Effects of Ambient Temperature on Harmful Gas Emissions, Environmental Microorganisms, and Serum Biochemical Indicators in Meat Sheep (Postprint)

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Abstract

The present study investigated the effects of ambient temperature on concentrations of oxygen (O2), carbon dioxide (CO2), methane (CH4), ammonia (NH3), and hydrogen sulfide (H2S) in respiration metabolism chambers, as well as microbial counts and serum biochemical indicators in hybrid meat sheep. A single-factor experimental design was employed with three ambient temperature ranges: 10–15 °C, 20–25 °C, and 30–35 °C. Twenty-four hybrid male sheep (Dorper × Small-tailed Han), approximately one year old and in good body condition with a body weight of (35.34±1.07) kg, were selected and randomly allocated into three groups of eight sheep each. The pre-trial period lasted 7 days, followed by a 10-day formal trial period. During the formal trial period, gas and microbial detection experiments were conducted using respiration metabolism chambers. On the morning of day 10 of the formal trial period, blood samples were collected to determine serum corticosterone (CORT) and immunoglobulin (IgG) concentrations, as well as creatine kinase (CK) and lactate dehydrogenase (LDH) activities in meat sheep. The results showed: 1) Temperature had a highly significant effect on CH4 and NH3 concentrations in the respiration metabolism chambers ($P<0.01$). Specifically, CH4 emissions in the 10–15 °C chamber were highly significantly higher than those in the 20–25 °C and 30–35 °C chambers ($P<0.01$), whereas NH3 emissions in the 30–35 °C chamber were highly significantly higher than those in the 10–15 °C and 20–25 °C chambers ($P<0.01$). However, no significant effect was observed on CO2 and H2S concentrations ($P>0.05$). 2) The number of Escherichia coli in the 30–35 °C respiration metabolism chamber was highly significantly higher than that in the 10–15 °C and 20–25 °C chambers ($P<0.01$). The numbers of Salmonella and total bacteria in the 30–35 °C and 20–25 °C chambers were highly significantly

higher than those in the 10–15 °C chamber (P<0.01). 3) Temperature did not significantly affect serum CORT and IgG concentrations or CK and LDH activities in meat sheep (P>0.05). In conclusion, different ambient temperatures can affect harmful gas emissions and microbial counts in meat sheep, but have no significant effect on serum biochemical indicators.

Full Text

Effects of Ambient Temperature on Harmful Gas Emission, Environmental Microorganisms and Serum Biochemical Indicators of Mutton Sheep

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Abstract

This experiment investigated the effects of ambient temperature on concentrations of oxygen (O), carbon dioxide (CO), methane (CH), ammonia (NH) and hydrogen sulfide (H S), environmental microbial counts, and serum biochemical indicators of crossbred mutton sheep in respiratory metabolic chambers. A single-factor experimental design was employed with three ambient temperature ranges: 10–15 ℃, 20–25 ℃, and 30–35 ℃. Twenty-four healthy crossbred rams (Dorper × Small-tailed Han sheep) approximately one year old with good body condition and body weight of (35.34±1.07) kg were randomly allocated into three groups of eight sheep each. The preliminary period lasted 7 days, followed by a 10-day formal experimental period. During the formal period, gas concentrations and microbial counts were measured using the respiratory metabolic chambers. On the morning of day 10, blood samples were collected to determine serum concentrations of corticosterone (CORT) and immunoglobulin G (IgG), and activities of creatine kinase (CK) and lactate dehydrogenase (LDH). The results showed: 1) Temperature had highly significant effects on CH and NH concentrations in the metabolic chambers (P<0.01). CH emissions at 10–15 ℃ were extremely significantly higher than those at 20–25 ℃ and 30–35 ℃ (P<0.01), while NH emissions at 30–35 ℃ were extremely significantly higher than those at 10–15 ℃ and 20–25 ℃ (P<0.01). However, no significant effects were observed on CO and H S concentrations (P>0.05). 2) The count of *Escherichia coli* at 30–35 ℃ was extremely significantly higher than at 10–15 ℃ and 20–25 ℃ (P<0.01). Counts of *Salmonella* and total bacteria at 30–35 ℃ and 20–25 ℃ were extremely significantly higher than at 10–15 ℃ (P<0.01). 3)

Temperature had no significant effect on serum CORT and IgG concentrations or CK and LDH activities (P>0.05). In conclusion, different ambient temperatures can influence harmful gas emissions and environmental microbial counts in sheep, but have no significant effect on serum biochemical indicators.

Keywords: temperature; mutton sheep; harmful gas; microorganism; serum biochemical indicator

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Introduction

Harmful gases such as carbon dioxide (CO), methane (CH), ammonia (NH) and hydrogen sulfide (H S) in livestock housing environments cause severe damage to animal growth performance, reproductive performance, and respiratory tract mucosa, while also threatening the health of farm workers. CO and CH are major greenhouse gases considered responsible for global temperature rise, with CH emissions from ruminants like cattle and sheep having 25 times the greenhouse effect of CO . Temperature significantly affects urease activity in livestock manure [1], promoting conversion of NH to NH and bacterial decomposition of sulfur-containing organic matter to produce H S. Temperature is also the dominant environmental factor influencing bacterial proliferation, fecal fermentation, decomposition of toxic harmful gases, and various odors in livestock housing. Elevated ambient temperature triggers behaviors such as coughing, sneezing, and fighting among livestock, which increase environmental suspended particulate matter content, typically ranging from 10^3 to 10 particles/m^3. Therefore, any factor that increases environmental particulates may elevate microbial counts, and the movement rate of airborne suspended particles correlates closely with temperature—higher temperatures accelerate particle movement. Moreover, excessive concentrations of harmful gases (CO , CH , NH , and H S) [2] or excessively high temperatures [3-4] in livestock housing environments can damage respiratory mucosa and reduce serum biochemical indicators (total protein, enzymes, glucocorticoids, etc.) and immune function. This experiment measured and analyzed changes in O , CO , CH , NH and H S concentrations, environmental microbial counts, and serum biochemical indicators of crossbred mutton sheep under different temperature conditions to provide theoretical basis and reference for effective control of harmful gas emissions and improvement of livestock housing environments.

Materials and Methods

1.1 Experimental Design and Animals The experiment employed a single-factor design with three ambient temperature ranges: 10–15 ℃, 20–25 ℃, and 30–35 ℃. Twenty-four crossbred rams (Dorper × Small-tailed Han sheep

) approximately one year old, in good body condition, with body weight of (35.34±1.07) kg were randomly divided into three groups of eight sheep each. The preliminary period lasted 7 days, and the formal experimental period lasted 10 days. Each sheep was weighed upon entering and exiting the respiratory metabolic chamber, with the average value used as the body weight during the measurement period.

1.2 Experimental Diet The experimental diet was formulated according to the nutrient requirements recommended in the Chinese agricultural industry standard "Feeding Standard of Mutton Sheep"(NY/T 816-2004). Its composition and nutrient levels are shown in Table 1 .

1.3 Animal Management Prior to the preliminary period, each experimental sheep was dewormed orally with ivermectin tablets. Sheep were individually housed in metabolic cages and fed twice daily (07:00 and 18:00) with free access to water.

1.4.1 Dietary Nutrient Analysis Dietary samples were analyzed for dry matter (DM), crude protein (CP), organic matter (OM), crude ash, ether extract (EE), calcium (Ca), phosphorus (P), neutral detergent fiber (NDF), and acid detergent fiber (ADF) according to GB/T 6435-2006, GB/T 6432-1994, GB/T 6438-2007, GB/T 6434-1994, GB/T 6433-2006, GB/T 6436-2002, and GB/T 20806-2006 methods, respectively.

1.4.2 Measurement of O , CO , CH , NH and H S Concentrations The gas flow circuit structure of the respiratory calorimetry device is described in references [5-6]. The system consists of air intake/exhaust equipment, respiratory chambers, high-precision online gas analyzers, data acquisition controllers, and temperature-humidity control systems. (1) The air intake/exhaust equipment provided airflow rates of 0.01–2.00 L/min into and out of the chambers. (2) The respiratory chambers had a volume of 3.20 m^3 and were constructed from organic glass, equipped with specialized feed troughs, water containers, and feces/urine collection facilities. Each chamber was connected to the electrical and gas circuit systems. (3) The high-precision online gas analyzer was independently developed by the Branch Academy of Animal Science, Jilin Academy of Agricultural Sciences. Analyzer parameters: O range 0–1.2%, accuracy ±0.2%, resolution 1.0 mg/m^3; CO range 0–1.5%, accuracy ±0.2%, resolution 1.0 mg/m^3; CH range 0–0.2%, accuracy ±0.02%, resolution 1.0 mg/m^3. Calibration: To ensure analyzer accuracy during analysis, gas sensors for O , CO and CH were calibrated using standard gases of different concentrations. (4) The data acquisition controller was computer-controlled and provided four collection modes: single-channel, dual-channel, three-channel, and four-channel according to experimental requirements. (5) The temperature-humidity control system maintained chamber temperature at -5–50 ℃ and humidity at 40%–85%.

The respiratory metabolic chambers could be controlled according to different experimental purposes and requirements.

Measurement of O , CO and CH : During the formal experimental period, individual sheep were placed in metabolic cages and moved into the respiratory metabolic chambers. Concentrations of O , CO and CH in each chamber were measured sequentially using probes for 10 consecutive days. Each chamber required 3 minutes per gas collection cycle. The system automatically calculated CH and CO production and O consumption based on indoor/outdoor gas concentrations and respiratory quotient, with results saved automatically.

O consumption (L/min) = [air intake flow (L/min) × outdoor O concentration] - [exhaust flow (L/min) × indoor O concentration]
CO production (L/min) = [exhaust flow (L/min) × indoor CO concentration] - [air intake flow (L/min) × outdoor CO concentration]
CH production (L/min) = [exhaust flow (L/min) × indoor CH concentration] - [air intake flow (L/min) × outdoor CH concentration]
Respiratory quotient (R.Q.) = CO production / O consumption

NH and H S measurement: NH concentration was determined by indophenol blue colorimetry (GB/T 18204.25-2000), and H S concentration by methylene blue colorimetry (GB11742-1989). During the formal period, measurements were taken daily at 11:00, 14:00 and 17:00. Before each measurement, 10 mL of standard absorption solution for NH and H S was drawn into large bubble absorption tubes. The sampling valves of the metabolic chambers were opened, and micro air pumps were adjusted to extract 5 L and 30 L of gas for NH and H S at flow rates of 0.5 and 1.5 L/min, respectively. Samples were stored in the dark and colorimetric analysis was completed within 6 hours, with temperature and atmospheric pressure recorded during sampling.

1.4.3 Microbial Count Determination During the formal experimental period, sampling points were designed according to environmental monitoring technical specifications before feeding (07:00, 18:00) using the five-point sampling method at 1.5 m above the chamber floor. Prepared culture plates were placed at fixed positions for 15 minutes, then removed and labeled with sampling point and chamber number. After incubation at 37 ℃ for 24 hours, colony counts were calculated and microbial numbers for *Escherichia coli* (MacConkey agar), *Staphylococcus aureus* (mannitol salt agar), *Salmonella* (SS agar), and total bacteria (nutrient agar NA) were determined using the 奥氏公式 [7]. All culture media were purchased from Qingdao Hope Bio-Technology Co., Ltd. The microbial count calculation formula was:

[Formula would appear here]

Where: C = airborne bacterial count (CFU/m³); N = number of colonies; A = plate area (cm²); T = sampling time (min).

1.4.4 Serum Biochemical Indicator Determination On the morning of day 10 of the formal period, 5 mL of jugular blood was collected from experimental sheep to determine serum concentrations of corticosterone (CORT) and immunoglobulin G (IgG), and activities of creatine kinase (CK) and lactate dehydrogenase (LDH). Assay kits purchased from Shanghai Enzyme-Linked Biotechnology Co., Ltd. were used.

1.5 Statistical Methods Experimental data were organized using Excel 2016 and analyzed using one-way ANOVA in SPSS 19.0 software. Duncan' s multiple comparison test was used for significant differences, with SEM and P-values reported. $P<0.05$ was considered significant and $P<0.01$ highly significant.

Results

2.1 Effects of Ambient Temperature on CO and CH Emissions from Mutton Sheep As shown in Tables 2 and 3 , ambient temperature had no significant effect on CO concentration in the metabolic chambers ($P>0.05$). From 08:00 to 16:00, CO concentration increased initially then decreased, with the lowest concentration at 08:00 (4,257.09 mg/m³, 220.96 mg/m³ · W ·) and peak concentration at 10:00 (10,275.89 mg/m³, 538.66 mg/m³ · W ·).

As shown in Tables 4 and 5 , CH concentration at 10–15 ℃ was extremely significantly higher than at 20–25 ℃ and 30–35 ℃ at all times except 08:00 ($P<0.01$). From 08:00 to 16:00, CH concentration increased initially then decreased, peaking at 11:00 (250.62 mg/m³, 13.04 mg/m³ · W ·) and reaching the minimum (119.19 mg/m³, 6.03 mg/m³ · W ·). Under 20–25 ℃ conditions, CH concentration was lower than at 10–15 ℃ and 30–35 ℃ at all time points, with the minimum value of 7.05 mg/m³ (0.05 mg/m³ · W ·).

2.2 Effects of Ambient Temperature on Total CO and CH Emissions from Mutton Sheep As shown in Table 6 , ambient temperature had no significant effect on total CO emissions ($P>0.05$) but had a highly significant effect on CH emissions ($P<0.01$). CH emissions at 10–15 ℃ were extremely significantly higher than at 20–25 ℃ and 30–35 ℃, being 2.13 and 1.83 times higher, respectively ($P<0.01$). CH emissions at 20–25 ℃ were lower than at 10–15 ℃ and 30–35 ℃. The respiratory quotient at 30–35 ℃ and 20–25 ℃ was extremely significantly higher than at 10–15 ℃ ($P<0.01$). Dry matter intake at 10–15 ℃ was significantly higher than at 30–35 ℃ ($P<0.05$).

2.3 Effects of Ambient Temperature on NH and H S Emissions from Mutton Sheep As shown in Table 7 , temperature had a highly significant effect on NH emissions ($P<0.01$). NH emissions at 30–35 ℃ were extremely significantly higher than at 10–15 ℃ and 20–25 ℃ ($P<0.01$). However, temperature had no significant effect on H S emissions ($P>0.05$).

2.4 Effects of Ambient Temperature on Microbial Counts in Respiratory Metabolic Chambers As shown in Table 8 , *Escherichia coli* counts at 30–35 ℃ were extremely significantly higher than at 10–15 ℃ and 20–25 ℃ ($P<0.01$). *Salmonella* and total bacterial counts at 30–35 ℃ and 20–25 ℃ were extremely significantly higher than at 10–15 ℃ ($P<0.01$), but showed no significant difference between 30–35 ℃ and 20–25 ℃ ($P>0.05$). Temperature had no significant effect on *Staphylococcus aureus* counts ($P>0.05$).

2.5 Effects of Ambient Temperature on Serum Biochemical Indicators of Mutton Sheep As shown in Table 9 , ambient temperature had no significant effect on serum CORT and IgG concentrations or CK and LDH activities ($P>0.05$).

Discussion

3.1 Effects of Ambient Temperature on CO and CH Emissions from Mutton Sheep Gas production from rumen fermentation in ruminants is fastest during feeding, comprising 40% CO and 30–40% CH . Therefore, CO and CH emissions follow an initial increase then decrease. Some CO is a fermentation byproduct, while another portion is generated from reactions between organic acids and salivary carbonates, but most CO is reduced to form CH , with some utilized by microorganisms. CO is the final product of normal physiological activities in animal cells, generated through the tricarboxylic acid (TCA) cycle and other oxidation-reduction reactions using glucose and oxygen as substrates to provide energy (ATP). The overall reactions are: anaerobic respiration: C H O → 2C H OH + 2CO + ATP; aerobic respiration: C H O + 6H O + 6O → 6CO + 12H O + ATP. This study found no significant effect of ambient temperature on CO emissions, likely because mammals can regulate body temperature relatively constantly through heat production and dissipation, so temperature does not affect physiological activities. Factors influencing animal CO emissions mainly include temperature, housing structure, and stocking density. Most published studies on temperature effects on CO emissions show similar results to this experiment. Chen [8] found that CO concentration remained relatively stable with ambient temperature changes in summer sheep housing, mainly distributed at animal height. Ouyang [9] reported that CO concentration in Xinjiang winter closed sheep houses remained between 1,785–2,582 mg/m³ with no significant diurnal variation. Ding [10] studied seasonal CO variation in large-scale sheep farms in Inner Mongolia and found the highest summer concentration in double-pitched roof houses was 1,323 mg/m³, but not significantly different from other seasons. Chen et al. [11] found strong correlation between stocking density and CO emissions but not temperature in three types of southern sheep houses during summer.

Approximately 98.7% of CH in ruminants is produced through rumen fermentation, with about 82% synthesized by rumen methanogens using CO and H

as substrates through a series of enzymes and coenzymes. Fiber-degrading bacteria anaerobically ferment dietary plant fiber and carbohydrates to produce volatile fatty acids (VFA), which also generate CH through chemical reactions. This study found highly significant effects of ambient temperature on CH emissions, with the lowest emissions at 20–25 ℃ (13.06 L/d) but highest at 10–15 ℃ (27.80 L/d), possibly because elevated temperature significantly reduced dry matter intake, shortening rumen fermentation time at low temperatures. Zhao [12] studied effects of different feed intake levels on CH emissions in meat sheep and found a highly significant linear relationship: CH (L/d) = 44.034 × DMI (kg) - 6.514 (R^2 = 0.6801, P = 0.0005). Cai [13] investigated effects of heat stress on rumen fermentation in goats and found decreased total VFA production, reduced acetate concentration, increased propionate and butyrate concentrations, and lower CH emissions under heat stress. Chen et al. [14] studied effects of summer high temperature on rumen metabolism in water buffalo and found increased respiration rate, rumen temperature, and rectal temperature, reduced feed intake, increased water consumption, slower rumen fluid flow rate, initially increased rumen microbial metabolic rate under high temperature that was suppressed by persistent heat, decreased acetate/propionate ratio, and consequently reduced CH emissions.

3.2 Effects of Ambient Temperature on NH and H S Emissions from Mutton Sheep NH is the most abundant and harmful gas in livestock housing, readily soluble in respiratory mucosa causing tissue damage, mucosal congestion, swelling, and even pulmonary hemorrhage and tissue hypoxia [15]. Indoor NH initially originates from microbial and extracellular enzymatic hydrolysis of carbohydrates, proteins, and fats in feces into monosaccharides, amino acids, and fatty acids, which are further decomposed into NH , amines, and nitrogen. Another portion comes from eructation, as rumen bacteria produce highly active urease that rapidly decomposes urea into NH , with small amounts excreted when rumen microorganisms cannot utilize it promptly. This study demonstrated highly significant effects of ambient temperature on NH concentration, with 30–35 ℃ showing significantly higher concentrations than 10–15 ℃ and 20–25 ℃, because urease activity in feces is optimal at 40–50 ℃ and relatively stable below 50 ℃. Since this experiment used 10–35 ℃, increasing temperature enhanced urease activity, accelerating microbial decomposition of protein substances and conversion of NH to NH , while also intensifying activities of ammonifying, nitrosifying, and nitrifying bacteria, resulting in higher NH emissions under high-temperature aerobic conditions. Chen and Shi [16] studied microbial characteristics during municipal waste composting and isolated 19 bacterial strains including *Bacillus* and *Enterobacter* at 45 ℃ and 55 ℃, finding higher average enzyme activities at 55 ℃ than at 45 ℃. Aarnink et al. [17] reported that each 1 ℃ increase in housing temperature increased NH emissions by 6–7%. Ni et al. [18] studied effects of temperature and ventilation rate on NH emissions and found that heating rapidly increased NH concentration from 94 to 167 g/h, dropping to 68 g/h one hour after heating ceased. Dai [19] investigated

daily NH emission patterns in pig houses and found that 33% of daily emissions occurred between 13:00–17:00, indicating that NH emissions mainly occur during high-temperature periods. NH emissions also show clear seasonal patterns, increasing with temperature. Liu et al. [20] measured NH concentrations in chicken houses across four seasons and found significantly higher concentrations in summer, confirming that high-temperature environments significantly elevate NH levels. However, this experiment found no significant effect of ambient temperature on H S emissions, possibly because ruminants consume mainly roughage with fewer sulfur-containing organic metabolites, while H S primarily originates from anaerobic degradation of sulfur-containing organics by fecal microorganisms, which is inhibited under aerobic conditions. Monteny et al. [21] monitored H S concentrations in pig houses and found levels below 7.5 mg/m³. Gao et al. [22] measured NH and H S in caged rabbit houses and detected NH at 1.4 mg/m³ but no H S, consistent with this study, indicating that NH is the primary harmful gas in livestock housing with low H S concentrations.

3.3 Effects of Ambient Temperature on Environmental Microorganisms in Respiratory Metabolic Chambers Airborne microbial counts are important indicators for evaluating livestock housing environmental hygiene quality. Animals themselves carry various pathogenic microorganisms, with ruminant rumen and digestive tracts containing numerous bacteria, protozoa, and fungi that are disseminated through respiration, sneezing, and feces, and can spread via air currents to distant locations, causing disease outbreaks and affecting human and livestock immunity and production performance [23]. For example, high *E. coli* counts in pig nursery environments significantly increase diarrhea incidence and mortality. This study showed that *E. coli*, *Salmonella*, and total bacterial counts increased significantly with temperature because high temperatures increased sheep activity, raising indoor suspended particulate matter, while elevated temperatures also accelerated particle movement rates, increasing microbial counts per unit volume [24-27]. Si et al. [27] reported that airborne microbial counts increase with temperature and stocking density. Wang et al. [28] detected airborne microorganisms in 10 livestock farms in Urumqi during winter and found positive correlation with temperature, with *E. coli* as the dominant genus. Xiong [29] found that airborne aerobic bacteria and *Staphylococcus* concentrations in cattle and sheep farms were relatively stable across seasons, following the trend spring < autumn < winter < summer, indicating that high temperature and humidity conditions favor bacterial proliferation, leading to higher harmful microbial counts in livestock environments.

3.4 Effects of Ambient Temperature on Serum Biochemical Indicators of Mutton Sheep Under high temperature and humidity conditions, animals exhibit heat stress responses including elevated body temperature and respiration rate, reduced feed intake and rumination frequency. Animals utilize their defense systems to overcome adverse effects of heat stress and maintain relative homeostasis. Song et al. [30] studied effects of heat stress on serum hormones

and physiological indicators in beef cattle and found significantly higher respiration rate, diarrhea rate, and serum LDH activity during heat stress, with serum cortisol (COR) concentration increasing significantly on day 20 then gradually returning to normal, indicating that physiological indicators correlate strongly with both heat stress and its duration. Li et al. [31] investigated effects of heat stress on serum biochemical indicators in beef breeding bulls and found highest serum GPT and GOT activities in summer, while CK activity tended to be lower. This experiment found no significant effect of ambient temperature on CO emissions, demonstrating that animal physiological activities remained relatively stable at 10–35 ℃ with no significant high-temperature effects. Serum CK activity decreased gradually with increasing temperature, differing from some reports but consistent with Yang et al. [32] who found that serum CK activity in Chinese Holstein cows fluctuated and decreased with rising temperature, being significantly lower at 25 ℃ than at other temperatures (5, 10, 15, 20 ℃), which differs from Song et al. [30] and Wang et al. [3]. CK is an important kinase directly related to intracellular energy metabolism, muscle contraction, and ATP regeneration, with elevated activity lasting only 2–4 days before returning to normal, indicating its association with heat stress duration. Luo et al. [33] and Cai et al. [34] found that serum CK activity in beef cattle increased with prolonged heat stress duration. These inconsistent changes may be influenced by heat stress duration and different breeds' sensitivity to heat stress, but the underlying mechanisms require further investigation.

Conclusions

1. Temperature had no significant effect on CO emissions from mutton sheep respiratory metabolic chambers. CH emissions at 10–15 ℃ were extremely significantly higher than at 20–25 ℃ and 30–35 ℃, while CH concentrations were lowest at all time points under 20–25 ℃ conditions. *Escherichia coli*, *Salmonella*, and total bacterial counts in metabolic chambers increased significantly with temperature, but *Staphylococcus aureus* counts showed no significant difference.

2. Temperature had no significant effect on serum CORT and IgG concentrations or CK and LDH activities in mutton sheep.

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Note: Figure translations are in progress. See original paper for figures.