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RESEARCH ARTICLE

Body condition scores and biochemical changes across gestation trimesters in Dorper ewes under free-grazing conditions

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ABSTRACT

The body condition score (BCS) and blood biochemical parameters are key indicators of the physiological and nutritional challenges for pregnant ewes. This study aims to evaluate the trimester-specific changes in BCS and blood biochemical parameters in Dorper ewes reared under a free grazing system. Twenty pregnant Dorper ewes were selected for this study, BCS and blood samples were collected during the first, second, and third trimesters. The BCS were assessed on a five-point scale, and the serum's hematological profiles and pregnancy-related biochemical parameters were analysed. Repeated measures of ANOVA were used to analyse the significant trimester-specific variations. Results show the BCS shifted as gestation progressed, with most ewes in BCS 3, whereas a decline in BCS 4 and 5 indicates increasing energy demands and the use of body reserves in the later trimester. The haematological profile has no significant deviations and is within physiological ranges across trimesters. The changes in biochemical parameters throughout the gestation period showed that blood urea nitrogen (BUN), albumin (ALB), and alanine aminotransferase (ALT) peaked in the third trimester, while the cholesterol (CHOL) significantly surged ($P<0.05$) in the third trimester. Energy-related metabolites such as glucose (GLU) are slightly decreased across trimesters from 102.633 ± 3.19 mg/dL to 100.967 ± 4.24 mg/dL, whereas calcium (Ca^{2+}) and magnesium (Mg^{2+}) levels progressively increase with fetal development. These findings align with the physiological demands of late gestation and the importance of nutritional strategies. Therefore, this study reveals the dynamic metabolic demands during gestation, which require nutritional management in free-grazing systems.

1. INTRODUCTION

Dorper sheep introduction in Malaysia has contributed to the diversification of livestock farming and has gained popularity in escalating local meat production since 2010 (Panadi et al., 2021). It can adapt and survive in various climates, including tropical environments. This breed was imported from Australia and is well-recognised for its high-quality meat and high-yield carcass characteristics (Norhayati et al. 2018). Due to these characteristics, local farmers raised this breed as primary livestock. Besides, this breed was propagated through breeding programs in commercial farms such as the Agropolitan Project in Pahang, Malaysia (Ramli et al. 2025). Nevertheless, the pregnancy rate was reported to be only around 55-60%, which requires more intervention to improve the breeding performance. Hence, understanding the physiology of livestock, particularly during the breeding period, in particular rearing systems, may increase the pregnancy rate.

Generally, Dorper was reared in extensive to semi-intensive systems that fed Napier as the main feed and also incorporated grazing (Ramli et al. 2025). However, the breeding performance was not similar to that of the original country. Particularly, local farmers usually reared this breed in extensive systems where the sheep were grown in free grazing, which exhibited low performance. Several factors may contribute to the low performance, which requires more research to alleviate this problem. Blood haematology and biochemical parameters are the methods to evaluate livestock health and productivity. These parameters indicate the physiological, metabolic, and nutritional status of the animal (Lamraoui et al. 2024). For example, glucose, total protein, albumin, alanine aminotransferase, and aspartate aminotransferase are important indicators of liver and kidney functions (Varanis et al. 2021). Consequently, it will detect early disease, reducing morbidity and mortality rates. Predominantly, specific blood biochemical parameters like calcium, phosphorus, and magnesium are usually gauged in pregnant and lactating females in reproductive performance.

The body condition score (BCS) is a valuable management practice for evaluating the nutritional and overall health of small ruminants. This method gauges the amount of muscle and fat covering specific anatomical parts surrounding the ribs and the loin area's spinous and transverse bones (Kenyon et al. 2014). Typically, BCS involves a scale from 1 to 5, where score 1 indicates emaciated, while score 5 indicates obese, and it is carried out regularly. The BCS assists in monitoring nutritional needs, is instantly alerted to significant losses in body condition, and is undetectable by outside appearance (Kenyon et al. 2014). The animals will mobilize the body reserves like adipose tissue or intramuscular fat to satisfy the physiological requirement when there is a low nutrient supply or high demand for feed (Kenyon et al. 2014). In the meantime, the BCS also influences the breeding of small ruminants. Ewes with a BCS of 3 to 4 at lambing exhibited heavier lambs than those with lower scores (Corner-Thomas et al. 2015). In reproduction, the BCS at 3 to 4 was ideal for breeding, and they tend to have higher ovulation rates and, consequently, higher lambing percentages (Kenyon et al. 2014). Contrarywise, ewes with BCS greater than four at breeding may experience a higher prevalence of infertility or dystocia (Everett-Hincks & Dodds, 2008; Corner-Thomas et al. 2015). On the other hand, the BCS also indicates the stress level, as Karaca et al. (2023) reported, where cortisol levels are elevated in pregnant ewes and are more severe in those bearing twins.

The blood biochemical parameters were modulated across the trimester due to metabolic demands during gestation. Concurrently, breed, age, parity, fetal growth, malnutrition, stress, and environment affect the biochemical parameters (Lamraoui et al. 2024). Monitoring the blood biochemicals throughout pregnancy gave insights into the normal and abnormal metabolic status (Lamraoui et al. 2024). Previous studies reported that glucose levels decrease from early pregnancy to lower levels in later stages (Varanis et al. 2021). This decline is due to the physiological requirement to maintain the pregnancy, where the need for nutrients increases with the rapid growth of the fetus (Lamraoui et al. 2024). Failure to provide the nutrient requirement may lead to energy depletion, and utilisation of body fat is a primary resource that needs to be mobilised. This incidence is indicated by serum lipid and lipoprotein profiles in conjunction with predicting periparturient problems and diagnosing metabolic disorders and nutrition status (Nazifi et al. 2002). Hence, biochemical parameter analysis is an important routine that can be carried out on farms to enhance nutritional management and production.

Free grazing systems are commonly practised in this region, where the livestock have unrestricted access to natural or cultivated forages. This cost-effective system promotes natural behaviour while exposing the livestock to variable nutrient availability *ad libitum*. Nevertheless, livestock in this system are prone to nutrient fluctuation and environmental conditions affecting the overall nutrient intake and adequate energy source. In the feedlot system, the feed can be formulated according to the physiological demands, whereas in the grazing system, the nutritional intake is subject to the age and state of the forages and animals. Inadequate forage can dent the energy balance, which leads to metabolic disorders such as pregnancy toxaemia (Varanis et al. 2021; Chikhaoui et al. 2023). Therefore, this study aims to evaluate the BCS, blood haematology, and biochemical parameters of Dorper ewes as metabolic indicators of pregnant ewes across trimesters reared under a free-grazing system.

2. MATERIALS AND METHODS

This study was conducted at Universiti Malaysia Kelantan, Jeli Campus, Kelantan (5° 44'49" N, 101° 52'21" E). The experimental period is from May to October 2024.

2.1. Ethics approval

The Institutional Animal Care and Use Committee (IACUC) of Universiti Malaysia Kelantan permitted the research procedures for this research under UMK/FIAT/ACUE/FYP/17/2024.

2.2. Animal husbandry and management

This study employed 25 adult dry ewes (3 years) in parity three and good clinical health. They were weighed at a mean of 45.78 ± 3.56 kg and had a body condition score (BCS) >3 before mating. These ewes were reared in a semi-intensive system, allowing them to graze at *Brachiaria humidicola* pasture with approximately 30% scattered legumes, *Arachis pintoi,* and freely access clean water at the grazing area and pen. The animals grazed *ad libitum* twice daily at 8.00-10.00 am and 4.00-6.00 pm. Commercial concentrates with molasses were supplemented to the ewes at 0.25 kg/head/day and provided after the ewes were back in the pen after evening grazing. During pre-breeding, the flushing program was carried out four weeks before the ram was introduced into the flock for the breeding program. Ewes were administered 1 ml of Ivermectin (subcutaneous) for parasite preventive measures, and vitamin ADE was administered (intramuscular, 1 ml/head). In addition, this routine was applied to free-grazing livestock every three months. However, this routine was halted during the gestation period until the lamb was born. The distance from the pen to the fodder is about 50 m, where the livestock can easily get in and out when complete.

In this farm, natural mating is practised with the assistance of systematic records. The breeding program started after flushing, and the selected ram joined the ewes. The ram stayed with the ewes for 40 days, which allowed all the ewes to be mated. Then, the ram was removed from the flock, and the pregnancy diagnosis was carried out at day 60. The pregnancy diagnosis was made using ultrasound (STT-B20P, China), where the non-pregnant ewes (n=5) were transferred to the other flock.

2.3. Body condition score evaluation

A total of 20 pregnant Dorper ewes underwent body condition scores (BCS) evaluation every trimester by using a scale ranging from 1 (emaciated) to 5 (obese), with 0.5 intervals (Everett-Hincks & Dodds, 2008; Corner-Thomas et al. 2015). All ewes with individual ID were evaluated using a hands-on palpation technique emphasising three anatomical landmarks: the lumbar region and ribs (Kenyon et al. 2014). Trained evaluators palpated restrained ewes to ensure consistency in scoring, and the BCS frequency was recorded.

2.4. Blood sampling

Whole blood samples were taken from all ewes in weeks 8, 14, and 18 of the pregnancy, representing the first, second, and third trimesters, respectively. The blood samples (5 mL) were withdrawn from the jugular vein of the animals before morning feeding using a sterile 18 mm-gauge needle attached to a vacutainer tube containing Ethylenediaminetetraacetic acid (EDTA). After that, the blood tubes were delivered to the Animal Research Laboratory, UMK Jeli Campus, and it took less than 30 minutes to get from the collection to the lab. Next, after 15 minutes of rest, 1000µL of blood from each sample was pipetted into microcentrifuge tubes and left to clot. The sera were collected through centrifugation at 3,000 x g and stored at -20°C for further analysis.

2.5. Haemotological analysis

After the blood collection, the tubes were cooled at 4°C before haematology evaluations were done. An automated haematology analyser was used to analyse the haematological parameters. The red blood cell count (RBC), haemoglobin concentration (HGB), hematocrit (HCT), red blood cell distribution width (RDW), platelet concentration (PLT), white blood cell count (WBC), lymphocytes (LYM), monocytes (MON), granulocytes (GRA), the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated using this equipment. The micro hematocrit reader measured the packed cell volume (PCV).

2.6. Serum biochemical parameters

A total of 11 serum biochemical parameters were evaluated from each ewe. Albumin (ALB), alanine aminotransferase (ALT), blood urea nitrogen (BUN), calcium ions (Ca^+), cholesterol (CHOL), creatinine (CREAT), glucose (GLU), lactic acid (LAC), lactate dehydrogenase (LDH), Magnesium ions (Mg^{2+}) and triglycerides (TRIG) were analysed for each period in the laboratory as described by the study of Lamraoui et al. (2024).

2.7. Statistical analysis

The body condition score (BCS) data were summarised using descriptive statistics, and the Chi-square determined if the BCS of ewes significantly changed across gestation trimesters. Mean values of the haematological profiles and serum biochemical parameters were calculated using repeated measures of ANOVA followed by the Bonferroni post hoc test to contrast the pregnancy-related means at different trimesters. The relationship between serum biochemical parameters was tested using a partial correlation, and all statistical analyses were carried out using SPSS 24.

3. RESULT AND DISCUSSION

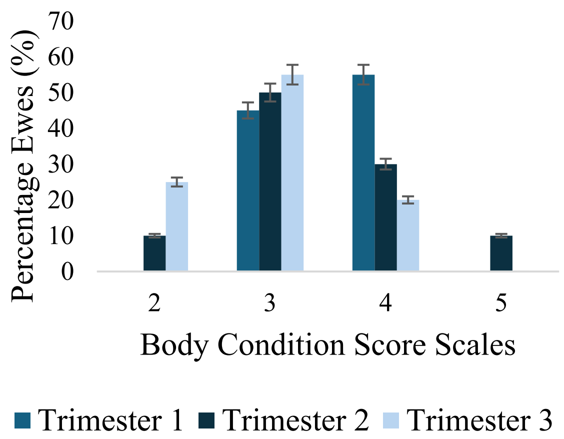

Figure 1: The body condition score (BCS) distribution of ewes across the three gestational trimesters. The percentage of ewes classified within each BCS category (2–5) can be seen in the bar graph.

Fig. 1 shows the distribution of body condition scores (BCS) among ewes across different trimesters of pregnancy. In general, 35% of ewes had a BCS of 2 (thin) in all trimesters, slightly increasing in Trimester 3. In average condition (BCS of 3), more than 45% of ewes fell into this score across all trimesters, where the percentages increased steadily from Trimester 1 to 3. Meanwhile, BCS 4 indicates a good condition, which was higher in Trimester 1 compared to later trimesters. A decreasing trend is observed as pregnancy progresses. Finally, only 10% of ewes had a BCS of 5 (Obese) and were only recorded in Trimester 2.

Table 1: Mean ± S.E. of haematological profile of free-grazing Dorper ewes throughout the gestation period.

Hemogram Parameters	Trimester 1	Trimester 2	Trimester 3	Range Reference
WBC ($10^3\mu l^{-1}$)	7.800±0.35^a	7.933±0.54^a	8.333±0.19^a	4.0-12.0
LYM	1.567±0.37^a	2.300±0.75^a	2.167±0.59^a	1.0-5.5
MON	0.500±0.00^a	0.500±0.17^a	0.867±0.06^a	0.1-1.0
GRA ($10^3\mu l^{-1}$)	5.733±0.03^a	5.133±0.35^a	5.300±0.53^a	2.0-8.0
LYM (%)	32.933±2.03^a	44.233±2.55^b	35.267±3.61^{ab}	25.0-50.0
MON (%)	7.633±1.89^a	7.167±0.98^a	7.167±0.98^a	2.0-10.0
GRA (%)	59.433±3.24^a	17.467±17.47^b	57.567±4.97^a	50.0-80.0
RBC ($10^6\mu l^{-1}$)	4.967±0.41 ^{a}	4.867±0.16^a	5.100±0.15^a	4.00-6.20
HGB (gdl^{-1})	12.600±0.06^a	11.867±0.68 ^{a}	10.200±2.50^a	11.0-17.0
HCT (%)	37.867±1.52^a	29.467±7.99^a	34.933±1.79^a	35.0-55.0
MCV (μm^3)	107.633±2.25^a	88.467±26.36^a	104.267±5.62^a	80.0-100.0
MCH (pg)	35.667±0.57^a	57.733±18.78^a	36.033±1.18^a	26.0-34.0
MCHC (gdl^{-1})	37.900±1.65^a	37.500±0.87^a	36.533±0.55^a	31.0-35.5
RDW (%)	13.667±0.60^a	15.100±1.76^a	24.067±8.47^a	10.0-16.0
PLT ($10^3\mu l^{-1}$)	166.000±50.30^a	127.433±56.97^a	194.333±32.94^a	150-400
MPV (μm^3)	7.433±0.23^a	7.367±0.12^a	7.533±0.29^a	7.0-11.0

*WBC: White Blood Cell Count, LYM: Lymphocytes, MON: Monocytes, GRA: Granulocytes, LYM%: Lymphocyte Percentage, MON%: Monocyte Percentage, GRA%: Granulocyte Percentage, RBC: Red blood cell count, HGB: Haemoglobin, HCT: Hematocrit, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration, RDW: Red cell distribution width, PLT: Platelet Count, MPV: Mean Platelet Volume.

*Superscript indicates significant different (P<0.05) between columns.

Table 1 shows that significant changes were recorded in some of the haematological parameters of free-grazing Dorper ewes. The White Blood Cell Count (WBC) had a small increase from 7.80 ± 0.35 × $10^3\mu l^{-1}$ during the first trimester to 7.93 ± 0.54 during the next trimester, finally settling at 8.33 ± 0.19 during the last trimester; however, this remained within the determined normal reference interval of 4.0–12.0 × $10^3\mu l^{-1}$. Lymphocyte (LYM) counts showed a linear increase from 1.567 ± 0.37 × $10^3/\mu l$ during the first trimester to 2.300 ± 0.75 during the next trimester, before a slight decrease to 2.167 ± 0.59 by the last trimester. These values also remained within normal levels of 1.0–5.5. The number of granulocytes (GRA) decreases from 5.733 ± 0.03 in the first trimester to 5.133 ± 0.35 in the second trimester and gradually rises to 5.300 ± 0.53 by the third trimester. On the other hand, the percentage of lymphocytes (LYM%) displayed a significant increase (P<0.05), rising from 32.93 ± 2.03% in the first trimester to 44.23 ± 2.55% in the second trimester before gradually declining to 35.27 ± 3.61% in the third trimester.

The granulocyte percentage (GRA%) shows a significant decrease in the second trimester (17.467 ± 17.47%) compared to that in the first trimester (59.433 ± 3.24%), followed by a rise in the third trimester (57.567 ± 4.97%). Regarding erythrocyte parameters, Red Blood Cell (RBC) counts remained at a consistent level of about 4.867–5.100 × $10^6\mu l^{-1}$ across the gestation period. On the other hand, Haemoglobin (HGB) and Hematocrit (HCT) levels decreased as gestation progressed, with HGB dropping from 12.600 ± 0.06 gdl^{-1} during the first trimester to 10.200 ± 2.50 gdl^{-1} during the third trimester, while HCT dropped from 37.867 ± 1.52% to 29.943 ± 1.79%. The MCV, MCH, and MCHC values showed variations within the reference ranges. Specifically, MCV showed a drop in the second trimester, followed by a rise, reflecting alterations in the size of erythrocytes throughout the pregnancy. Red cell distribution width (RDW) also increased from 13.667 ± 0.60% in the first trimester to 24.067 ± 8.47% in the third trimester. Platelet counts (PLT) showed a uniform drop from 166,000 ± 50.34 × $10^3\mu l^{-1}$ in the first trimester to 94,333 ± 32.94 × $10^3\mu l^{-1}$ in the third trimester; however, these values stayed within the wide range of reference from 150–400 × $10^3\mu l^{-1}$. Mean Platelet Volume (MPV) displayed stability across all different trimesters.

Pregnancy significantly affected the serum biochemical parameters in the Dorper ewes. Table 2 shows the serum biochemical parameters across three trimesters with the progression of the pregnancy. The albumin (ALB) and blood urea nitrogen (BUN) were found to be significantly different (P<0.05) between earlier trimesters and the third trimester. Creatinine (CREAT) and lactate dehydrogenase (LDH) were significantly lower (P<0.05) in the second trimester compared to the first and third trimesters. Alanine aminotransferase (ALT) and cholesterol (CHOL) increased significantly (P<0.05) in the third trimester compared to earlier trimesters. Then, glucose (GLU), lactic acid (LAC), and triglycerides (TRIG) were found steadily in all trimesters, and

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calcium ions (Ca^{2+}) and magnesium ions (Mg^{2+}) varied in trimesters, as revealed in Table 2.

Table 3 shows the total mean of serum biochemical parameters at different trimesters in pregnant Dorper ewes. The earlier trimesters show non-significant differences (P>0.05). Meanwhile, the serum biochemical parameters in the final trimester increased significantly differently from those in earlier gestation.

Table 2: Mean ± S.E. of serum biochemical parameters concentration at three pregnancy trimesters in pregnant Dorper ewes.

Serum biochemical parameters	Pregnancy Trimester (mean ± S.E)		
	1	2	3
ALB (g/dL)	2.858 ± 0.12^{a}	2.741 ± 0.06^{a}	2.913 ± 0.07^{b}
ALT (U/L)	73.280 ± 2.08^{a}	75.800 ± 2.42^{a}	82.053 ± 6.40^{b}
BUN (mg/dL)	16.727 ± 0.46^{a}	16.973 ± 0.56^{a}	21.640 ± 1.07^{b}
CHOL (mg/dL)	91.413 ± 4.75^{a}	83.633 ± 5.26^{a}	219.067 ± 22.05^{b}
CREAT (mg/dL)	1.273 ± 0.08^{a}	0.907 ± 0.11^{b}	1.160 ± 0.13^{a}
GLU (mg/dL)	102.633 ± 3.19^{a}	101.867 ± 3.56^{a}	100.967 ± 4.24^{a}
LAC (mmol/L)	1.720 ± 0.11^{a}	1.660 ± 0.12^{a}	1.613 ± 0.11^{a}
LDH (U/L)	440.000 ± 14.32^{a}	412.600 ± 12.57^{b}	426.800 ± 11.63^{a}
TRIG (mg/dL)	45.033 ± 2.57^{a}	42.567 ± 2.75^{a}	47.053 ± 2.86^{a}
Ca^{2+} (mg/dL)	7.453 ± 0.36^{a}	9.047 ± 0.29^{b}	9.780 ± 0.37^{b}
Mg^{2+} (mg/dL)	2.173 ± 0.03^{a}	2.373 ± 0.05^{a}	2.607 ± 0.04^{b}

* Albumin (ALB), alanine aminotransferase (ALT), blood urea nitrogen (BUN), cholesterol (CHOL), creatinine (CREAT), glucose (GLU), lactic acid (LAC), lactate dehydrogenase (LDH), triglycerides (TRIG), calcium ions (Ca^{2+}), Magnesium ions (Mg^{2+}). Superscripts with different letters indicate significant differences (P<0.05) detected between groups.

Table 3: The total mean of serumbiochemical parameters at different trimesters in pregnant Dorper ewes and their BCS

Trimester	Mean± S.E	BCS
First	71.324 ± 1.37^{a}	3
Second	68.637 ± 1.21^{a}	3.5
Third	82.801± 2.52^{b}	3.5

*Superscripts with different letters indicate significant differences (P<0.05) detected between groups.

4. DISCUSSION

Local farmers commonly practice conventional systems like free grazing, and a lack of information regarding the nutritional requirements tends to cause metabolic diseases in pregnant ewes, leading to the loss of lambs and ewes. The most practical assessment method is the BCS evaluation, which can be directly employed in farm management to predict and assess the nutritional status, general health, and signs of metabolic diseases, such as pregnancy toxaemia (PT). Small ruminants scored on a scale from 1 to 5 with 0.5 increments, whereas healthy animals should have a BCS between 3 and 4 (Karaca et al. 2023). In a recent study, most ewes had a BCS of 3, which is ideal for pregnancy maintenance. The number of ewes with BCS 4 decreased over time due to energy demand as pregnancy progressed. On the other hand, the BCS 2 increase in Trimester 3 indicated the ewes lost body condition, which also signifies the early metabolic diseases. The findings from this study suggested metabolic status and nutritional demands of fetal growth while maintaining the health of ewes. Pregnancy induces significant metabolic adaptations, and pregnant ewes have a risk for negative energy balance, particularly in the late gestation period (Ji et al. 2023). The energy required increases during gestation and is also affected by the number of bearing lambs (Chikhaoui et al. 2023; Lamraoui et al. 2024).

The haematological parameters of free-grazing ewes throughout gestation indicate that all the animals in the study were in good health. However, some parameters tended to rise as gestation progressed, clarifying physiological and health-related changes. The litter size significantly influences the haematological profile, as ewes with twins showed higher RBC, PCV, MCH, and MCHC values than ewes with singletons. In addition, twin pregnancies are associated with increased LYM count, reflecting an increased immune response during gestation (Kadhem & Al‐Thuwaini, 2022). The changes detected in the haematological profile indicate that the physiological requirements of carrying more than one fetus require more support for fetal growth (Kadhem & Al‐Thuwaini, 2022). Current study results reveal that LYM levels were significantly higher in Trimester 2 (P<0.05), possibly due to the immune response stimulation caused by antigen exposure. The immunological status of the ewes was found to be enhanced, as reflected by the rise in monocytes detected in Trimester 3, probably due to their involvement in placental functions.

The increased immune response also correlated with WBC count, as seen in Table 1. An increase in WBC during Trimester 1 reflected an initial immune reaction to the implantation of the fetus. Conversely, Trimester 2 showed a decline in granulocyte levels due to a temporary shift to adaptive immunity from innate immunity in mid-gestation (Oli, 2022). Subsequently, granulocyte levels increase in Trimester 3. The occurrence indicates that the organism readies its immune function in preparation for lambing. A decline in hemoglobin concentration has affirmed physiological distress caused by developing and maturing fetuses (El-Shahawy, 2016). The recent study found that HGB and hematocrit HCT levels reached their minimum in Trimester 2 but then recovered in Trimester 3 due to compensatory processes in the bone marrow and increased erythropoiesis. Last but not least, a declining PLT in Trimester 2 indicates hemodilution, while a PLT in Trimester 3 increased to imply readiness for clotting during lambing.

During the first trimester of gestation, ewes face increased metabolic demands with the onset of placental growth. In this gestational stage, ewes' physiology triggers adaptations in terms of increasing blood volume, even in the absence of significant alterations in haematological indicators at this time (Cal-Pereyra et al. 2015). Typically, HGB levels and RBC counts are within standard parameters, reflecting the increased oxygen demand of early pregnancy. Likewise, GRA counts also fall in expected parameters, suggesting that there has been no significant inflammation or even infection. These parameters are advantageous because an early pregnancy-related stressor, represented by heat stress or even a subclinical infection, would be detectable (Kulka et al. 2022). Secondly, haematological indicators offer insight into whether ewes consumed enough energy and protein for fetal implantation, especially in that free-grazing animals consume forages of varying grades. Lack of anaemia in all the ewes in this study indicates that nutrient consumption was adequate to meet gestation needs (Cal-Pereyra et al. 2015). Besides that, pre-breeding anthelmintic administration would reduce parasitic infestation and improve overall animal health and reproductive efficiency.

Rapid fetal growth begins in Trimester 2, leading to increased nutrient demand. The RBC, HGB, and HCT indicate a reduction due to the blood volume expansion caused by dilutional anaemia. These reduction parameters in the ewes may be due to mid-pregnancy and a struggle to meet metabolic demands (Mulvaney et al. 2012). In this trimester, the MCV values are above 100 fL, indicating a nutrient deficiency, leading to larger RBCs commonly found in free-grazing animals with poor-quality pasture. The WBC was found stable, but the LYM indicates a slight increase in this trimester, suggesting the immune activation is likely due to parasitic exposure in free-grazing systems (Stadalienė et al. 2014).

The rapid growth of the fetus occurs during the second trimester and increases the ewe's nutritional needs. Throughout this time frame, there is generally a noted reduction in RBC, HGB, and HCT levels due mainly to expanded blood volume andilutional anaemia (Mulvaney et al. 2012). The reduction suggests that ewes can have difficulty providing for the increased metabolic demands of mid-gestation. In addition, MCV over 100 fL signals that RBCs are being enlarged and typically points to a nutrient deficiency, commonly found in free-grazing sheep graze in poor-quality pastures. WBC tend to be stable through this time frame, yet a minimal rise in LYM levels likely results from elevated exposure to parasites in the free-grazing system (Cal-Pereyra et al. 2015).

During the third trimester, fetal development reached 70% of the period. In this duration, the ewe's physiology is geared towards the nutritional and metabolic requirements of the developing fetus, frequently at the expense of its own nutrient stores, often aggravating anaemia and greater metabolic stress (Cal-Pereyra et al. 2015). Consequently, haematological parameters such as RBCs, HGB, and HCT will suffer a slight decline due to mechanisms such as hemodilution, poor dietary intake levels, or ongoing parasitic infection. Ewes may manifest a heightened susceptibility to pregnancy toxaemia, a metabolic disorder involving hypoglycemia, raised blood ketone concentration, and a considerable decline in body condition caused by the increasing demands of late gestation. The disorder can be identified through clinical features such as lethargy, decreased appetite, muscular tremors, and in severe cases, recumbency (Crilly et al. 2021).

Glucose is the most important energy source for growing the placenta and fetus. In late gestation, in the third trimester, the glucose demand in ewes increases significantly due to the growing fetus, which relies solely on glucose. In the recent study, consistent glucose levels were found adequate in all trimesters, indicating sufficient energy reserves without signs of pregnancy toxaemia, characterised by low blood glucose and elevated ketones (Thorn et al. 2012). A previous study reported a decline in glucose across the trimesters of Ouled Djellal ewes reared in semi-arid conditions (Lamraoui et al. 2024). The glucose level is highly influenced by fetal development and maternal glucose allocation to the fetal circulation, especially when the liver and muscle glycogen increase rapidly in the fetus (Lamraoui et al. 2024). On the other hand, gluconeogenesis significantly increases in the liver to satisfy the glucose demands of the developing fetuses, particularly in late gestation (Thorn et al. 2012). If the nutritional status of ewes is inadequate or they experience severe energy deficiency, it can lead to a condition known as pregnancy toxaemia, characterised by hypoglycaemia and ketosis (Thorn et al. 2012). Therefore, it is vital to maintain healthy ewes and lambs to ensure the glucose level is stable across the gestation period.

Then, cholesterol levels in non-pregnant and pregnant ewes exhibit significant changes due to the physiological demands of gestation. In non-pregnant ewes, cholesterol levels are typically lower than those of pregnant ewes. Meanwhile, during pregnancy, the increase in cholesterol is attributed to several factors, including decreased insulin sensitivity and enhanced lipid metabolism (Chalmeh et al. 2019; Chikhaoui et al. 2023). As pregnancy progresses, ewes become less responsible for insulin, intensifying cholesterol circulation and triglycerides (Chalmeh et al. 2019). On the other hand, cholesterol also increases due to the synthesis of progesterone, which serves as a substrate for progesterone production by the placenta and corpus luteum to support fetal development (Varanis et al. 2021; Lamraoui et al. 2024). A similar finding in this research is that cholesterol increases significantly in the third trimester compared to earlier trimesters. A previous study reported a significant cholesterol surge towards the end of pregnancy (Berkani et al. 2018). This increase may be due to fatty acid mobilisation because of the energy deficit in the final trimester, which necessitates lipoproteins, mainly cholesterol and its ester, for passage (Lamraoui et al. 2024).

In addition, a recent study found that triglycerides are consistent across trimesters (Table 2). Triglycerides are the plasma lipids used by ewes when the energy necessities increase, although a previous study reported that plasma triglyceride concentration was significantly decreased in the third trimester (Lamraoui et al. 2024). Meanwhile, other reports mentioned that the plasma triglyceride level fluctuates across the trimester and surges near parturition (Lotfollahzadeh et al. 2016). To elucidate this finding, the elevation of triglycerides in late gestation is probably associated with the decline in insulin efficiency due to hormonal changes influencing ewes to increase cholesterol and triglyceride levels.

Pregnancy in small ruminants such as sheep and goats indicates significant physiological changes affecting energy metabolism, protein turnover, and lipid reserves. These modulations are important for supporting fetal development and maternal health. During the gestation period, the energy metabolism undergoes adaptations across trimesters, where the energy is relatively stable with minimal caloric intake intensification in the first trimester. At this moment, the body primarily depends on existing energy stores. Meanwhile, during the second and third trimesters, there is a noticeable increment in energy requirements due to embryonic growth. For instance, a previous study found that ewes have increased energy needs during fetal growth, particularly in the last two months of gestation, when approximately 80% fetal growth occurs (Wang et al. 2022). Pregnant ruminants often face a negative energy balance, particularly in the later stages of pregnancy. This negative energy balance occurs due to decreased dry matter (DM) consumption and the physical limitation of the growing fetus on the rumen. As a result, both sheep and goats mobilised body fat reserves to meet their energy requirement during this period (Nawito et al. 2016; Wang et al. 2022). Furthermore, in breeding ewes, a higher body condition score (BCS>4) can be considered obesity, which is prone to negative energy balance. In consequence, pregnant ewes tend to experience ketosis due to fat mobilisation that leads to the excessive release of non-esterified fatty acids (NEFAs) and other metabolic byproducts (Lisuzzo et al. 2022). Research suggests that the average daily energy intake during the second and third trimesters should be around 10-13 MJ and 11.5-16 MJ per day for single and twin-bearing lambs, respectively (Keady & Hanrahan, 2009; Wang et al. 2022).

Lactic acid levels in energy-related metabolites in pregnancy increased rapidly in the third trimester. This increment is due to metabolic demands increasing as growing fetuses were significantly observed in ewes carrying multiple lambs. Furthermore, the higher fetal demand increases lactate production due to anaerobic glycolysis when energy needs exceed oxygen supply (Goldansaz et al. 2022). Inadequate nutrition during pregnancy usually contributes to lactate levels since ewes do not have enough energy to supply the fetus's needs. In this study, the lactate was found to be steady across the trimesters, indicating sufficient energy supply to the fetus and not indicative of inadequate nutrition, which can lead to liver damage.

Blood chemical analysis gauged the protein-related metabolite, where the albumin indicates liver function and nutritional status across the trimesters. In this study, the albumin increased significantly in the third trimester. This metabolite is considered a sensitive status indicator of protein, where low albumin concentrations show scarce protein intake (Cardoso et al. 2010; de Oliveira et al. 2014). Albumin is significant in carrying non-esterified fatty acids to be utilised by peripheral tissues as an energy source (Varanis et al. 2021). In this study, the albumin was in the range where fluctuation was recorded and slightly higher in the third trimester. However, a previous study elucidated that albumin levels are in the physiological range, although in pregnancy, toxaemia was observed in Santa Inês, Dorper, and their crossbreds (Santos et al. 2011). Earlier studies revealed that the albumin range was reported in the broad range of 1.56 to 5.01 g/dL in pregnant sheep in Ouled Djellal sheep (Varanis et al. 2021), 3.64 g/dL in Awassi ewes (Gürgöze et al. 2009), and Makouei sheep (4.91 g/dL) at the last trimester (Mohammadi et al. 2016). Therefore, the albumin level varied in different breeds, ages, feed intakes, geography, and climates, nevertheless, it was significant in protein nutritional

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status indicators in pregnant ewes.

In addition, the BUN indicates protein metabolism during pregnancy and kidney function. A recent study found an increment pattern in trimester 3, suggesting increased nitrogen excretion. This finding corroborates the increase in BUN at the end of the gestation period due to decreasing feed intake, which leads to proteolysis of endogenous amino acids for energy (Feijó et al. 2014). A previous study reported that the BUN concentration was found to range from 40.48 mg/dL to 49.9 mg/dL in the late pregnancy of Santa Inês ewes (Cardoso et al. 2010; Morsy et al. 2016). These findings were slightly higher than in a recent study where the BUN concentration was recorded at 21.64 mg/dL, a marginal increase compared to the physiological range. Conversely, the serum creatinine level decreased in the second trimester and slightly increased (Table 2) in the third trimester in this study. Creatinine is primarily produced in the kidneys and liver and then transported through the bloodstream to the muscles and brain, where it is converted into phosphocreatine (Varanis et al. 2022). A previous study concluded that the increases in creatinine over the physiological ranges were caused by the lessened glomerular filtration rate in the kidneys (Raoofi et al. 2013). Particularly, the creatinine concentration within the uterine lumen is generally low but surges during the implantation phase of pregnancy and early embryonic development, demonstrating its role in early embryonic development (Sah et al. 2022). In this case, in the first trimester, the creatinine level is significantly higher in Dorper ewes, which may indicate the early embryonic stages. Hence, monitoring creatinine levels in pregnant ewes can be useful in detecting potential kidney issues, especially when significant deviations from normal ranges are observed.

ALT levels and LDH are liver enzymes that reflect liver activity in blood chemistry. Particularly, the ALT reflects liver enzyme activity, where increases in the level may indicate stress or metabolic changes. Meanwhile, fluctuation of the LDH may suggest metabolic adjustments during pregnancy. In recent studies, these enzymes are in the normal physiological range. A significant increase in these enzymes can be a marker of pregnancy toxaemia in ewes due to a deficit of significant energy, leading to the breakdown of muscle tissue and potential liver cells due to gluconeogenesis related to gestation (Lamraoui et al. 2024). Usually, these enzymes rise in late pregnancy, especially in ewes carrying multiple lambs. While liver damage is the primary concern, LDH can also be elevated due to muscle breakdown, which can occur in severe cases of pregnancy toxaemia (Lamraoui et al. 2024). Therefore, interpreting ALT and LDH levels requires considering the overall health of ewes, pregnancy stage and other clinical signs before any intervention can be done.

Minerals such as calcium and magnesium are important for gestation and lactating ewes because they are vital in many physiological processes, including energy metabolism, immune system, muscle contractions, and bone structure (Ji et al. 2023). In pregnancy, these minerals are transferred to foetuses and decreases in the plasma indicate higher foetal demands (Lamraoui et al. 2024). Deficiencies in calcium and magnesium can cause ewe and lamb mortality. Clinical deficiencies of calcium (hypocalcaemia or milk fever) and magnesium (hypomagnesemia or grass tetany) can cause ewes' death. Ewes with multiple lambs require higher requirements and a high risk of deficiency.

These results highlight the importance of the nutritional management of pregnant ewes within free-grazing systems prevalent in peninsular Malaysia. In agreement with earlier findings across breeds and productive systems, an optimal BCS of 3 and 3.5 across gestation is desirable for ewe health and avoids the risk of metabolic disease such as pregnancy toxemia (Ji et al. 2023). The decline of ewes with BCS of 4 and the rise of ewes with BCS of 2 during the second half of gestation indicate energy deficiencies that necessitate corrective measures, a trend also recorded among tropical grazing systems elsewhere (Lamraoui et al. 2024). Compared to controlled feeding systems, free grazing offers difficulties with maintaining regular nutrient intake, and resultant haematological and biochemical fluctuations characteristic of metabolic stress and adaptation. The RBC, PCV, and immune parameters rise in twin-bearing ewes, which agrees with other research emphasizing the higher physiological demands with litter size (Kadhem & Al‐Thuwaini, 2022).

5. CONCLUSION

In conclusion, while free-grazing systems benefit pregnant ewes, they pose challenges in continuously meeting nutritional demands, particularly during late gestation. Blood biochemical and haematological changes reflect intensified metabolic stress and physiological demands. Maintaining an optimal body condition score (BCS) of 3–3.5 throughout pregnancy is significant to prevent energy deficits and metabolic disorders like pregnancy toxemia. Therefore, strategic pasture management, including legume integration, appropriate forage maturity, targeted concentrate supplementation, and early pregnancy monitoring, can mitigate nutritional deficiencies and improve lamb production outcomes. Hence, these findings align with broader indications highlighting the necessity for proactive nutritional management in tropical grazing systems.

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