed significantly higher ALT and AST activity. The study found that sea transport significantly impacts liver enzyme activity in sheep, with sheep in the 40-49 kg group experiencing more changes. The location of the sheep on the ship, particularly on the upper deck, also influenced the results, suggesting that sea transport can negatively impact liver function. Sea transport can significantly impact liver health in sheep, particularly in light-weight animals, by increasing levels of liver enzymes, particularly ALT and AST, which could be influenced by discomfort from motion sickness, heat, noise and crowding. The study compared the liver function of sheep transported on two ships, finding that the weight and location on the ship affected liver function. Both sheep experienced discomfort during transport, potentially contributing to physiological changes in liver enzymes. The effect of sea transport was more pronounced in sheep (G1), with higher AST/ALT ratios on the upper deck. Both ships altered their physiological parameters.

Cortisol

Sea transport for sheep can be challenging due to elevated cortisol levels, which indicate a physiological response to environmental challenges. Deck effects, such as deck effects, may contribute to this physiological change, with all sheep on upper decks showing lower cortisol levels. It can therefore be said that the sheep on the upper deck were less physiologically changed, this is consistent with the results of (Miranda-de *et al.*, 2018) who showed that the animals at the lower location and those at the front of the transporter had much higher cortisol levels and differs from what (Collins *et al.*, 2018) said sheep transferred on the ship's upper deck had more stress than sheep transported on the lower deck. This may be due to several factors such as reduced movement as the upper deck may be exposed to less kinetic vibrations during navigation. Also, the quality of ventilation, as the upper decks often have better airflow, which may positively impact on animal comfort. In addition, the upper deck may be less exposed to noise in general. In contrast to the results of (da Cunha Leme *et al.*, 2012) that cortisol concentrations in sheep carried in the totally enclosed section were lower than those in animals transported in an open section, indicating that animals transferred in a closed environment experienced less stress. The enclosed section (the lower section) was more Physiological change in cortisol level in this study. In summary, the differences observed between G1 and G2 sheep in terms of cortisol levels could be due to transport conditions, which include factors such as loading practices and voyage duration. Longer transport periods can lead to changed cortisol levels, for the longer the transport period. As noted by (El Khasmi *et al.*, 2015) who discussed that serum cortisol levels during medium-distance travel are substantially greater than those obtained during short-distance travel and these values increase during long-distance transportation. Also, Dalmau *et al.* (2013) found higher cortisol concentrations in blood after long-term sheep transfer, possibly due to handling practices during loading and transport. This agrees with the statement of Pascual-Alonso *et al.* (2017) said that cortisol levels rose during the transfer and animal unloading. While on the contrary it differs from the statement Othman *et al.* (2021) who mentioned that before and after transit and unloading, there was no discernible difference in the plasma cortisol concentrations. The 40-49 kg group showed a greater increase in cortisol levels, suggesting that smaller sheep are more vulnerable and responsive to physiological change. This may be due to their smaller size and potential greater sensitivity to environmental changes or factors such as duration and conditions of transport, which is consistent with Marcato *et al.* (2021) stated that cortisol levels in young animals are affected by a variety of factors, including transportation conditions and the duration of the journey.

In general, one of the most important factors affecting cortisol levels is the duration of transport, as longer transport periods can lead to increased cortisol levels, as previous researchers have reported. van Dijk *et al.* (2023) reported that cortisol concentrations varied over time. Many transported animals exceeded the upper reference limits. El Khasmi *et al.* (2015) concluded that serum cortisol levels during medium-distance travel are much greater than those obtained during short-distance travel and these values increase during long-distance transport. Caroprese *et al.* (2020) showed that long distance transport leads to increased cortisol levels, which indicates that transport may cause stress in lambs, as cortisol is a reliable biomarker for assessing stress responses in animals. Dalmau *et al.* (2013) found that blood cortisol concentrations were higher after long-term transport than before short-term transport.

Pro and anti-inflammatory cytokines

Based on the results, the study found levels of IL-10, anti-inflammatory cytokine, were significantly elevated in both weight groups in the upper deck. This further supports the idea of a less inflammatory environment for sheep in the upper deck. This result is in line with what (Park *et al.*, 2021) reported, that some breeds of Livestock show an increase in IL-10 in response to heat stress. (Li *et al.*, 2019) mentioned that all breeds of cattle experienced an increase in the anti-inflammatory cytokine IL-10 due to transport stress. Also, (Ciliberti *et al.*, 2017) stated that after both acute and chronic stress, the amount of IL-10 was affected by cortisol concentrations, compared to acute stress, chronic stress led to increased IL-10 production. Avila-Jaime *et al.* (2021) found that sheep with different stress responses, particularly those with high and medium stress reactivity, produced more IL-10. Immunological variables can be used as stress indicators since the immune system is very sensitive to stress. Immune function is impacted by a variety of factors, including disease, movement and transportation as noted by (Jain and Shakkarpude, 2024). The results for IL-17, a pro-inflammatory cytokine, were in contrast to IL-10, which were higher in the lower decks for both weights compared to the upper decks and were particularly significantly higher in the lighter sheep in the lower deck. which is inconsistent with (Naylor *et al.*, 2020) who said that stress did not elicit any IL-17 cytokine response. The study by Naylor *et al.* (2021) found no differences in IL-17 stress response between sheep groups during sea transport. The upper deck environment caused less physiological change, possibly due to better ventilation and natural light. However, the 40-49 kg group showed a more pronounced response, suggesting smaller sheep may be more susceptible to environmental changes. The study found that IL-10 levels were similar across weight groups, but higher IL-17 levels in the basement of heavier sheep, 50-59 kg, suggest a more pronounced Th17 immune response, possibly due to their greater sensitivity to environmental physiological responses. The study found that lighter sheep on Romanian and Spanish ships were more susceptible to physiological changes, with higher levels of IL-17. Upper deck sheep had higher IL-10 responses, while Romanian sheep showed higher levels in lower decks, indicating a more pronounced Th17 immune response. The lower deck environment is more susceptible to physiological change during sea transport.

CONCLUSION

The findings of this study are noteworthy because they demonstrate that sea journey impacts physiological parameters and that there are considerable changes in physiological response among sheep following long-distance sea transport, with the lower decks exhibiting the most marked alterations. It is important to note that in order to support future research concentrating on more diverse conditions to study physiological parameters, the study employed comparison analysis to illustrate physiological differences depending on distance, weight and location within the vessel. Though it is unable to conclusively identify stress levels, this study offers insights into the relative changes that occur in physiological parameters after long-distance sea transport.

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Disclaimers

The authors' views and conclusions in this study are solely their own and do not necessarily reflect the views of their affiliated institutions. They are responsible for the accuracy and completeness of the information provided, but they accept no liability for any direct or indirect harm resulting from the use of this content.

Informed consent

On September 27, 2023, the Bioethics certificate was obtained from King Abdulaziz City for Science and Technology's National Committee for Bioethics. The research procedure used in the current study was approved by the Standing Committee for Scientific Research Ethics at Qassim University, No. 24-07-02, dated October 1, 2024.There were no operations conducted that may cause pain, suffering, or discomfort to the animals. Blood samples were collected via jugular vein. Vital parameters were measured using electronic instruments that did not cause harm to the animal.

Conflict of interest

The authors of this work state that they have no conflicts of interest related to its publication. There was no impact from funding or support on the study's design, data collection, analysis, manuscript preparation, or publication decision.

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