Effect of supplemental rumen-protected methionine on reproduction and production of Awassi ewes

Hosam H. Titi, Mufeed A. Alnimer and Mohamed A. Abedal-majed

Department of Animal Production, School of Agriculture, University of Jordan, Amman, Jordan

ABSTRACT
The effect of rumen-protected methionine (RPM) was investigated in Awassi ewes (0, 3 or 5 g/head/d, n = 20) on reproductive and productive performance (initial body weight [BW] = 70.9 – 71.8 kg, 3 – 5 years). Supplementation started with the flushing feeding regime (at the breeding time) and lasted for 42 d. RPM was reused during the last 60 d of pregnancy and the first 60 d of lactation. RPM increased (p < .05) progesterone concentration with a higher lambing rate at the 5RPM level. The number of lambs born for each ewe exposed to the ram was also higher (p < .05) with 3RPM and 5RPM. Methionine levels in the plasma were greater (p < .05) with RPM mainly at 5RPM level. Birth weights of the lambs were higher (p < .05) with RPM regardless of the level. Contrary to this, weaning weights and rate of gain for lambs were reduced (p < .05) with RPM addition. RPM did not affect milk production of ewes during the first 60 d of lactation or on energy corrected milk and feed to milk ratio. However, milk protein content was increased (p < .05) at the 5RPM level while milk fat content was decreased. Regardless of the level of RPM addition, milk casein content increased (p < .05). At all levels, the effect of RPM on milk fatty acid profile was minor. It can be concluded that supplementing RPM might be a valuable tool to enhance the reproductive and productive performance of ewes during their breeding season.

ARTICLE HISTORY
Received 5 August 2021
Revised 17 February 2022
Accepted 21 February 2022

KEYWORDS
Awassi ewes; milk production; pregnancy; birth weight; RPM

Introduction
Methionine has been identified as one of the most limiting amino acids, mostly in ewes and goats (NRC 2007). Feeding a rumen-protected methionine (RPM) supplement improved milk production in lactating dairy cows (Ardalan et al. 2009, 2010; Titi et al. 2013), lactating goats, or ewes (Adriana et al. 2009). Previous research found that supplementing Shami goats’ diets with RPM during late pregnancy (last 60 d) had little impact on birth and weaning weights, or average daily gain of Shami kids (Titi 2017).

Few studies reported the effects of RPM supplementation on reproductive performance. Reproductive performance of dairy cows fed RPM was improved (Ardalan et al., 2010; Liu et al. 2013). Moreover, feeding a RPM supplement to lactating dairy cows raised circulating methionine concentrations and appeared to improve fertility. Most of the published literature on the effect of supplementation with RPM on reproductive performance in sheep was in late pregnancy (Baldwin et al. 1993; Sevi et al. 1998; Goulas and Papadopoulos 2003).

At the beginning of the breeding season and the pre-lambing periods of sheep are characterised by dramatic changes in nutrient demand that necessitate meeting the requirements for energy and amino acid (NRC 2007). Dietary supplementation with various AA, like Met, improved embryonic and foetal survival and development by regulating essential signalling and metabolic pathways (Acosta et al. 2016). Availability of methionine may be essential for oocyte maturation and early embryonic growth (Bonilla et al. 2010). A recent study in dairy cows found that Met is abundant in uterine and embryonic fluids, implying that elevated uterine Met plays a role in normal embryonic growth and survival (Toledo et al. 2017).

Livestock, including sheep, suffer from infertility or subfertility, which reduces their lifetime productivity and the number of offspring that a ram or ewe could produce (Toledo et al. 2017). In sheep production, reproductive success is a critical factor, particularly when milk and meat production are the primary goals.
RPM could improve the reproductive and productive performance of Awassi sheep.

In this study, we hypothesise that feeding RPM would enhance reproduction and production in Awassi ewes. This study aimed to investigate the effects of RPM supplements on Awassi ewes during their breeding season on the reproductive performance in terms of ovulation rate, conception rate and pregnancy rate, during late pregnancy terms of body weight and birth weight of their newborns, on milk production and composition of lactating Awassi ewes during the early lactation period (up to day 60 post lambing), and on growth performance of suckling Awassi lambs.

**Material and methods**

The research was carried out at the University of Jordan’s Agricultural Research Station in the Jordan Valley (32 100 N, 35 370 E, 230 m below sea level). The Research Animal Care and Use Committee gave its approval to the experimental design and procedures. Sixty adult Awassi ewes were used in this experiment with initial body weight averaged between 70.8 and 73.4 ± 1.3 kg, and aged 3 – 5±0.7 years. Ewes were randomly assigned to three dietary groups of 20 ewes each. Groups were control with no supplement (0 g/head/d RPM), and a supplement with 3 g/head/d RPM (3RPM) or 5 g/head/d (5RPM), respectively (0 g/head/d RPM). The RPM source was top-dressed in the daily dietary allowances offered. Phase 1 of the experiment started at the beginning of the breeding season with a flushing feeding regime (20 d before mating to 20 post the first oestrous), followed by Phase 2 started from the last 60 d of pregnancy through the first 60 d of lactation.

Animals were held in covered loose pens with adjacent pen yards (11.5 m/head) at natural day length and ambient temperature. Dams were fed a concentrate mixture presented separately once daily at 0700 h in addition to alfalfa hay offered *ad libitum*. The diet was formulated according to the NRC (2007) recommendation. Nutrient composition and proximate analysis of the concentrate ration are presented in Table 1. Feed was provided individually at a rate of 0.5 kg concentrate/head/d during phase 1 (flushing time). During phase 2, the feed was offered at 1.0 kg concentrate/head/d up to lambing date, and 1.5 kg/head/d to the end of the experiment. Concentrate feed was mixed twice weekly with samples obtained and kept frozen for further composition analysis. Animals had free access to shade, water and mineral blocks all the time.

Two weeks after starting the feeding of the flushing diet, dams were oestrus synchronised utilising progesterone vaginal sponges (40 mg FGA, Ceva Sante Animale, Libourne, France) for 14 d to synchronise oestrous, combined with an intramuscular injection of eCG (600 U) on the day of sponge withdrawal. Ewes were mated naturally with eight adults and tested Awassi rams. Rams were joined for 7 d beginning on the day of eCG administration and allowed to re-join with ewes 10 d later (beginning of the second cycle) for another 7-d mating period. By the beginning of the third cycle, males were left with females to the end of the breeding. Breeding dates were recorded for each ewe. None returned to the oestrus for two consecutive cycles and an Ultra-sound Doppler Pregnancy Scanner (Medata, Medata Systems, Chichester, West Sussex, England) were used for pregnancy diagnosis.

Individual blood samples were taken at 3 – 4 h post-feeding to assess plasma progesterone levels as an indication of the initiation of the corpus luteum function. Blood samples were drawn at the start of the experiment (day 7, 7 d before sponge insertion), on the day of sponge insertion (day 14), and at sponge removal (day 28). Further samples were collected every 3 d until day 35, and on the day of

| Table 1. Percent ingredients composition and chemical analysis of the concentrate ration offered to Awassi ewes supplemented with increasing levels of rumen-protected methionine. |
|----|----|----------|
| Ingredient | % | Chemical composition |
| Barley | 70.0 | Dry matter, % as fed |
| Soybean meal | 15.0 | Crude protein % dry matter |
| Wheat bran | 13.0 | Ether extract, % dry matter |
| Limestone | 0.5 | Ash, % dry matter |
| Dicalcium phosphate (DCP) | 1.0 | Neutral detergent fibre, % dry matter |
| Salt | 0.4 | ADL (%) |
| Vitamins and mineral premix | 0.1 | Metabolizable Energy, MJ/kg dry matter |

*Vitamin A: 1500 U, vitamin D3: 150 U, Vitamin E 50%; 2 mg, vitamin B1: 300 μg, 300 μg vitamin B2: 300 μg, vitamin B6: 300 μg, 300 μg vitamin K3 50%; 300 μg, Manganese Oxide: 218 μg, Ferrous Sulphate: 435 μg, Copper Oxide: 15.5 μg Zinc Oxide 138.5 μg, Potassium Iodine,2.2 μg, Sodium Selenite: 0.9 μg, Cobalt Carbonate: 0.43 μg, calcium carbonate: 1 g |

*Calculated using NRC (2007). |
NRC: National Research council; ADF: Acid Detergent Fibre.
lambing. Samples were drawn from the jugular vein and placed in lithium heparinised vacutainer tubes (BD Vacutainer TM; LH 119 I.U., Belliver Industrial Estate, UK). Plasma was separated from blood samples by centrifuging them at 1000 × g for 15 min and storing them at −20 °C for examination.

Progesterone concentrations were determined by commercial radioimmunoassay kits (RIA Progesterone, IM1188; IMMUNOTECH SA, Marseille, France). Concentrations less than 1.0 ng/mL, accompanied by a prolonged progesterone concentration greater than 1.0 ng/mL before the completion of blood sampling, were used to predict ovulation and pregnancy (Rekik et al. 2016). The average number of eggs released in ovulating ewes is known as the ovulation rate (Scaramuzzi and Radford 1983; Schoenian and Burfening 1990), while conception rate is normally defined as the number of lambed ewes divided by the number of ewes exposed to the ram (Schoenian and Burfening 1990). To determine amino acid content, plasma was treated with sulfosalicylic acid and tested using ion-exchange chromatography with an amino acid auto-analyser (835-50, HITACHI, Japan) (Yang et al. 2010). The average number of eggs released in ovulating ewes is known as the ovulation rate (Scaramuzzi and Radford 1983; Schoenian and Burfening 1990), while conception rate is normally defined as the number of lambed ewes divided by the number of ewes exposed to the ram (Schoenian and Burfening 1990). To determine amino acid content, plasma was treated with sulfosalicylic acid and tested using ion-exchange chromatography with an amino acid auto-analyser (835-50, HITACHI, Japan) (Yang et al. 2010).

Body weight was recorded at the beginning of the experiment, at lambing time, and again at the end of the experiment. Lambs birth weight and weaning weight at 60 d of age were also recorded. Immediately after giving birth, pregnancy rate, litter size, birth weight and type of birth (single or twin) were recorded. Suckling lambs were left with their dams in the same yard for the whole duration of the experiment. No special diet was given to the lambs during the trial, and dam milk was the only feed source of nutrition. During concentrate feeding time, dams were separated from their lambs.

Milk production was measured biweekly and individually by hand-milking. The double oxytocin injection protocol of Goulas et al. (2003) was used to estimate milk production over a 4-h period and then adjusted for daily milk production. At 0800 h, ewes were intramuscularly injected with 1.5 mL of oxytocin (Dila-part, Cenavisa, Tarragona, Spain), accompanied by full udder emptying. Another injection was administrated at 1200 h to milk dams for the second time and milk production was recorded as production over a 4-h interval. During oxytocin treatment, lambs were isolated from their dams. Energy-corrected milk (ECM) was determined following the equation of Adriana et al. (2009), as they reported: ECM = (0.327 × milk yield) + (12.86 × fat yield) + (7.65 × protein yield). Feed to milk ratio was determined as daily dry matter (DM) intake (kg): daily milk production (kg). Collected feed samples were ground by IKA mill via a 1-mm screen (MF 10, IKA GMBH, Staufen, Germany). Samples were analysed according to (AOAC 2006) for DM, crude protein (CP), ether extract and ash. CP was determined by the Kjeldahl method for N analysis (N × 6.25) utilising a 1031 Kjeltec analyser unit (Tecator, Tocator AB, Hoganas, Sweden). Neutral detergent fibre and acid detergent fibre were measured (Georing and Van Soest 1970) using Ankom fibre analyser (Ankom220, ANKOM Technology, Fairport, NY) and expressed on an ash-free basis. Milk samples were analysed for fat (Gerber method), CP (Kjeldahl method), total solid and casein concentrations using the standard procedures of (AOAC 2006). Protein values were determined by the Kjeldahl method (N × 6.38). For milk fatty acid analysis, the fat was separated following the Roese–Gottlieb method (Schoenian and Burfening 1990; AOAC 2006), transesterified into fatty acid methyl esters by methylation at room temperature with KOH (2M) in methanol, and analysed by gas chromatography using a Shimadzu 2010 equipped with flame ionisation detector (Trinacty et al. 2006). Gas chromatography conditions were column oven temperature 70 °C for 1 min, then increased to 165 °C at 20 °C/min, and kept at 165 °C for 8 min, then increased to 180 °C at 1 °C/min and 220 °C at 3 °C/min, for 10 min. The carrier gas was helium. Injector and detector temperatures were 250 °C and the helium flow rate was 1.1 mL/min.

SAS GLM procedure (SAS 2004) was used in a completely randomised design to test the fixed effect of supplementing the diet with RPM on studied variables. The analysis model included treatment (0RPM, 3RPM or 5RPM), time, and their two-way interactions. A repeated-measures design was used to examine DM intake, milk yield and composition and progesterone concentration. Dams’ initial and lambing body weights were used as covariates for final body weight, while birthweight was used as a covariate for weaning weight and lambs average daily gain. Lamb sex and type of birth (single or twin) were included in the analysis model. In addition, the age and parity of dams were also used as covariates during analysis. The least square mean analysis of variance was separated for significance (p < .05) using Fisher’s protected least significant difference test. Data for lambing rate and multiple births were analysed using the chi-square test at (p < .05) level.

Results

The daily allowance of RPM was top-dressed for individual ewes diet, allowing individual feeding of RPM,
keeping the ewe as the experimental unit, and increasing statistical power so that the effects of nutrition on ewe reproductive performance could be evaluated. To our knowledge, this is the first study that evaluated the effects of feeding RPM on reproductive efficiency and progesterone concentrations combined with the milking efficiency of ewes. Therefore, it is hard to compare our results with other experiments in the same line.

Progesterone profiles of the ewes in the experimental dietary groups from the beginning of sampling through day 35 are shown in Figure 1. There were no significant differences in progesterone concentrations before sponge insertion and removal among ewes of the different groups. Progesterone secretion reached the peak on day 29 in all treatments with a profound increase \((p < .05)\) was observed on day 20–29 of the experiment. On day 29, progesterone secretions were greater \((p < .05)\) in 5RPM than in 3RPM and control. Likewise, the overall progesterone concentration (ng/mL) was increased \((p < 0.05)\) as the RPM level increased (Table 2).

Reproductive parameters and the overall lambing data in Awassi ewes are shown in Table 2. Compared to the other groups, the 5RPM had a higher lambing rate to first oestrous \((p < .05)\). RPM increased the number of lambs born per ewe exposed to the ram \((p < .05)\). However, lambs/ewe exposed to the ram was greater \((p < .05)\) in the RPM groups with no difference between the 5RPM group and 3RPM group (Table 2).

The plasma concentration of methionine was measured at three different phases of this experiment. At the start of the experiment (day −7) as a baseline

![Figure 1. Progesterone concentration (ng/mL) of Awassi ewes fed increasing levels of rumen-protected methionine (RPM) in their flushing diets during their breeding season; 0RPM: 0 g RPM/head/d; 3RPM: 3 g RPM/head/d; 5RPM: 5 g RPM/head/d.](image)

| Table 2. Reproductive response and overall lambing data of Awassi ewes supplemented with increasing levels of rumen-protected methionine in their diet. |
|---------------------------------|-----------------|----------------|----------------|----------------|
| **Treatment**                  | **0RPM**        | **3RPM**       | **5RPM**       | **SE**         |
| Progesterone concentration, ng/mL | 1.71\(^a\)       | 2.22\(^b\)       | 2.76\(^a\)       | 0.16           |
| Overall lambing rate, %        | 150             | 180            | 175             |                |
| Lambing rate to first oestrous, % (of ewes)\(^b\) | 45\(^a\) (9) | 40\(^b\) (8) | 65\(^c\) (13) | 3.17           |
| Lambs/ewe exposed to the ram   | 1.50\(^b\)      | 1.80\(^a\)      | 1.75\(^c\)      | 0.11           |
| Total number of lambs born     | 30              | 36             | 35              |                |
| Male lambs born                | 13              | 20             | 23              |                |
| Female lambs born              | 17              | 16             | 12              |                |
| Twinning rate, %\(^c\)         | 16.6            | 50.0           | 28.5            |                |
| Lambs born as single births    | 25              | 18             | 25              |                |
| Lambs born as twin births      | 5               | 18             | 10              |                |
| Plasma methionine, \(\mu\)M    |                  |                |                 |                |
| At start of experiment         | 2.10            | 2.32           | 2.00            | 0.78           |
| At day of sponge insertion     | 3.25\(^b\)      | 4.76\(^b\)     | 5.33\(^a\)      | 0.83           |
| At day of first oestrous       | 3.34\(^b\)      | 4.40\(^b\)     | 5.56\(^a\)      | 0.57           |
| At day of lambing              | 5.10\(^b\)      | 7.74\(^a\)     | 8.08\(^a\)      | 1.20           |

\(^{a,b,c}\) Within rows, means with a common superscript were not different at probability \(p = .05\), 0RPM: 0 g rumen-protected methionine (RPM)/head/d, 3RPM: 3 g RPM/head/d, 5RPM: 5 g RPM/head/d.

\(^{a}\)Overall lambing rate: # of lambs born/# of ewes exposed to the ram\(^a\) 100%.

\(^{b}\)Lambing rate to first oestrous %: # of lambs born/# of ewes exposed to the ram of 1\(^{st}\) oestrous\(^b\) 100%.

\(^{c}\)Twinning rate %: # of lambs born twins/# of lambs born for each group.
measurement, at days of progesterone sponge insertion, removal, and first oestrous cycle (days 0, 14 and 17) to determine the effect of RPM on reproductive performance, and at the day of lambing to determine the effect of RPM during late pregnancy on growth rate as indicated by birth weights as well as milking performance of ewes. When RPM was added, plasma methionine concentrations increased \( (p < .05) \) over time (Table 2). A substantial increase in methionine concentration was noted when 5RPM was added, but there was no difference with the control group in plasma methionine concentrations when 3RPM was added on the day of sponge insertion and the day of the first oestrous (Table 2).

The effect of supplemental RPM on birth weights and growth performance (in kg) of lambs born to ewes in the experiment is shown in Table 3. Lambs born to ewes fed diets containing RPM had higher average birth weights \( (p < .05) \) than those for ewes fed the control diet. Males and twins birth weights were also higher \( (p < .05) \) for lambs of the RPM groups. However, no differences in birth weight were observed among female and single lambs. Average weaning weight as well as body weight gain of suckling lambs were reduced \( (p < .05) \) following RPM supplementation. Similar results were observed for female lambs. Control male lambs had best \( (p < .05) \) weaning weights and body weight gain during suckling but not different from that of the 5RPM group. However, males of the 3RPM group had the lowest \( (p < .05) \) weaning weight but statistically similar to those of the 5RPM lambs. When expressed as single or twins, single lambs from the 3RPM group had the best \( (p < .05) \) weaning weight and weight gain, while those for twins were reduced \( (p < .05) \) following supplementation. Milk to gain ratio (milk consumed (suckled) during 60 d: body weight gain, kg/ kg) was negatively \( (p < .05) \) affected following RPM supplementation.

Milk production (kg/d) did not differ among treatments (Table 4). However, the addition of 5RPM reduced \( (p < .05) \) milk fat content with no difference in the 3RPM diet from the control. Milk protein content was increased \( (p < .05) \) with the RPM supplementation. Energy corrected milk and feed to milk ratio followed the same trend as milk production with RPM addition, meanwhile, casein content was increased \( (p < .05) \).

In terms of fatty acids (FAs) percentage composition of milk, no significant differences were seen through dietary treatments (Table 5). Following RPM supplementation, an increase in C8, C13, C16 and C16:1 was observed \( (p < .05) \). On the other hand, C17 and C20 were decreased \( (p < .05) \) with supplementation of RPM.

### Table 3. The average birth weight, weaning weight, and weight gain of lambs born to Awassi ewes supplemented with increasing levels of rumen-protected methionine in their diets.

| Treatment | 0RPM | 3RPM | 5RPM | SE |
|-----------|------|------|------|----|
| Ewe body weight (kg) | | | | |
| At start of experiment (day – 7) | 71.31 | 70.92 | 71.83 |
| At breeding (day 0) | 72.15 | 71.85 | 72.70 | 1.10 |
| 60 d before lambing | 73.80 | 72.66 | 74.23 | 2.30 |
| At lambing | 79.51 | 81.22 | 80.75 | 2.30 |
| Birth weight, kg | 3.34b | 4.47a | 4.05a | 0.21 |
| Male | 2.91b | 4.71a | 4.24a | 0.27 |
| Female | 3.78 | 4.22 | 3.86 | 0.29 |
| Single | 4.54 | 4.68 | 4.90 | 0.33 |
| Twin | 2.15a | 4.26a | 3.21b | 0.24 |
| Weaning weight, kg | 23.31a | 20.09b | 18.52b | 1.39 |
| Male | 24.69a | 21.01b | 22.22ab | 1.35 |
| Female | 21.93a | 19.15b | 14.78c | 1.35 |
| Single | 20.31b | 22.81a | 19.66b | 0.96 |
| Twin | 26.31c | 17.34b | 17.40b | 0.90 |
| Weight gain, kg | 18.80b | 15.57b | 14.01b | 1.40 |
| Male | 20.17a | 16.45b | 17.76b | 1.81 |
| Female | 17.42b | 14.46ab | 10.27b | 1.73 |
| Single | 15.80b | 18.30a | 15.14b | 1.03 |
| Twin | 21.80a | 12.83b | 12.89b | 2.94 |
| Milkgain ratio (kg/kg)\(a\) | 3.58b | 4.47b | 5.33b | 0.41 |

\(a\) Within rows, means with a common superscript were not different at probability \( p = .05 \).

### Table 4. Milk yield and composition of Awassi ewes supplemented with increasing levels of rumen-protected methionine in their diets.

| Traits | 0RPM | 3RPM | 5RPM | SE |
|--------|------|------|------|----|
| Milk production, kg/d | 1.16 | 1.22 | 1.26 | 0.17 |
| Fat content, % | 10.81a | 10.41bc | 9.58a | 0.52 |
| Protein content, % | 4.76a | 5.71a | 5.92a | 0.08 |
| Energy corrected milkc, kg/d | 2.25 | 2.31 | 2.28 | 0.24 |
| Feed: Milk ratio, kg/kg | 1.19 | 1.19 | 1.19 | 0.14 |
| Casein, g/ protein | 3.69b | 4.50a | 4.72a | 0.11 |
| % of protein content | 77.62 | 78.80 | 79.82 |

\(a\) Within rows, means with a common superscript were not different at probability \( p = .05 \).

\(b\) Energy-corrected milk = (0.327 * milk yield) + (12.86 * fat yield) + (7.65 * protein yield) (Adriana et al. 2009).

### Discussion

The progesterone profiles show cycling activity (progesterone concentrations greater than 1.0 ng/mL) at the time of sampling which may suggest the existence of their luteal role (Husein and Kridli 2002). Progesterone levels in the RPM treated ewes steadily increased relative to the control ewes during the experimental period. Within 3–4 d of sponge removal, progesterone concentrations increased in all
treatments. Properly, ovulation happened in these ewes due to a subsequent rise in progesterone concentration (Husein and Kridli 2002). The reduction in progesterone concentration after day 29 in all treatments would be due to the beginning of the subsequent cycle (Husein and Kridli 2002). The reduction in concentration (Husein and Kridli 2002). The reduction in plasma progesterone concentration observed in the RPM groups might be the reason for the improvements in lambing rate and the number of lambs born observed in these groups. Progesterone plays a vital role in pregnancy maintenance until the placenta takes over its function at 60 d (Kuźnicka et al. 2016). Dairy cows supplemented with RPM had higher lipid accumulation in embryos in a way that enhances their capacity for survival (Acosta et al. 2016, 2017). RPM supplementation to dairy cows shows a fundamental function in embryo development from the early stages of morula to blastocyst and can improve embryo preimplantation survival capacity by utilising the lipid reserves as the energy source for the pre-implanted embryos in membrane synthesis that increases their survivability (Lopes et al. 2019). The same reference stated that RPM affected the follicular fluid of the first postpartum follicle and changes in mRNA expression in follicular cells of methionine supplemented cows, demonstrating that RPM supplementation aids in the establishment of ovarian development and follicular dynamics.

Pregnancy is a critical period during which the foetus growth may be influenced by nutrition (Alharthi et al. 2018). Maternal nutrient supply and placental transport efficiency are major intrauterine factors affecting foetal development. Previous research has shown that feeding RPM is responsible for the abundance of nutrient transporters; primarily glucose and AA, in dairy cow placental tissue, as well as improving the efficiency of nutrient usage by the foetus, which may be responsible for the increase in birth weight (Waterman et al. 2012; Alharthi et al. 2018). According to the same reference, methionine supplementation in late pregnancy resulted in increased transport of nutrients through the utero-placental tissues and increased growth in the uterus as well as during the pre-weaning and early post-weaning periods. Unfortunately, no placental data were collected in the present study.

Methionine levels in plasma were higher when RPM was mostly at 5RPM. The current findings of plasma methionine levels are consistent with other findings.

### Table 5. Milk fatty acid composition of Awassi ewes supplemented with increasing levels of rumen-protected methionine in their diets.

| Fatty acids (g/100g) | 0RPM | 3RPM | 5RPM | SE |
|----------------------|------|------|------|----|
| Butyric acid, C4:0   | 3.69 | 3.45 | 3.49 | 0.21|
| Caproic acid, C6:0   | 3.01 | 2.85 | 2.88 | 0.16|
| Caprylic acid, C8:0  | 2.65<sup>a</sup> | 3.17<sup>a</sup> | 2.72<sup>b</sup> | 0.20|
| Capric acid, C10:0   | 8.79 | 7.68 | 7.78 | 0.71|
| Lauric acid, C12:0   | 4.13 | 3.40 | 4.19 | 0.55|
| Tridecanoic acid, C13:0 | 0.19<sup>b</sup> | 0.16<sup>b</sup> | 0.21<sup>a</sup> | 0.05|
| VMyristic acid, C14:0 | 11.01 | 10.22 | 10.36 | 0.36|
| Tetradecanoic acid, C14:1 | 0.39 | 0.27 | 0.27 | 0.12|
| Pentadecanoic acid, C15:0 | 1.09 | 0.97 | 0.98 | 0.32|
| Palmitic acid, C16:0 | 23.23<sup>a</sup> | 25.85<sup>a</sup> | 25.67<sup>a</sup> | 1.08|
| Palmitoleic acid, C16:1 | 1.20<sup>b</sup> | 1.82<sup>b</sup> | 1.02<sup>b</sup> | 0.11|
| Heptadecanoic acid, C17:0 | 0.81<sup>b</sup> | 0.65<sup>b</sup> | 0.62<sup>b</sup> | 0.07|
| Heptadecenoic acid, C17:1 | 0.89 | 0.90 | 0.94 | 0.09|
| Stearic acid, C18:0 | 9.37 | 9.40 | 9.52 | 0.57|
| Oleic acid, C18:1 cis-9 | 20.79 | 20.45 | 20.99 | 1.01|
| Linoleic acid, C18:2 cis-cis | 3.14 | 3.32 | 3.22 | 0.23|
| Conjugated linoleic acid (CLA) | 2.10 | 1.57 | 1.71 | 0.30|
| Alpha-linolenic acid, C18:3 | 0.78 | 0.86 | 0.79 | 0.11|
| Arachidic acid, C20:0 | 0.47<sup>a</sup> | 0.46<sup>a</sup> | 0.38<sup>b</sup> | 0.03|
| Eicosatrienoic acid, C20:1 | 0.41 | 0.48 | 0.44 | 0.03|
| Behenic acid, C22:0 | 0.09 | 0.08 | 0.08 | 0.005|
| Lignoceric acid, C24:0 | 0.17 | 0.19 | 0.10 | 0.06|

<sup>a,b</sup>Within rows, means with a common superscript were not different at probability p=0.05. 0RPM: 0 g rumen-protected methionine (RPM)/head/day, 3RPM: 3 g RPM/head/day, 5RPM: 5 g RPM/head/d.
that recorded an elevated plasma concentration of methionine while supplying RPM, but not in all instances (Trinacty et al. 2006; Yang et al. 2010). The concentration of Met in plasma increased when PRM was fed to lactating dairy cows (Yang et al. 2010; Toledo et al. 2017). Likewise, plasma methionine was increased in ewes fed a source of RPM (Pisulewski and Kowalski 1999) or encapsulated amino acids including methionine and lysine (Lynch et al. 1991). Liu S et al. (2016) reported a 25% insignificant higher concentration of plasma methionine at lambing in RPM treated ewes compared to controls. Plasma Met concentrations were higher in lambs and steer fed an RPM-containing diet relative to those fed a control diet (Oke et al. 1986), and in late-gestating primiparous and winter-grazing range, heifers receiving wheat middling-based diet (Waterman et al. 2012). When adequate amounts of rumen-protected amino acids are fed, plasma amino acid concentrations can be used to estimate post-ruminal delivery of rumen-protected amino acids (Papas et al. 1984). Yang et al (2010) reported that the absorption of methionine can be determined by measuring amino acid content as the levels of serum-free methionine are positively related to the levels of methionine in the small intestine. The increase in plasma Met indicates that the amino acid was released and was available to ewes. According to Chow et al. (1990), the concentrations of limiting amino acids remain relatively constant until tissue requirements are met.

Lack of a linear increase in plasma methionine may demonstrate that the levels tested of RPM were below requirements (Oke et al. 1986). This could explain why no difference existed between the control diet and diets containing 3RPM in our study. The lack of response in plasma methionine concentrations may be attributed to partial ruminal protection, incomplete release for absorption in the small intestine, or insufficient RPM supplementation since milk and milk protein production were not improved (Papas et al. 1984).

Birth weights were used as a covariate in the statistical analysis of weaning and growth performance. In this study, the lambs’ birth weights were greater with RPM regardless of the level. Supplementation of pregnant ewes with RPM during late pregnancy tended to increase the birth weight of lambs (Liu S et al. 2016). No differences were observed in the birth weights of kids born to dams supplemented with RPM regardless of the sex of kids (Al-Qaisi and Titi 2014; Titi 2017), or beef calves (Clements et al. 2017). Work on dairy cows found that late gestation methionine supplementation would increase calf birth weight (Batistel et al. 2017). Similarly, Toledo et al. (2017) pointed out that dairy cows feeding RPM had larger embryos than non-supplemented cows.

Again, RPM supplementation in dairy cow increased nutrient transporters (glucose and AA) in placental tissue and improved the efficiency of nutrient usage by the foetus (Waterman et al. 2012; Alfarthi et al. 2018). The increase in essential AAs within the uterine lumen are transported through the vascular wall and the endometrial tissue to support and nourish the development of the conceptus which might result in increased birth weight as shown in sheep (Groebner et al. 2011).

RPM supplementation decreased lamb weaning weights and rate of growth. Differences in weaning weights and the growth rate of kids are mostly attributed to the milk production of dams. The lack of differences in milk production following RPM supplementation can explain the differences observed in weaning weights. Ewes fed RPM diets had more lambs and more twins than dams fed on the control diet (Table 2). As a result, lambs suckled control dams had more milk compared to lambs of the RPM dams which might restrict their growth and result in higher weaning weight for control lambs. This conclusion is supported by the higher weaning weight for single and male lambs that did not compete for available milk. Variations in weaning weights might also be attributed to improved milk composition mainly milk fat content. Ewes fed the control diet had higher fat content in their milk. Increased milk fat production has been positively associated with increased calf daily gain in beef cows (Clements et al. 2017).

In this study, RPM had no effect on ewe milk production during the first 60 d of lactation, as well as on energy corrected milk and feed to milk ratio. However, milk protein content rose and milk fat content dropped. Previous studies have shown that RPM supplements have a major effect on milk yield and milk protein concentration, and this positive response in milk protein has been linked to a significant increase in casein synthesis (Yang et al. 2010; Titi 2017; Toledo et al. 2017). However, the work of Yang et al. (2010) reported that the percentage of milk protein may be a more sensitive index than milk yield to estimate the effect of RPM and the lack of effect of PRM on protein percentage in milk may be due to low bioavailability of methionine from PRM for protein synthesis. Present findings of casein might explain the higher concentration of milk protein observed when RPM was supplemented to ewes. The addition of RPM resulted in greater milk casein in primiparous dairy cows (Chow
et al. 1990). Higher milk protein content was also reported in dairy cows supplemented with the RPM source (Papas et al. 1984; Zhou et al. 2016; Toledo et al. 2017). It appears that RPM can provide more available methionine to the mammary gland to increase milk protein production (Toledo et al. 2017). Our findings are opposing other results suggested that supplementary dietary RPM was associated with an increase in milk fat production. Differences in results from these experiments might have been caused by differences in the status of Met or other AA of the cows, the amount of Met supplied in the protected product and the efficacy of the protection scheme in delivering Met to the small intestine (Yang et al. 2010).

The analysis of milk fatty acid content was not the primary goal of this research, which was carried out to investigate any improvements in milk from the standpoint of human nutrition. Despite the scarce published literature concerning the effect of RPM on milk FAs of milking ewe, lacking the response in milk FAs composition following RPM is in line with results obtained in milking a goat or dairy cows (Chow et al. 1990; Al-Qaisi and Titi 2014; Titi 2017).

The addition of RPM to diets of lactating ewes resulted in a decrease in the proportion of C4:0 to C12:0 FAs and an increase in the proportion of C16:0 to C18:3 FAs, leading to methionine role in milk secretion (Titi 2017). Providing a limiting amino acid source can increase the liver ability to synthesise lipoproteins needed for lipid transport to the mammary gland or methionine, as a methyl donor, can influence milk fat synthesis in lipid transmethylation reactions (Chow et al. 1990). Supplementing dairy cows with RPM increased the C18 content while decreasing C18:2 content in milk lipids (Chow et al. 1990; Al-Qaisi and Titi 2014). Reducing the content of saturated FAs along with the increased content of the long-chain unsaturated FAs have been associated with increase healthfulness of milk.

Conclusions

Supplementing RPM for Awassi ewes was found to improve the milk protein and milk casein. This work suggests that feeding RPM to ewes during their breeding time can improve their reproductive performance and result in improving their lambing rate, and the number of lambs born. RPM increased the progesterone concentration which is responsible for embryo survival. At the same time, this supplement at late pregnancy resulted in increasing the birth weight of born lambs but did not improve lamb performance in the growing phase and their weaning weights. Male and twin lambs born to ewes supplemented with RPM were heavier than lambs from control ewes. Evidence from the current study can draw attention to the positive animal response to RPM supplementation, especially during their breeding season when protein needs are greatest.

However, further research is required to gain a better understanding of RPM and its importance in various stages of animal life.

Acknowledgements

The authors would like to appreciate the Deanship of Scientific Research at the University of Jordan for funding this work through the research group project No:1704.

Disclosure statement

The authors whose names are listed in this article certify that they have NO affiliations with or involvement in any organisation or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

All authors listed have made a substantial, direct and intellectual contribution to the work and have approved it for publication.

Funding

This work was supported by the Deanship of Scientific Research at the University of Jordan through the research group project No:1704.

ORCID

Hosam H. Titi http://orcid.org/0000-0003-3594-6413
Mohamed A. Abedal-majed http://orcid.org/0000-0003-3088-9528

Data availability statement

The data that support the findings of this study are available from the corresponding author (H. H. Titi) upon reasonable request.

References

Acosta DAV, Denicol AC, Tribulo P, Rivelli MI, Skenandore C, Zhou Z, Luchini D, Corrêa MN, Hansen PJ, Cardoso FC. 2016. Effects of rumen-protected methionine and choline supplementation on the preimplantation embryo in Holstein cows. Theriogenology. 85(9):1669–1679.
Acosta DAV, Rivelli MI, Skenandore C, Zhou Z, Keisler DH, Luchini D, Correa MN, Cardoso FC. 2017. Effects of rumen-
protected methionine and choline supplementation on steroidogenic potential of the first postpartum dominant follicle and expression of immune mediators in Holstein cows. Theriogenology. 96:1–9.

Adriana F, German M, Juan MP-R, Fernando P, Salvador V, Ricardo B. 2009. Effects of rumen-protected methionine on milk production of dairy goats. Ital J Anim Sci. 8: 271–275.

Alharthi AS, Batistel F, Abdelmegeid MK, Lascano G, Parsy C, Helmbrecht A, Trevisi E, Loor JJ. 2018. Maternal supply of methionine during late-pregnancy enhances rate of Holstein calf development in utero and postnatal growth to a greater extent than colostrum source. J Anim Sci Biotechnol. 9:83.

Ali A, Derar DR, Alshahed M. 2020. Management strategies, reproductive performance and causes of infertility in sheep flocks in the central region of Saudi Arabia. Trop Anim Health Prod. 52(4):1691–1697.

Al-Qaisi M, Titi HH. 2014. Effect of rumen-protected methionine on production and composition of early lactating Shami goats milk and growth performance of their kids. Arch Anim Breed. 57(1):1–11.

AOAC. 2006. Official method of analysis. 18th ed. Washington (DC): Association of Officiating Analytical Chemists.

Ardalan M, Rezayazdi K, Dehghan-Banadaky M. 2009. Investigation on the effect of supplementing rumen-protected forms of methionine and choline on health situation and reproductive performance of Holstein dairy cows. Pak J Biol Sci. 12(1):69–73.

Ardalan M, Rezayazdi K, Dehghan-Banadaky M. 2010. Effect of rumen-protected choline and methionine on physiological and metabolic disorders and reproductive indices of dairy cows. J Anim Physiol Anim Nutr (Berl). 94(6):e259-265–e265.

Ayat MS, Al-Sagheer A, Noreldin AE, Abd El-Hack ME, Khafaga AF, Abdel-Latif MA, Swelum AA, Arif M, Salem AZM. 2021. Beneficial effects of rumen-protected methionine on nitrogen-use efficiency, histological parameters, productivity and reproductive performance of ruminants. Anim Biotechnol. 32(1):51–66.

Baldwin JA, Horton GMJ, Wohlt JE, Palatini DD, Emanuelue SM. 1993. Rumen protected methionine for lactation, wool and growth in sheep. Small Rumin Res. 12(2): 125–132.

Batistel F, Alharthi AS, Wang L, Parsy C, Pan YX, Cardoso FC, Loor JJ. 2017. Placenta nutrient transporters and mammalian target of rapamycin signaling proteins are altered by the methionine supply during late gestation in dairy cows and are associated with newborn birth weight. J Nutr. 147(9):1640–1647.

Bonilla L, Luchini D, Devillard E, Hansen PJ. 2010. Methionine requirements for the preimplantation bovine embryo. J Reprod Dev. 56(5):527–532.

Chow JM, DePeters EJ, Baldwin RL. 1990. Effect of rumen-protected methionine and lysine on casein in milk when diets high in fat or concentrate are fed. J Dairy Sci. 73: 1051–1061.

Clements AR, Ireland FA, Freitas T, Tucker H, Shike DW. 2017. Effects of supplementing methionine hydroxyl analog on beef cow performance, milk production, reproduction, and preweaning calf performance. J Anim Sci. 95(12): 5597–5605.

Georghi HK, Van Soest P.J. 1970. Forage fiber analysis. Agriculture handbook. Vol. 379. Washington (DC): USDA.

Goulas and Papadopoulos 2003 is present in the reference list Kassim, H.W., Omar Almallah, Saeb Abdulrahman. 2019. Effect of protected methionine and lysine supplementation to Awassi ewes ration at flushing period on productive performance. Iraqi J. Vet. Sci. 33:105–109.

Goulas C, Zervas G, Papadopoulos G. 2003. Effect of dietary animal fat and methionine on dairy ewes milk yield and milk composition. Anim Feed Sci Technol. 105(1–4):43–54.

Grobner AE, Rubio-Alia I, Schulke K, Reichenbach HD, Daniel H, Wolf E, Meyer HHD, Ulbrich SE. 2011. Increase of essential amino acids in the bovine uterine lumen during preimplantation development. Reproduction. 141(5): 685–695.

Husein MQ, Kridli RT. 2002. Reproductive responses following royal jelly treatment administered orally or intramuscularly into progesterone-treated Awassi ewes. Anim Reprod Sci. 74(1–2):45–53.

Kuźnicka E, Witold R, Aurella RR, Malgorzata KS, Marek B. 2016. The ovulation rate, plasma progesterone and estradiol concentration, and litter size of a local ewe breed kept in a barn vs. those kept under an overhead shelter. Arch Anim Breed. 59:145–150.

Liu S, Lei J, Hancock S, Scanlan V, Broomfield S, Currie A, Thompson A. 2016. Lamb survival, glutathione redox state and immune function of neonates and lambs from periparturient Merino ewes supplemented with rumen-protected methionine. Arch Anim Nutr. 70(5):389–401.

Liu YG, Peng HH, Schwab CG. 2013. Enhancing the productivity of dairy cows using amino acids. Anim Prod Sci. 53(11):1156–1159.

Lopes MG, Jose HED, Marcio NC, Eduardo S, Geferson F. 2019. Rumen-protected methionine in cattle: influences on reproduction, immune response, and productive performance. Arq Inst Biol. 86:1–9.

Lynch GP, Elsasser TH, Jackson C, Jr., Rumsey TS, Camp MJ. 1991. Nitrogen metabolism of lactating ewes fed rumen-protected methionine and lysine. J Dairy Sci. 74(7):2268–2276.

NRC. 2007. Nutrient requirements of small ruminants: sheep, goats, cervids, and new world cameldids. 6th ed. Washington (DC): National Academy Press.

Oke BO, Loerch SC, Deetz LE. 1986. Effects of rumen-protected methionine and lysine on ruminant performance and nutrient metabolism. J Anim Sci. 62(4):1101–1112.

Papas AM, Vicini JL, Clark JH, Peirce-Sandner S. 1984. Effect of rumen-protected methionine on plasma free amino acids and production by dairy cows. J Nutr. 114(12): 2221–2227.

Pisulewski PM, Kowalski ZM. 1999. The effect of protected methionine on milk yield and its composition in lactating dairy cows fed grass silage-based diets. J Anim Feed Sci. 8(3):341–353.

Rekik M, Haile A, Abebe A, Muluneh D, Goshme S, Ben Salem I, Hilali ME, Lassoued N, Chanyalew Y, Rischkowsky B. 2016. GnRH and prostaglandin-based synchronization protocols as alternatives to progestogen-based treatments in sheep. Reprod Dom Anim. 51(6):924–929.
Scaramuzzi RJ, Radford HM. 1983. Factors regulating ovulation rate in the ewe. J Reprod Fertil. 69(1):353–367.

Schoenian SG, Burfening PJ. 1990. Ovulation rate, lambing rate, litter size and embryo survival of Rambouillet sheep selected for high and low reproductive rate. J Anim Sci. 68(8):2263–2270.

Sevi A, Rotunno T, Di Caterina R, Muscio A. 1998. Rumen-protected methionine or lysine supplementation of Comisana ewes’ diets: effects on milk fatty acid composition. J Dairy Res. 65(3):413–422.

Titi HH, Azzam SI, Alnimer MA. 2013. Effect of protected methionine supplementation on milk production and reproduction in first calf heifers. Arch Anim Breed. 56(1):225–236.

Toledo MZ, Baez GM, Garcia-Guerra A, Lobos NE, Guenther JN, Trevisol E, Luchini D, Shaver RD, Wiltbank MC. 2017. Effect of feeding rumen-protected methionine on productive and reproductive performance of dairy cows. PLoS One. 12(12):e0189117.

Trinacty J, Krizova L, Hadrova S, Hanus O, Janstova B, Vorlova L, Drackova M. 2006. Effect of rumen-protected protein supplemented with three amino acids on milk yield, composition and fatty acid profile in dairy cows. J Anim Feed Sci. 15:3–15.

Waterman RC, Ujazdowski VL, Petersen MK. 2012. Effects of rumen-protected methionine on plasma amino acid concentrations during a period of weight loss for late gestating beef heifers. Amino Acids. 43(5):2165–2177.

Yang WR, Sun H, Wang QY, Liu FX, Yang ZB. 2010. Effects of rumen-protected methionine on dairy performance and amino acid metabolism in lactating cows. Am J Anim Vet Sci. 5:1–7.

Zhou Z, Vailati-Riboni M, Trevisi E, Drackley JK, Luchini DN, Loor JJ. 2016. Better postpartal performance in dairy cows supplemented with rumen-protected methionine compared with choline during the peripartal period. J Dairy Sci. 99(11):8716–8732.