A Thesis Submitted for the Degree of Doctor of Philosophy at

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Factors affecting the response of pregnant and lactating ewes to vitamin E supplementation

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PhD **April, 2019**

Factors affecting the response of pregnant and lactating ewes to vitamin E supplementation

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Abstract

A series of experiments were carried out to investigate the effect of body tissue reserves and source of vitamin E on the response of pregnant and lactating ewes to supra-nutritional levels of vitamin E. In experiment one, at day 77 of pregnancy 48 twin-pregnant ewes (Suffolk x Mule) with a live weight (LW, 82.89 kg) and condition score (C, 3.32) were allocated to one of two groups, and fed to achieve a mean C of either < 2.5 (LC) or > 3.0 (HC) prior to housing. At day 103 (week -6) ewes were individually allocated to one of two concentrates containing either a low vitamin E (LV) (74 mg/day) or high vitamin E (HV) (513 mg/day) level and offered straw *ad-libitum*. The results indicated that there was no significant interaction between nutritional restriction and vitamin E supplementation. High condition score ewe had a higher C at week -6 and lost more C *pre-partum*. Ewe C had no effect on *post-partum* C loss, but LC ewe had a higher LW loss. They also had a higher litter weight. Level of vitamin E had no impact on ewe or lamb performance, but ewes fed HV had higher plasma and colostrum α-tocopherol concentration. In experiment two, at day 103 of pregnancy (week -6), 44 twin bearing Suffolk x Mule ewes with a mean live weight (LW) of 77 kg and body condition score (C) of 2.9 were allocated by parity, LW and C to either a grass silage (GS) or straw (S) based diet supplemented with either a low (L, 240 mg/day) or high (H, 750 mg/day) vitamin E concentrate to provide 4 treatments; GSLV, GSHV, SLV and SHV. Forages were offered *ad-libitum*. The results show that *pre-partum*, ewes offered GS had a higher LW gain and tended to have a lower C loss than those offered S. In addition, they had a higher placental weight. However, the forage source had no effect on litter weight. *Post-partum*, lambs from ewes offered S were heavier at week +8. Ewes offered high vitamin E (HV) had a higher placental weight, cotyledon weight, litter weight and were heavier at week +8 than those offered low vitamin E (LV). In experiment three, 12 castrate lambs (LW, 25 kg) were housed in raised floor pens (1.5 x 2.0 m) in a sheep metabolism room and allocated to either a grass silage (GS) or straw (S) based diet supplemented with either a low (LV, 5.3 mg/kg LW) or high (HV, 10.0 mg/kg LW) vitamin E concentrate to provide 4 treatments; GSL, GSH, SL and SH. The experiment was run over two periods with three lambs being allocated to each treatment in each period, giving six replicates per treatment. The results showed that lambs' offered grass silage as a natural source of vitamin E had higher blood plasma α-tocopherol concentration than those offered α-tocopherol acetate from concentrates. In addition, high vitamin E supplementation reduced diet DM and fibre digestibility resulting in a reduction in the digestible energy (DE) and metabolisable energy ME content of the diet.

Declaration

I declare that this thesis is my original work and it has been written by myself and that it has not been accepted in any previous application for a degree. I have acknowledged all the sources of information which have been used in this thesis.

Araz Abbi, 2019

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Table of contents

Abstract I

Declaration II

Acknowledgements III

Statement of publications and conferences attended IV

List of tables IX

List of figures XII

1. Literature review 1

1.1. General introduction 1

1.2. Vitamin E 3

1.2.1. Vitamin E structure and sources 3

1.2.1.1. Vitamin E chemical classification 3

1.2.1.2. Dietary sources 5

1.2.2. The antioxidant defence system 7

1.2.3. Vitamin E metabolism 10

1.2.3.1. Rumen metabolism 10

1.2.3.2. Intestinal digestion and absorption 11

1.2.3.3. Transport, storage and excretion 12

1.3. Vitamin E requirements of sheep 16

1.3.1. Introduction to vitamin E requirements 16

1.3.2. Nutritional standards and calculation of requirements 16

1.3.2.1. Historical requirements 16

1.3.2.2. Current requirements and allowances 17

1.3.2.3. Inclusion rates in UK sheep diets 19

1.3.3. Deficiency and toxicity 19

1.3.3.1. Assessment of vitamin E status 19

1.3.3.2. Vitamin E and selenium deficiency 20

1.3.3.3. Vitamin E toxicity 21

1.3.3.4. Selenium toxicity 21

1.3.4. Supra-nutritional vitamin E supplementation of sheep 25

1.3.4.1. Effects on ewe performance 25

1.3.4.2. Effects on lamb performance 27

1.3.4.3. Ewe to lamb transfer 29

1.3.4.4. Effects on immunity 31

1.4. Responses of ewes to supra-nutritional supplementation of vitamin E 32

1.4.1. Factors affecting responses to vitamin E 33

1.4.1.1. Body composition of the ewe 33

1.4.1.2. Vitamin E source and absorption 35

1.4.1.3. Rumen environment 37

1.5. Conclusions 38
Chapter Two **40**
General Materials and Methods **40**
2.1. Samples proximate analysis 40
2.1.1. Dry matter 40
2.1.2. Ash and organic matter 40
2.1.3. Crude protein (CP) 40
2.1.4. Ether extract (EE) 41
2.1.5. Neutral detergent fibre 41
2.1.6 Acid detergent fibre (ADF) 42
2.1.7. Gross energy determination (GE) 43
2.1.8. Whole tract digestibility determination by acid insoluble ash method 43
2.2. Blood sampling 44
2.3. Ewes and lambs weighting 44
2.4. Ewe body condition scoring (C) 44
2.5. Ewe back fat and eye muscle depth scanning 45
2.6. Ewe milking 46
2.7. Colostrum and milk analysis 47
2.8. Vitamin E analysis 48
2.8.1. Vitamin E analysis in the plasma and colostrum 48
2.8.2. Vitamin E analysis in Feedstuff 49
2.8.3. HPLC conditions and quantification 49
2. 9. Plasma immunoglobulin (IgG) 50
Chapter 3 .... **52**
Effect of nutritional restriction during mid pregnancy on the response of ewes to vitamin E supplementation during late pregnancy and early lactation **52**
3.1. Introduction 52
3.2. Materials and methods 54
3.2.1. Experimental design 54
3.2.2. Diet formulation and treatments 54
3.2.3. Experimental Routine 57
3.2.4. Chemical analysis 57
3.2.5. Statistical analysis 58
3.3. Results 59
3.3.1. Animal health 59
3.3.2. Diet composition 59
3.3.3. Ewe and lamb performance 59
3.3.3.1. Ewes live weight and weight gain (LW) 59
3.3.3.2. Ewe body condition score (C) 61

3.3.3.3. Ewe back fat thickness 63
3.3.3.4. Ewe muscle depth 64
3.3.3.5. Colostrum yield and composition 66
3.3.3.6. Milk yield and composition 66
3.3.3.7. Litter weight performance 66
3.3.4. Ewe plasma Vitamin E 70
3.3.5. Lamb plasma vitamin E and immunoglobulin (IgG) 70
3.3.6. Blood plasma metabolites 71
3.3.6.1. Plasma glucose 71
3.3.6.2. Plasma BHB 72
3.3.6.3. Plasma NEFA 72
3.3.6.4. Plasma total protein 73
3.3.6.5. Plasma albumin 74
3.3.6.6. Plasma urea 75
3.4. Discussion 77
3.5 Conclusion 82
Chapter 4 83
Effect of source and level of vitamin E supplementation on ewe and lamb performance during late pregnancy and early lactation 83
4.1. Introduction 83
4.2. Materials and methods 84
4.2.1. Experimental design 84
4.2.2. Grass silage, diet formulation and treatments 84
4.2.3. Experimental Routine 87
4.2.4. Chemical analysis 88
4.2.5. Statistical analysis 88
4.3. Results 90
4.3.1. Animal's health 90
4.3.2. Diet composition 90
4.3.3. Food intake 90
4.3.3.1 Forage and total dry matter intake (DMI) 90
4.3.4. Ewes performance 92
4.3.4.1 Ewe live weight and weight gain (LW) 92
4.3.4.2 Ewes body condition score (C) 93
4.3.4.3 Ewe back fat 95
4.3.4.4 Ewe eye muscle depth 96
4.3.4.5. Placenta 97
4.3.4.6. Colostrum yield and composition 100
4.3.4.7. Milk yield and composition 100

4.3.4.8. Litter weight and lamb growth 100
4.3.5. Ewes and lamb plasma vitamin E and IgG 102
4.3.5.1. Ewe plasma vitamin E 102
4.3.5.2. Lamb's plasma vitamin E and IgG 103
4.3.6. Blood plasma metabolites 104
4.3.6.1. Plasma glucose 104
4.3.6.2. Plasma beta-hydroxybutyrate (BHB) 104
4.3.6.3. Non-esterified fatty acids (NEFA) 105
4.3.6.4. Total protein 106
4.3.6.5. Albumin 107
4.3.6.6. Urea 108
4.3.7. Diet digestibility 109
4.4. Discussion 111
4.5. Conclusion 116
Chapter 5 117
Effect of source and level of vitamin E supply on vitamin E absorption and availability in growing lambs 117
5.1. Introduction 117
5.2. Materials and methods 118
5.2.1. Experimental design 118
5.2.2. Digestibility Trial Diets 119
5.2.3. Experimental routine: 121
5.2.4. Chemical analysis: 122
5.2.5. Calculations and statistical analysis: 122
5.3. Results 123
5.3.1. Animal health 123
5.3.2. Diet composition 123
5.3.3. Lamb live weight, dry matter intake and faecal output. 123
5.3.4. Vitamin E intake 125
5.3.5. Nutrients intake and faecal nutrient output 125
5.3.6. Diet apparent digestibility 129
5.3.7. Lambs blood plasma metabolites and vitamin E concentration 131
5.3.8. Lamb plasma vitamin E concentration throughout the experiment 131
5.4. Discussion 133
5.5. Conclusion 136
Chapter 6 137
General Discussion 137
6.1. General Discussion 137
6.2. General Conclusion 144

6.3. Areas for further research 145
References **146**

List of tables

Chapter one

Table 1. 1. Alpha-tocopherol stereoisomers 4

Table 1. 2. Vitamin E content in some ruminant feedstuffs. 6

Table 1. 3. Antioxidant vitamins and minerals and their location of action and activity. 9

Table 1. 4. Some clinical and subclinical symptoms of vitamin E and selenium deficiency in ruminants 23

Table 1. 5. Symptoms of extremely high supplementation of vitamin E in some animal species. 24

Chapter two

Table 2. 1. Sheep body condition scoring system. 45

Chapter three

Table 3.1. Raw material and predicted chemical composition of the experimental concentrate diets (g/kg) 55

Table 3. 2. Concentrate feeding levels (kg/day) for twin bearing ewes offered straw ad-libitum during late pregnancy and early lactation. 56

Table 3. 3. The chemical composition (g/kg DM) of experimental feeds. 59

Table 3. 4. Effect of nutritional restriction and vitamin E supplementation on ewe live weight (kg) and live weight changes (%) 61

Table 3. 5. Effect of nutritional restriction and vitamin E supplementation on ewe condition score (unit) and condition score changes (%) 62

Table 3. 6. Effect of nutritional restriction and vitamin E supplementation on ewes back fat (BF) and back fat changes (mm) 64

Table 3. 7. Effect of nutritional restriction and vitamin E supplementation on ewes muscle depth (MD) and muscle depth changes (mm). 65

Table 3. 8. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on colostrum yield, composition and components yield. 67

Table 3. 9. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on milk yield, composition and components yield. 68

Table 3.10. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on the litter weight gain during post-lambing. 69

Table 3. 11. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on ewe plasma vitamin E (µg/ml). 70

Table 3. 12. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on lamb plasma vitamin E (µg/ml) and IgG (mg/ml). 71

Table 3. 13. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on ewe plasma glucose concentration (mmol/l) 71

Table 3. 14. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on ewe plasma BHB (mmol/l). 72

Table 3. 15. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on ewe plasma NEFA (mmol/l). 73

Table 3. 16. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on ewe plasma total protein concentration (g/l). 74

Table 3. 17. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on ewe plasma albumin (g/l) 75

Table 3. 18. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on ewe plasma urea (mmol/l). 76

Chapter four

Table 4. 1. Raw material and predicted chemical composition of the experimental diets . 85

Table 4. 2. Chemical composition (g/kg DM) of forages 86

Table 4. 3. Predicted forage dry matter intake for 75 kg twin-bearing ewes producing 3.0 litres/ day of milk during early lactation according to (AFRC, 1993) 86

Table 4. 4. Concentrate allocation for 75 kg twin-bearing ewes producing 3.0 litres/ day of milk during early lactation or 2.0 litres/ day of milk during late lactation 86

Table 4. 5. Total dry matter intake (TDMI) and nutrient supply for 75 kg twin-bearing ewes producing 3.0 litres/ day of milk during early lactation 86

Table 4. 6. Relative proportions of the low and high vitamin concentrates required by ewes offered the straw based diets to provide similar levels of vitamin E intake to those offered the grass silage based diets. 87

Table 4. 7. Predicted vitamin E supply (mg/day) for 75 kg twin-bearing ewes producing 3.0 litres/ day of milk during late pregnancy early lactation 88

Table 4. 8. The chemical composition of experimental feedstuff (g/kg DM) 90

Table 4. 9. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on ewe live weight and live weight change. 93

Table 4. 10. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on ewe body condition score (C) and body condition score change. 94

Table 4. 11. The effect of forage type and vitamin E supplementation during late pregnancy and early lactation on ewe back fat depth (mm) and back fat change (mm). 96

Table 4. 12. The effect of forage type and vitamin E supplementation during late pregnancy and early lactation on the ewe eye muscle depth (mm) and eye muscle depth change (mm). 97

Table 4. 13. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on the placental weight and placental efficiency. 99

Table 4. 14. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on colostrum yield, colostrum composition and component yields........... 101

Table 4. 15. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on milk yield, milk composition and component yields. 101

Table 4. 16. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on the ewe litter weights (kg) and litter weight changes (kg)................... 102

Table 4. 17. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on the ewe blood plasma vitamin E (µg/ml). 103

Table 4. 18. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on lamb blood plasma vitamin E and IgG at week +2. 103

Table 4. 19. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on the ewe blood plasma glucose (mmol/ l). 104

Table 4. 20. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on the ewe blood plasma beta-hydroxybutyrate (BHB) (mmol/ l). 105

Table 4. 21. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on the ewe blood plasma non-esterified fatty acids (NEFA) (mmol/ l). 106

Table 4. 22. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on the ewe blood plasma total protein (g/ l) 107

Table 4. 23. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on the ewe blood plasma albumin (g/ l).. 108

Table 4. 24. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on the ewe blood plasma urea (mmol/ l) 109

Table 4. 25. The effect of forage type and vitamin E supplementation in late pregnancy on diet digestibility 110

Chapter five

Table 5. 1. Raw material and predicted chemical composition of concentrate diets. 120

Table 5. 2. Analysed chemical composition (g/kg DM) of grass silage (GS) and straw (S) 120

Table 5. 3. Raw material and chemical composition of lamb diets. 121

Table 5. 4. Daily fresh feeding offered levels (g/day) and offered nutrient supply for 30 kg growing lamb 121

Table 5. 5. Chemical composition (g/kg DM) of the forages and concentrates used in the experiment. 123

Table 5. 6. The effect of forage type and vitamin E level on live weight (kg), dry matter intake (g/ day) (g) and faecal output (g) of lambs used in the experiment. 124

Table 5. 7. The effect of forage type and vitamin E level on vitamin E intakes of lambs used in the experiment. 127

Table 5. 8. The effect of forage type and vitamin level on nutrient intake and faecal nutrient output (g/day) of lambs used in the experiment. 128

Table 5. 9. The effect of forage type and vitamin E level on apparent diet digestibility and energy values 130

Table 5. 10. The effect of forage type and vitamin E level on lamb blood metabolites ... 131

List of figures

Chapter one

Figure 1. 1. Vitamin E chemical structure; Source: (DellaPenna, 2005) 4

Figure 1. 2. Vitamin E metabolism. HDL: high density lipoprotein; VLDL: very low density lipoprotein; LDL: low density lipoprotein. 15

Chapter two

Figure 2. 1. Eye muscle area and fat layer of lamb. 46

Figure 2. 2. Standard calibration curve of rabbit-anti-sheep IgG for lamb’s plasma IgG determination 51

Chapter three

Figure 3. 1. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on the ewe live weight (kg) and live weight change (kg) during the experiment. 60

Figure 3. 2. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on the ewe condition score (unit) and condition score changes (unit) during the experiment. 62

Figure 3. 3. Effect of nutritional restriction and vitamin E supplementation on ewes back fat (mm) 63

Figure 3. 4. Effect of nutritional restriction and vitamin E supplementation on ewe muscle depth 65

Chapter four

Figure 4. 1a. Ewes forage dry matter intake (DMI) in late pregnancy and early lactation 91

Figure 4. 1b. Ewes total dry matter intake (TDMI) in late pregnancy and early lactation 92

Figure 4. 2. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on ewe live weight. 93

Figure 4. 3. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on the ewe body condition score (C). 94

Figure 4. 4. The effect of forage type and vitamin E supplementation during late pregnancy and early lactation on ewe back fat depth 95

Figure 4. 5. The effect of forage type and vitamin E supplementation during late pregnancy and early lactation on ewe muscle depth. 97

Chapter five

Figure 5. 1. The effect of forage type and vitamin E level on plasma vitamin E concentration throughout the experiment 132

Chapter six

Figure 6. 1. Relationship between dietary vitamin E supply (mg/day) and plasma vitamin E concentration (µg/ml) for ewes in late pregnancy offered the diets fed in experiments 1 and 2. 139

Figure 6. 2. Relationship between dietary vitamin E supply (mg/day) and plasma vitamin E concentration (µg/ml) for ewes in early lactation offered the diets fed in experiments 1 and 2. 140

Figure 6. 3. Relationship between ewes plasma vitamin E (µg/ml) during late pregnancy and litter weight (kg) for ewes offered the diets fed in experiments 1 and 2. 141

Figure 6. 4. Relationship between ewe vitamin E intake (mg/day) and colostrum vitamin E concentration (mg/kg) for ewes offered the diets fed in experiments 1 and 2. 142

Figure 6. 5. Relationship between colostrum vitamin E concentration (mg/kg) and lambs' plasma vitamin E concentration (µl/ml) for ewes offered the diets fed in experiments 1 and 2. 143

1. Literature review

1.1. General introduction

The productivity of a sheep enterprise depends on lamb output (Merrell *et al.,* 1998; Capper *et al.,* 2007). Over the last forty years lamb mortality rate has remained relatively constant at approximately 15% in many countries including the UK (Dwyer *et al.*, 2015; AHDB, 2016). Lamb mortality occurs due to the effect of breed (Nash *et al.*, 1996; Mukasa-Mugerwa *et al.*, 2000), weather conditions (Nash *et al.*, 1996), hypothermia (Dwyer *et al.*, 2015) and type of birth. For example, lamb mortality is higher in ewes bearing triplets or twins, than single lambs (Nash *et al.*, 1996). Lamb birth weight can also affect lamb mortality, with lambs with a low birth weight being weak and lacking vigour (Dalton *et al.*, 1980; Nash *et al.*, 1996). Finally, lamb mortality can be increased by poor colostrum production, milk production or suckling (Binns *et al.*, 2002). The highest proportion of lamb mortality (up to 50%) takes place during the first 24 hours to 3 days of life (Hatfield *et al.*, 2000; Dwyer *et al.*, 2015). The transition of lambs from intrauterine to environmental oxygen induces oxidative stress and the production of free radicals (Mutinati *et al.*, 2013; Liu *et al.*, 2014). This increase of free radicals, causes oxidative stress for the neonate and precipitates the development of pathological changes which potentially lead to lamb death (Mutinati *et al.*, 2014). Vitamin E is characterised by an antioxidant function, which refers to its ability to protect animal's cells from oxidative damage and potentially increase immunity (Burton *et al.*, 1980). Alpha-tocopherol inhibits peroxidation of polyunsaturated fatty acids (PUFA) in the cell membrane of animal tissues by preventing the oxidation of lipoprotein into hydro-peroxides (Bramley *et al.*, 2000). As an antioxidant, vitamin E supplementation might reduce the lamb's oxidative stress (Liu *et al.*, 2014). It has been reported that vitamin E supplementation during late pregnancy significantly reduces neonatal mortality (Kott *et al.*, 1998; Dønnem *et al.*, 2015).

Evidence suggests that supplementation of ewes during late pregnancy above currently accepted vitamin E requirements may enhance lamb birth weight and neonatal lamb survival (Merrell *et al.,* 1998; Capper *et al.,* 2005). It also has a positive effect on neonatal immunity, colostrum and milk production in sheep (Capper *et al.,* 2005; Rosales Nieto *et al.,* 2015). However, these responses are inconsistent (Rooke *et al.,* 2008) and several authors have reported no benefits (Dafoe *et al.*, 2008; Rooke *et al.,* 2009). This inconsistency might be due to the variations between studies in term of vitamin E supplementation levels, the availability of vitamin E in body tissues or vitamin E form from natural and synthetic sources.

The National Research Council NRC (1985) recommended that the vitamin E requirement of ruminants should be between 10 to 60 mg/kg DM, and suggested a dietary requirement of 20 mg/kg DM. To protect animals from white muscle disease (WMD), the National Research Council NRC (2007) recommended that vitamin E requirements should be increased to maintain plasma α-tocopherol level above a critical values of ≥2 µg/ml. To maintain plasma α-tocopherol concentration ≥ 2.0 µg/ml, they recommended that animal requirements should be increased to 5.3 IU/kg live weight. For an 85 kg ewe, this equates to approximately 450 mg/day. In the UK, EBLEX (2014b) recommended that the vitamin E requirements of the pregnant and lactating ewe should be 100-150 mg/kg DM to protect lambs from white muscle disease and enhance performance. These feeding levels are lower than (NRC, 2007), but higher than previous recommendations (ARC, 1980 and NRC, 1985). Vitamin E supplementation above recommended levels may enhance ewe and lamb performance (Capper *et al.* 2005; Capper *et al.* 2006; Nieto *et al.* 2016) .

Liver, skeletal muscle, and adipose tissue are able to accumulate around 90% of the total vitamin E in the body (Machlin and Gabriel, 1982; Bjorneboe *et al.,* 1986; Bjørneboe *et al.*, 1990). As a fat-soluble vitamin, the majority of vitamin E is stored in adipose tissue (Bjørneboe *et al.*, 1990; Bramley *et al.,* 2000). In addition, vitamin E is assimilated into adipose tissues more avidly but is depleted at a similar rate to muscle (Hidiroglou *et al.,* 2003). Moreover, the concentration of vitamin E in adipose tissue is highly variable and depends upon the previous diet (Ochoa *et al.*, 1992). For example, the concentration of vitamin E in sheep tissues increased by increasing supplementation of vitamin E in the diet (Ochoa *et al.*, 1992; Njeru *et al.*, 1994b). Inconsistent results have been published as to whether tocopherol is mobilised from adipose tissue during nutritional restriction (Machlin *et al.,* 1979; Schaefer *et al.,* 1983; Fry *et al.,* 1993). The mobilisation of α-tocopherol has been documented in sheep (Fry *et al.,* 1993). However, a limited mobilisation of α-tocopherol has been documented in guinea pigs (Machlin *et al.,*1979) and humans (Schaefer *et al.,* 1983; Janneke Brouwer *et al.,*1998). Therefore, ewes with low fat reserves at the end of mid pregnancy may show a higher response to supra-nutritional vitamin E supplementation during late pregnancy. This could be due to mobilisation of adipose tissue and vitamin E during mid pregnancy or limited adipose tissue capacity for vitamin E accumulation during late pregnancy.

Naturally occurring vitamin E from forages is biologically more active and more efficiently absorbed in the small intestine than the synthetic form of vitamin E generally included in concentrate feeds (Burton and Traber, 1990; Kasapidou *et al.*, 2009). However, the mechanism is poorly understood. The form of vitamin E and rumen environment may be

important factors in explaining responses to additional vitamin E supplementation (Chikunya *et al.* 2004; Kasapidou *et al.*, 2009). Therefore, the response of pregnant and lactating ewes may be higher to natural vitamin E.

The objective of this series of experiments was to investigate the effect of body tissue reserves and source of vitamin E on the response of pregnant and lactating ewes to supra-nutritional levels of vitamin E supplementation.

1.2. Vitamin E

1.2.1. Vitamin E structure and sources

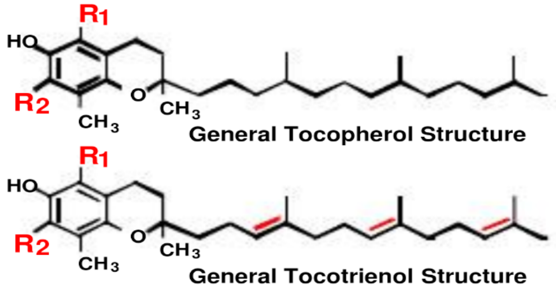
1.2.1.1. Vitamin E chemical classification

Vitamin E is a powerful fat-soluble antioxidant vitamin (Fritsche *et al.*, 2017). Vitamin E was first discovered in 1922 when Evans and Bishop found an unknown component in the oil of vegetables that was a necessity for reproduction in female rats and protected their foetuses from death when the rats were fed rancid fat (McDowaell, 2000; DellaPenna and Mène-Saffrané, 2011).

Vitamin E (tocochromanols) can be categorised into eight biologically active isomers; four are tocopherols (α, β, γ, and δ) and the remaining four are tocotrienols (α, β, γ, and δ) (DellaPenna, 2005; Fritsche *et al.*, 2017) (Figure 1.1). Among them, α-tocopherol is considered to be the most important biologically active form in animal tissues and is often added to animal diets as α-tocopherol acetate (Scherf *et al.*, 1996). The difference between tocopherols and tocotrienols is the presence of a phytol chain in the tocopherols structure, which is replaced by an unsaturated chain due to the presence of a double bond in the positions 3,7 and 11 of the hydrocarbon tail in tocotrienols (Schaffer *et al.*, 2005; Fritsche *et al.*, 2017). Furthermore, the analogues (α, β, γ, and δ) differ from each other by the location of methyl (CH_3) groups at position 5, 7 or 8 of the chromanol ring (Scherf *et al.*, 1996; Reboul, 2017; Desmarchelier and Borel, 2018)

Tocopherols are classified as either natural or synthetic, depending on their sources (Table 1.1). Firstly, the naturally occurring *RRR*-α-tocopherol is considered the most bioactive among the four available tocopherols that can be found in grains and forages (Azzi, 2018). Secondly, dl-α-tocopherol (all-*rac*-α-tocopherol) is the synthetic form of α-tocopherol that is used for feed supplementation and consists of a racemic mixture of the eight stereoisomers (*RRR,RRS,RSR,RSS,SSS,SRS,SSR and SRR*) (Meglia *et al.*, 2006; Wilburn *et al.*, 2008 and Yang, 2003). Naturally occurring *RRR*-α-tocopherol is more bioavailable than synthetic

all-*rac*-α-tocopherol (Yang, 2003). The activity of vitamin E can be expressed in international units (IU), which refer to the biological efficiency of these compounds (Yang, 2003). The bioavailability of each 1 mg of *RRR*-α-tocopherol is equal to 1.49 IU α-tocopherol (Yang, 2003). The bioavailability of all stereoisomers is summarised in Table 1.1. The active hydroxyl site of α-tocopherol is easy for oxidation (Wilburn *et al.*, 2008). Therefore, succinate esters or acetate forms of α-tocopherol are synthesised to be more stable and prevent alcohol oxidation when added to animal diets (Wilburn *et al.*, 2008; Vagni, 2011).



Form	R_1	R_2	*Relative Activity (toco vs trienol)
α-tocopherol / trienol	$-CH_3$	$-CH_3$	100% vs 30%
β-tocopherol / trienol	$-CH_3$	-H	50% vs 5%
γ-tocopherol / trienol	-H	$-CH_3$	10% vs 0%
δ-tocopherol / trienol	-H	-H	3% vs 0%

Figure 1. 1. Vitamin E chemical structure; Source: (DellaPenna, 2005)

Table 1. 1. Alpha-tocopherol stereoisomers

α-tocopherol stereoisomers	Bioavailability IU/mg
(*RRR*) *d*-α-tocopherol	1.49
(*RRR*) *d*-α-tocopherol acetate	1.36
(*RRR*) *d*-α-tocopherol succinate	1.21
(*all-rac*) *dl*-α-tocopherol	1.10
(*all-rac*) *dl*-α-tocopherol acetate	1.00
(*all-rac*) *dl*-α-tocopherol succinate	0.89

Source: Adapted from (Yang, 2003)

1.2.1.2. Dietary sources

Ruminants depend on fresh forages as an important natural source of vitamin E (Elgersma *et al.*, 2012). Naturally, photosynthetic eukaryotes (plants and algae) and other oxygenic photosynthetic organisms such as cyanobacteria and algae are the main natural organisms responsible for vitamin E biosynthesis (Fritsche *et al.*, 2017). As an antioxidant, vitamin E has an essential role in maintaining the photosynthesis process in photosynthetic organisms via protecting cell membranes from oxidative damage, lipid peroxidation and photo-oxidation (Chaudhary and Khurana, 2013). As mentioned above, to protect cells from oxidative damage and lipid peroxidation, plants synthesise and accumulate tocochromanols (tocopherols + tocotrienols) in growing tissues that undergo new cell divisions, fruits and seed oil (Colombo, 2010).

Vitamin E can be found in different amounts in animal feedstuffs. These amounts may be three to ten times higher in some feedstuffs compared to others (Mcdowell, 2000) (Table 1. 2). Cereal grains are a rich source of vitamin E, especially the germ, and thus by-products containing the germ are particularly high (McDowell *et al.*, 1996). The alpha-tocopherol level is high in leaves and green plant tissues, whereas the seeds contain a high concentration of γ-tocopherol (Fritsche *et al.*, 2017). Consequently, the best sources of natural α-tocopherol are green forage, good-quality hay and Lucerne (McDowell *et al.*, 1996). All-*rac*-α-tocopherol (dl-α-tocopherol acetate) is the most available synthetic form of α-tocopherol used widely as a source of vitamin E for animal feed supplementation (McDowell *et al.*, 1996). The acetate ester is resistance to *in vitro* oxidation, and it is easily hydrolysed to non-esterified (free tocopherol) in the gut of the animal (McDowell *et al.*, 1996).

Several factors affect the concentration of vitamin E in plants, such as processing, storage conditions, wilting, and diet type. McDowell *et al.* (1996) documented that the poor stability of naturally occurring tocopherols affects the amount of vitamin E in feedstuff. Therefore, there is a substantial reduction in α-tocopherol activity during processing and storing. In green forages, the vitamin E content depends on the grass species and the leaf to stem ratio; for example, leaves contain 20-30 times more vitamin E than the stem. Stage of maturity is also important with mature grass containing less vitamin E than immature grass (McDonald *et al.*, 2011). In a study to investigate the tocopherol concentration of seven herb species and perennial ryegrass, Elgersma *et al.* (2012) reported that the tocopherol concentration varied between 21 and 85 mg/kg DM over the growing season and was higher in caraway, chicory, birdfoot trefoil, salad burnett and plantain than it was in lucerne and yellow sweet clover. In addition, the vitamin E concentrations were highest in October.

Table 1. 2. Vitamin E content in some ruminant feedstuffs.

Feedstuff	Regions	α-tocopherol (mg/kg DM)
Maize	USA	1.4-14.7
Maize Seed	Undefined	60
Maize seed oil	Undefined	1000
Barley	USA	7.9-9.6
Barley	Australia	0.6-6.9
Barley straw	Australia	2.5-12.6
Wheat	USA	8.4-12.1
Wheat, brane	Undefined	15-19
Wheat Seed	Undefined	50
Wheat germ oil	Undefined	2700
Soybean seed oil	Undefined	1200
Sunflower seed oil	Undefined	700
Lucerne dehydrated or meal	USA	27.5-83.6
Lucerne meal, dehydrated 17% protein	Undefined	28-121
Lucerne meal, sun-cured 13% protein	Undefined	18-61
Lucerne Hay	Undefined	23-102
Pasture (March, August)	South Australia	26-166
Grass (Leafy)	Undefined	29-172
Grass Silage	Undefined	4-145
Maize Silage	Undefined	0-9
Hay	Undefined	1-85

Source: Adapted from (McDowell, 2000; Liu *et al.*, 2014; DellaPenna, 2005)

Over two years research using four mixtures of five different herbs to measure α-tocopherol harvested in four cuts, Lindqvist *et al.* (2014) found that the highest α-tocopherol concentration was recorded in birdsfoot trefoil in most harvests (86 mg/kg DM), while the mean α-tocopherol concentration in red clover and white clover were 34 and 37 mg/kg DM respectively. In addition, Lindqvist *et al.* (2014) documented that the average for timothy and perennial ryegrass were 46 and 50 mg/kg DM respectively. The natural α-tocopherol is unstable; therefore, the concentration reduces when the forages and cereals are harvested, artificially dried and stored for an extended period. Vitamin E losses can be 50% in one

month, lucerne stored for 12 months at 33°C lost 54-73% vitamin E, and commercial dehydrated lucerne lost 5-33% (McDowell *et al.*, 1996). In a study on the effect of wilting, additive type and ensiling on the concentration of α-tocopherol in legume-grass mixtures, Lindqvist *et al.* (2012) found that wilting reduced α-tocopherol quantity by 30% in timothy+ birdsfoot trefoil, ensiling raised α-tocopherol content of timothy+ birdsfoot trefoil from 41.1 mg/kg DM in wilted herbage to 56.8, 56.9 and 65.2 mg/kg DM in acid treated silage, untreated and inoculated respectively. Alpha-tocopherol concentration was higher in red clover silage (50.1 vs 34.2 mg/kg DM) that was treated with an inoculant, compared with untreated red clover silage. A short wilting period (three hours) might be the reason for the effect of wilting on α-tocopherol reduction only in timothy+ birdsfoot trefoil (Lindqvist *et al.*, 2014). The vitamin E content of concentrate diets also decreases as a result of oxidation, which occurs during the processes of milling, and pelleting (Mcdowell., 2000).

1.2.2. The antioxidant defence system

Free radical production is a normal process that happens in living organisms during oxidative damage of lipids, particularly polyunsaturated fatty acid (PUFA) oxidation, which is called lipid peroxidation (Yamauchi, 1997; Mutinati *et al.*, 2014; Chauhan *et al.*, 2014). Free radicals are molecules that have one or more uncoupled electrons which are characterised as being high reactive (Mutinati *et al.*, 2014). These free radicals try to pair their electrons by giving or obtaining an electron from other molecules to produce more stable types (Pourova *et al.*, 2010). Free radicals are created as by-products of non-enzymatic and enzymatic reactions during normal metabolic processes in living organisms (Pourova *et al.*, 2010; Yaribeygi *et al.*, 2019). Free radicals are divided into two main groups; reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Mutinati *et al.*, 2014). The main biological active ROS are hydroperoxyl ($HO_2^{\bullet}$), alkoxyl ($RO^{\bullet}$), peroxyl ($ROO^{\bullet}$), hydroxyl radical ($OH^{\bullet}$) and superoxide anion ($O^{-}{}_2{}^{\bullet}$) (Pourova *et al.*, 2010; Rizzo *et al.*, 2012; Mutinati *et al.*, 2014; Niki, 2014). When production is within normal physiological levels, ROS are essential for the activity of all cells. However, excessive production of ROS can lead to oxidative stress and damage to tissue and cell (Rizzo *et al.*, 2008). ROS cause DNA damage, peroxidation of lipid in the cell membrane, and protein damage (Rizzo *et al.*, 2012).

The reactive nitrogen species (RNS) include several free radicals, and non-free radicals including nitroxide (NO), nitric dioxide ($NO_2\cdot$), peroxynitrite and its protonated form (peroxynitrous acid, ONOOH), alkylperoxynitrite (ROONO) and nitrosil (NO+) (Pourova *et al.*, 2010). Antioxidants are divided into two types: enzymatic antioxidant and non-enzymatic antioxidant (Sugino, 2006). The metalloenzymes that include glutathione peroxidase (Se),

superoxide dismutase (Zn and Cu) and catalase (Fe), are considered as intracellular antioxidant enzymes that convert hydrogen peroxide (H_2O_2) to water and alcohol and protect intracellular components from oxidative damage (McDowell *et al.*, 2007; Spears and Weiss, 2008). Whereas, several vitamins and mineral are considered to act as non-enzymatic antioxidants (McDowell, 2000; McDowell *et al.*, 2007; Wu *et al.*, 2019). Non-enzymatic antioxidant are also recognised as a synthetic or dietary supplemental antioxidant which includes vitamins (E, C and β-carotene), and minerals that are work via enzymes include selenium(Se), iron, copper, zinc and manganese (Rizzo *et al.*, 2012).

Vitamin E is characterised as being a strong natural lipid-soluble antioxidant (Fritsche *et al.*, 2017). Vitamin E can react with polyunsaturated acyl groups and extinguish free radicals action by scavenging lipid peroxyl radicals (Fritsche *et al.*, 2017).

Alpha-tocopherol is considered to be the main antioxidant in plasma and mammalian tissues as it prevents the oxidation of polyunsaturated fatty acids (PUFA) and lipoproteins in cell membranes to hydroperoxides and protects animal cell membrane from oxidative damage (Bramley *et al.*, 2000; Mcdowell, 2000). The inhibition of lipid peroxidation occurs as a consequence of the donation of hydrogen atoms (H) from the phenolic hydroxy group in the chromanol nucleus of vitamin E to produce an alpha-tocopherol radical which is totally stable (Debier and Larondelle, 2005).

Vitamin E is the first line of defence which protects phospholipids from peroxidation while the second line of defence is the enzyme glutathione peroxidase (GPx) that contains selenium (Se), which works on the destruction of peroxides and hydroperoxides (McDowell *et al.*, 2007). Selenium (Se) is an essential trace element for humans and animals, which plays an essential role in the protection of animal's tissue cells from oxidative damage (Lu and Holmgren, 2009; McDowell, 2000). Selenium is the most essential mineral that has an antioxidant role (McDowell *et al.*, 2007). Selenium is a constituent of several selenoproteins antioxidant enzymes including, glutathione peroxidases (GPxs) and thioredoxin reductases (TrXR1) (Lu and Holmgren, 2009; McDowell *et al.*, 2007).

Glutathione peroxidase enzymes can be categorised to five main groups including the ubiquitous cytosolic glutathione peroxidase (GPx1), gastrointestinal glutathione peroxidase (GPx2), plasma glutathione peroxidase (GPx3), phospholipid hydroperoxide glutathione peroxidase (GPx4) and the olfactory epithelium and embryonic tissue-specific glutathione peroxidase (GPx6) (Surai, 2007; Lu and Holmgren, 2009; Rayman, 2012; Chauhan *et al.*, 2014). The above glutathione peroxidases (GPxs) works as antioxidants by removing hydrogen peroxide (Chauhan *et al.*, 2014).

Glutathione peroxidase (GPx1) works on retroviral virulence reduction via preventing viral mutations (Rayman, 2012; Chauhan *et al.*, 2014). Glutathione peroxidases (GPx4) is an essential antioxidant against lipid peroxidation that reduces phospholipid and cholesterol hydroperoxides (Lu and Holmgren, 2009; Mehdi and Dufrasne, 2016). Whereas, GPx1–3 catalyse organic hydroperoxides and the reduction of hydrogen peroxide (Lu and Holmgren, 2009). Plasma glutathione peroxidase (GPx3) is an extracellular fluid antioxidant that protects the thyroid's follicular lumen and thyrocytes from hydrogen peroxide (Rayman, 2012; Chauhan *et al.*, 2014). Glutathione peroxidase enzymes (GPx) have a synergistic action with α-tocopherol to protect tissue cells membranes from the oxidative damage (Van Metre and Callan, 2001). Selenium works synergistically with vitamin E and delays the signs of vitamin E deficiency, In addition, sulphur containing amino acids may act as precursors for glutathione peroxidase and may play an important role, together with vitamin E, in delaying symptoms of Se deficiency. (Mcdowell, 2000).

Table 1. 3. Antioxidant vitamins and minerals and their location of action and activity.

Nutrients	position in the cell (Component)	Activity
Vitamin E	Membranes (α-tocopherol)	Prevent fatty acid peroxidation
Selenium	Cytosol (Glutathione peroxidase GPx1, GPx3)	An enzyme that converts hydrogen peroxide to water
Vitamin C	Cytosol (Ascorbic acid)	Reaction with various kinds of RNS and ROS
β-carotene	Membranes (β-carotene)	Prevent starting peroxidation of fatty acids chain reaction
Zinc and copper	Cytosol (superoxide dismutase)	Transformation of superoxide to hydrogen peroxide
Zinc and Manganese	Mitochondria (superoxide dismutase)	Transformation of superoxide to hydrogen peroxide
Copper	Blood plasma (ceruloplasmin)	An antioxidant protein, maybe prevent iron and copper from participating in oxidation reactions
Iron	Cytosol (catalase)	Transformation hydrogen peroxide to water

Source: Modified from (McDowell *et al.*, 2007; Wu *et al.*, 2019)

Low tissue α-tocopherol content is often correlated with high GPx concentration, which suggests that there is a compensational relationship between vitamin E and GPx to protect lipids from peroxidation (Daun *et al.*, 2001). Nevertheless, although these two antioxidants work to complement each other (Mehdi and Dufrasne, 2016), the surplus of one does not recompense for a deficiency of the other (Mcdowell, 2000). Vitamin C (ascorbic acid) reduces vitamin E requirements by scavenging free radicals and vitamin E (Rucker *et al.*, 2001). Vitamin C can donate an electron to tocopheryl free radicals after being oxidised to regenerate vitamin E (McDowell, 2000). All antioxidant vitamins and minerals, and their location in the animal's body and activity are summarised in Table (1.3).

1.2.3. Vitamin E metabolism

1.2.3.1. Rumen metabolism

In ruminants, there is little evidence to suggest that vitamin E is degraded in the rumen, or absorbed through the rumen wall. The majority of ingested vitamin E is absorbed in the small intestine. Early researchers reported significant degradation of vitamin E in the rumen, with the amount degraded increasing with concentrate proportion. Alderson *et al.* (1971) found that there was a pre-intestinal disappearance of vitamin E of 8.4, 22.2, 25.0 and 42.4% when diets containing 20, 40, 60 and 80% corn respectively. Therefore, Alderson *et al.* (1971) proposed that the small intestine absorbed only a proportion of the dietary vitamin E, and the amount degraded in the rumen was positively correlated with the proportion of concentrate in the diet. Concentrate supplementation changes the characteristics of the rumen microbial population, and this change might favour microorganisms that have a high α-tocopherol degradation propensity. However, Belanche *et al.* (2017) found that vitamin E supplementation with concentrate *in vitro* had a positive effect on fermentation by increasing gas production, total VFA, protozoal activity and resulted in a small increase in feed degradability. Increases in microbial activity suggest that the rumen microbes require vitamin E. It does not necessarily indicate that vitamin E is lost in the rumen, and might be incorporated into the microbes themselves.

Shin and Owens (1990) reported rumen vitamin E losses of 16 to 44% using duodenal cannulas in adult and young calve steers. Likewise, Chikunya *et al.* (2004) reported rumen α-tocopherol losses of either 21% or 9% when cannulated sheep were supplemented with either 100 or 500 mg/kg DM α-tocopherol.

In contrast, Leedle *et al.* (1993) found that vitamin E concentration did not change during *in vitro* ruminal fermentation. Leedle *et al.* (1993) supported the supposition that rumen

microorganisms degrade vitamin E using a high concentrate corn based diet fed to cannulated cattle in a series of *in vitro* experiments. Rumen contents were supplemented with α-tocopherol acetate, equivalent to 450 mg/animal/day, and after 24 hours of incubation, the amount remaining was recorded. Vitamin E recovery varied from 85 to 50% during 24 hours of incubation using three different methods of vitamin E extraction. In later experiments, using one extraction method, the results indicated that vitamin E recovery was 96% and that there was no vitamin E degradation and no biological activity of esterase enzymes upon α-tocopherol acetate. Anaerobic degradation or bio-hydrogenation of α-tocopherol is not easy as a result of the presence of a double bond within the aromatic ring (Leedle *et al.*, 1993). Poor extraction of α-tocopherol from the digesta might be the reason why other authors have reported ruminal destruction of vitamin E. In an *in vitro* study to determine free α-tocopherol disappearance and α-tocopherol acetate hydrolysis in the rumen, Han and Owens., (1999), used rumen fluid collected from beef steers fed on high roughage or concentrates. Both tocopherol and tocopherol acetate were added to rumen fluid to achieve two tocopherol concentrations (5 or 10 μl/L), and incubated in bottles at 39 ºC for 24 hours. After incubation, the free α-tocopherol concentration was unchanged, which support the theory that α-tocopherol is not degraded in the rumen. Overall, current evidence suggests that there is negligible degradation of α-tocopherol in the rumen.

1.2.3.2. Intestinal digestion and absorption

The process of vitamin E digestion and absorption takes place in association with digestion and absorption of fats (Bramley *et al.*, 2000; Baldi, 2005; Vagni, 2011). The availability of vitamin E varies between 30 to 70%, which indicates that a high proportion of ingested vitamin E is excreted in the faeces (Bramley *et al.*, 2000; Debier and Larondelle, 2005). During digestion, vitamin E is emulsified with fat globules to smaller particles (Bramley *et al.*, 2000). Biliary salts produced in the liver and released into the duodenum from the gallbladder act as emulsifying agents, which break down fat to smaller particles (Hollander *et al.*, 1975; Borel *et al.*, 2001). Triglycerides are converted to diacylglycerols, monoglycerides, free fatty acids and glycerol by pancreatic lipase, which acts as a fat digesting enzyme (Hollander *et al.*, 1975; Borel *et al.*, 2001; Nelson and Cox, 2011). Together (bile salts, fatty acids, and vitamin E), are incorporated into a water-soluble micellular solution of bile salts and fatty acids (Bramley *et al.*, 2000). Micelles then move through the unstirred water layer, towards the microvilli membrane where they are absorbed across the brush border into the proximal small intestine (Hollander *et al.*, 1975; Hollander, 1981and Bramley *et al.*, 2000).

Vitamin E and fatty acid absorption take place together. Therefore lipid digestion inefficiency or disruption also affects vitamin E absorption efficiency (Herdt and Stowe, 1991). Fat quantity and quality in the stomach has an important effect on vitamin E absorption (Baldi, 2005; Vagni, 2011). Vitamin E absorption rate in the small intestine increases with medium-chain triglycerides, whereas absorption efficiency reduces with long-chain polyunsaturated fatty acids (PUFA) (Mcdowell, 2000; Hollander, 1981). Vitamin E absorption efficiency decreases with increasing level of PUFA supplementation (Hollander, 1981). This may due to increased micelles size or the enhanced negative surface charge of micelles containing a high vitamin E concentration (Hollander, 1981). Variation in intestinal absorption may be the main reason for vitamin E losses between ingestion and integration into plasma. This was attributed to an interaction between dietary PUFA and vitamin E supplementation (Bramley *et al.*, 2000). The study of Capper *et al.* (2005) indicated that dietary PUFA supplementation decreased plasma vitamin E at day 14 *post-partum* in pregnant sheep, Capper *et al.* (2005) linked this to an increase of oxidative stress in the animal's body due to additional PUFA supplementation. However, it could be due to the poor absorption of vitamin E associated with PUFA supplementation.

1.2.3.3. Transport, storage and excretion

Following absorption of micelles into the intestinal mucosa, vitamin E together with other particles such as phospholipids, free and esterified cholesterol, triglyceride, fat-soluble vitamins, carotenoids, apolipoproteins and proteins are re-assembled and incorporated into chylomicrons (Bramley *et al.*, 2000)(Figure 1.2). Chylomicrons are then released into the circulation after being transported through the lymphatic pathway from the intestine (Bramley *et al.*, 2000). In the circulation, catabolism of chylomicrons by lipoprotein lipase takes place to produce chylomicron remnants (Debier and Larondelle, 2005). In addition, some of the vitamin E is incorporated into high-density lipoproteins (HDL) which can transfer vitamin E to several body tissues including adipose tissue, muscle and skin as a transferring protein (Traber *et al.*, 1985). Tissues can synthesise lipoprotein lipase (adipose tissue) (Bramley *et al.*, 2000). Therefore, this mechanism is used by most tissues for obtaining vitamin E (Bramley *et al.*, 2000). However, chylomicron remnants containing the majority of absorbed tocopherol, are collected by liver parenchymal cells (Bramley *et al.*, 2000). During chylomicron metabolism in the blood, vitamin E is rapidly taken up by the liver (Herdt and Stowe, 1991; Azzi and Stocker, 2000; Bramley *et al.* (2000). The liver packages the chylomicron remnants, including vitamin E (Debier and Larondelle, 2005) . The liver has a major role in tocopherol release into the circulation and movement toward peripheral tissues (Traber *et al.*, 1990; Traber *et al.*, 1992). Alpha-tocopherol uptake into very low-density

lipoprotein (VLDL) from hepatic cells is under the control of a cytosolic protein named α-tocopherol transfer protein (α-TTP) (Kuhlenkamp *et al.*, 1993). The predominance of α-tocopherol in tissues and plasma compared with other tocopherol isomers could be due to the preference of α-TTP to bind with α-tocopherol over the γ-homologue (Bramley *et al.*, 2000; Schubert *et al.*, 2018). Alpha-TTP can differentiate between the number and location of the methyl groups on the chromanol ring resulting in over 80% of plasma vitamin E being in the α-form of tocopherols (Bramley *et al.*, 2000). Even though absorption of both α- and γ-tocopherol is similar and they assimilate to chylomicrons in the same quantity, α-tocopherol is preferentially kept while γ-tocopherol is excreted in bile (Hosomi *et al.*, 1997; Bramley *et al.*, 2000; Azzi and Stocker, 2000). In addition, Burton *et al.* (1998) documented that the bioavailability of synthetic vitamin E (all rac α-tocopherol) was half that of natural vitamin E (*RRR-* α-tocopherol).

Following release from the liver lipoprotein lipase in the circulation catabolises very low-density lipoprotein (VLDL) (containing vitamin E) to low-density lipoprotein (LDL) and intermediate density lipoprotein IDL (VLDL remnants), VLDL catabolism results in the transfer of α-tocopherol to LDL, HDL or IDL (VLDL remnants) (Herrera *et al.*, 2001) . The majority of VLDL remnants returns to the liver (Bramley *et al.*, 2000). Facilitation of α-tocopherol exchange between low-density lipoprotein (LDL) and high-density lipoprotein (HDL) fractions in the plasma is facilitated by phosphatidylglycerol transfer protein (PLTP), which transports vitamin E to target cells (Azzi and Stocker, 2000).

Most body tissues including adipose tissue, skeletal muscles and liver are able to accumulate vitamin E (Bramley *et al.*, 2000; Rucker *et al.*, 2001; Debier and Larondelle, 2005). As a fat-soluble vitamin, α- tocopherol is transferred to the body tissues by the same mechanism as fat delivery (Debier and Larondelle, 2005). However, body tissues vary in their rate of vitamin E uptake. The slowest vitamin E uptake can be found in adipose tissue, brain and spinal cord and the quickest in liver, lungs, kidney, spleen and red blood cells (Hidiroglou *et al.*, 1992; Debier and Larondelle, 2005). Parenchymal cells store 75% of hepatic α-tocopherol, while, non-parenchymal cells stores only 25% (Bjørneboe *et al.*, 1990). Azzi *et al.* (2002) reported that in addition to α-TTP, there is a family of tocopherol-associated proteins (TAPs) in all cell types in body tissues including liver, prostate and brain that prefer to bind with α-tocopherol compared with other tocopherols. These TAPs control α-tocopherol transfer into the cells in these tissues (Azzi *et al.*, 2002). In total body tissues, adipose tissue, muscle and liver store about 90% of the total quantity of alpha-tocopherol in the body (Bjørneboe *et al.*, 1990). In addition, vitamin E assimilates into adipose tissues more avidly but is depleted at a similar rate to muscle (Hidiroglou *et al.*, 2003). Bramley *et*

al. (2000) reported that the majority of tocopherols are stored in adipose tissue. In addition, Bjornebeo *et al.* (1990) documented that the metabolism of α-tocopherol is very slow from adipose tissue when vitamin E is deficient.

The liver is the primary location of vitamin E metabolism (Schmölz, 2016). Due to poor specify of α-TTP for an excess amount of α-tocopherol and the other vitamin E isomers (Herrera *et al.*, 2001), high levels of vitamin E supplementation, leads to high levels of vitamin E excretion via bile and faeces. Therefore, vitamin E is unlikely to cause problems if provided in excess (Bramley *et al.*, 2000; Debier and Larondelle, 2005).

Alpha-TTP protects α-tocopherol from degradation. Therefore, all forms of vitamin E are catabolised faster than α-tocopherol (Schubert *et al.*, 2018). Degradation of vitamin E (tocopherols and tocotrienols) produce shortened side-chain and vitamin-specific physiological metabolites with an entire chromanol ring (Schubert *et al.*, 2018). Regardless of their rates of catabolism, all forms of vitamin E follow a similar route of metabolism (Schubert *et al.*, 2018), confirming that all the end products of vitamin E catabolism vary, but they are excreted via faeces and urine (Chiku *et al.*, 1984; Swanson *et al.*,1999; Traber, 2013; Schubert *et al.*, 2018).

Alpha-tocopherol in the liver or tissues is oxidised to α-tocopheryl quinone (Bramley *et al.*, 2000). Further reduction then converts α-tocopheryl quinone to hydroquinone, which may be excreted in the bile after being conjugated with glucuronic acid, or degraded to α-tocopherol acid in the kidneys and excreted in the urine (Bjørneboe *et al.*, 1990; Herrera *et al.*, 2001; Debier and Larondelle, 2005). However, excretion of α-tocopherol via the urinary pathway is undetectable, with less than 1% of absorbed α-tocopherol being excreted by the kidneys (Hidiroglou *et al.*, 1992; Bramley *et al.*, 2000; Debier and Larondelle, 2005).

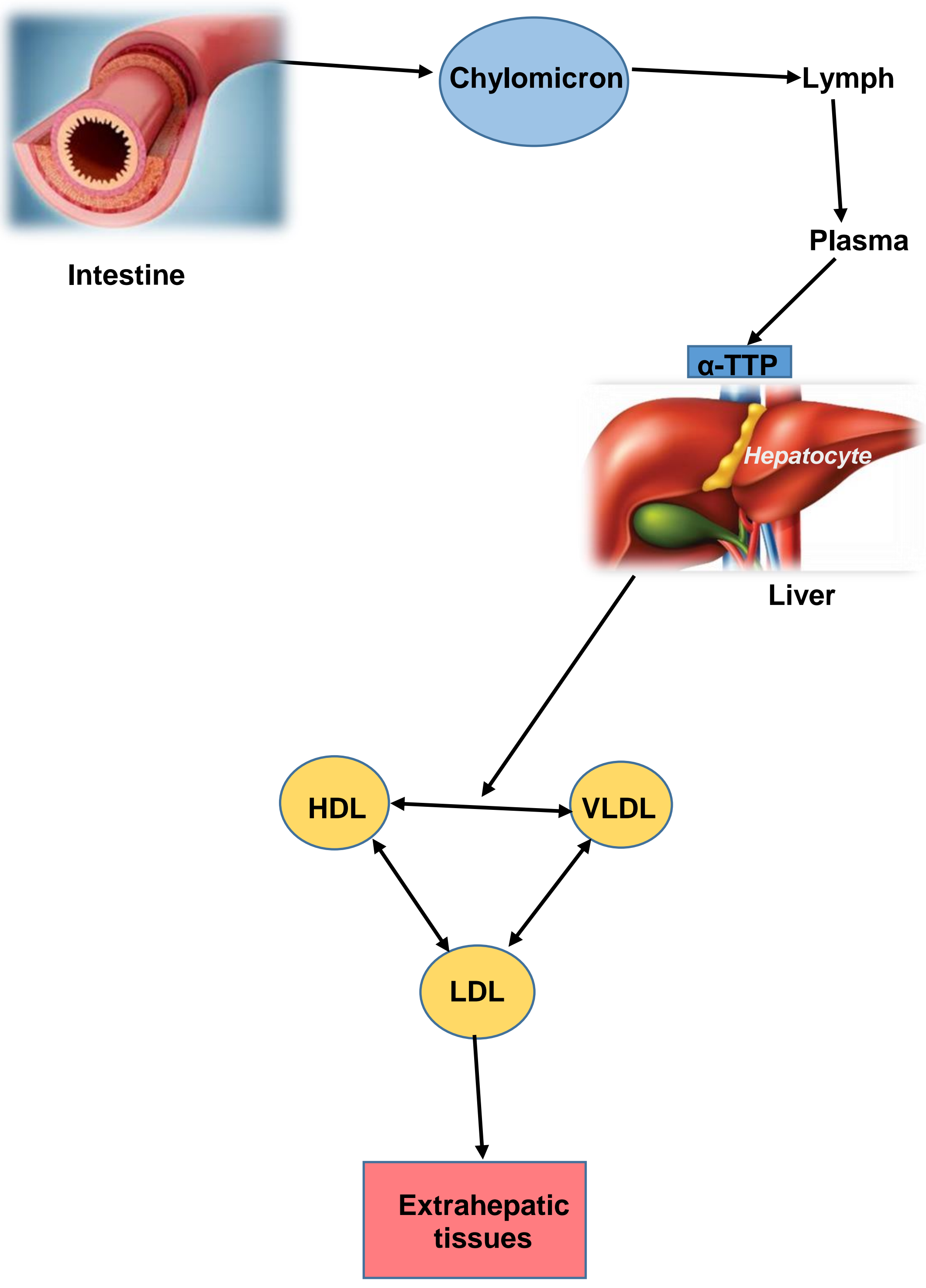


Figure 1. 2. Vitamin E metabolism. HDL: high density lipoprotein; VLDL: very low density lipoprotein; LDL: low density lipoprotein.

Source: Adapted from (Azzi and Stocker, 2000)

1.3. Vitamin E requirements of sheep

1.3.1. Introduction to vitamin E requirements

In ruminants, vitamin E requirements are defined as the quantity required to protect animals from the clinical or subclinical development of myopathies (Liu *et al.*, 2014). Unlike most other vitamins, the animal's body cannot store vitamin E in appreciable amounts (Rammell, 1983). There is an interrelationship between vitamin E and other dietary factors; therefore, it is extremely difficult to determine vitamin E requirements in isolation (Mcdowell, 2000; NRC, 2007). Increasing PUFA, vitamin A, oxidising agents, carotenoids and trace minerals (Cu, Fe or Zn) may increase vitamin E requirements ,while, it may be reduced by increasing selenium (Se), fat-soluble antioxidants and sulfur amino acids (Dove and Ewan, 1990; Hidiroglou *et al.*, 1992; Franklin *et al.*, 1998). Unsaturated oils that contain PUFA such as soybean oil, linseed oil and sunflower oil increase the requirements for vitamin E (Mcdowell, 2000). This is especially true when these oils undergo oxidative rancidity in feedstuff or during consumption by the animal (Mcdowell, 2000). Feedstuffs require 0.6 mg of α-tocopherol per 1g of PUFA to prevent peroxidation (Harris and Embree, 1963; Mcdowell, 2000; Raederstorff *et al.*, 2015). However, this ratio could be increased depending on the PUFA source, with fatty acids that have a longer carbon chain and a higher number of double bonds requiring more vitamin E (Mcdowell, 2000).

During maintenance or weight loss, there is a reduction in vitamin E requirements while the requirements may increase with increasing of growth rate (Liu *et al.*, 2014). The vitamin E requirements to maintain immunity functions is higher than that required for adequate growth and reproduction (Weiss, 1998). Factors such as exercise, stress, tissue trauma and infection have been shown to increase vitamin E requirements (Mcdowell, 2000). Other factors could also affect the vitamin E requirements, for example, level of productivity and physiological status. Consequently, the National Research Council (NRC 2007) recommend that animal diets should be supplemented with vitamin E when they are fed on conserved forage for long periods.

1.3.2. Nutritional standards and calculation of requirements

1.3.2.1. Historical requirements

The Agricultural Research Council ARC (1980) calculated vitamin E bio-potency as 1mg of dl- α-tocopherol acetate (synthetic vitamin E) is equal to 1 IU of vitamin E, while the bio-potency of 1 mg of (*RRR*) d-α-tocopherol (natural vitamin E) is equal to 1.5 IU. Both ARC

(1980) and the National Research Council (NRC, 1985) determined the minimum requirements of vitamin E to protect from nutritional muscular dystrophy (NDM) and white muscle disease (WMD). According to ARC (1980), the minimum, dietary vitamin E requirements of pregnant sheep or growing lambs were 10 to 15 mg/kg dry matter (DM), however, when there is a dietary deficiency of selenium (Se) the requirements are 15 to 30 mg/kg DM. The National Research Council NRC (1985) estimated vitamin E requirements for ruminants as between 10 to 60 mg/kg DM, and NRC (1985) recommended supplementing 20 IU/ kg DM of vitamin E for lambs less than 20 kg weight and 20 IU/ DM for the other sheep categories with diets containing sufficient selenium.

These requirements were determined from previously conducted research (Rousseau *et al*., 1957; Ewan *et al*., 1968; Jenkins *et al*., 1974). Vitamin E supplementation above 10-13 mg/kg DM with adequate selenium can protect sheep from clinical or subclinical myopathy (Schubert *et al*., 1961; Jenkins *et al*., 1974). Rousseau *et al*. (1957) documented that there were no vitamin E deficiency signs in lambs supplemented with 51.3 mg/kg DM of *d*-α-tocopherol acetate. Ewan *et al*. (1968) reported that dietary supplementation of *dl*-α-tocopherol acetate *at* 11 mg/kg live weight, protected lambs from death due to nutritional muscular dystrophy, but growth rate was slightly reduced, whereas lambs that were not supplemented with *dl*-α-tocopherol acetate had clinical signs of nutritional muscular dystrophy (NMD) and did not survive. Sharman (1973) found that diets containing 20 IU/kg dl-tocopherol protected rapid growing lambs (< 300 g/day) from nutritional muscular dystrophy.

1.3.2.2. Current requirements and allowances

The National Research Council (NRC, 2007) recommended that vitamin E requirements should be increased to keep plasma α-tocopherol levels above a critical value (≥2 µg/ml), which is necessary to protect animals from white muscle disease (WMD) (NRC, 2007). Hidiroglou *et al*. (1992b) reported that serum α-tocopherol increased to over 2 µg/ml within one week of sheep being supplemented with 300 mg/day *dl*- α-tocopherol acetate. Njeru *et al*. (1994a) documented that lamb's serum α-tocopherol reduced to ≤ 1 µg/ml over four weeks when they were fed a vitamin E deficient diet.

To keep plasma α-tocopherol concentration ≥ 2.0 µg/ml, it is necessary to supply more than 5.3 IU/kg live weight (NRC, 2007). Therefore, a 50kg sheep requires at least 265 mg vitamin E per day. This level agrees with Hidiroglou and Charmley (1990) who recorded plasma α-tocopherol value of 2.1 µg/ml when lambs weighing 45 to 55 kg were supplemented with 200 mg/day *dl*-α-tocopherol acetate. However, Njeru *et al*. (1994b) found that lambs

weighing 42 to 60 kg, supplemented with 60 IU/d *dl*-α-tocopherol acetate had a serum α-tocopherol concentration of 3.18 µg/ml. This quantity should be considered a minimum, based on recommendations determined from limited data (Hidiroglou *et al.*, 1992a). Hidiroglou *et al.* (1992b) supplemented 35 kg lambs with 300 IU/lamb/day of vitamin E, serum tocopherol increased to a peak after 15 to 21 days, then showed a plateau with a fluctuation in serum vitamin E within the minimum values proposed by Hidiroglou *et al.* (1992b). Consequently, NRC (2007), recommended that the minimum level of vitamin E required to maintain plasma vitamin E above the critical value (2.0 µg/ml) was 5.3 mg/kg LW per day.

In light of this recommendation, for *RRR*-α-tocopherol the level should be 3.74 mg/kg LW α-tocopherol per day because the bioavailability of each 1 mg of *RRR*-α-tocopherol is equal to 1.49 IU α-tocopherol (Yang, 2003). The results of studies on the effect of oral vitamin E supplementation of ewes in late pregnancy indicate that plasma α-tocopherol concentration can be increased by vitamin E supplementation. For example, Merrell, (1998) allocated 270 twin-bearing ewes eight weeks before lambing into two treatments on two upland and hill farms (Pwllpeiran or Redesdale). Ewes were fed on silage *ad libitum* at Pwllpeiran farm and restricted hay at Redesdale farm, concentrates were fed to supplement ewes with either 50 or 200 IU/ewe/day of vitamin E. At lambing, the highest plasma α-tocopherol concentration was recorded in the high supplemented treatment at Redesdale (3.19 µg/ml) and lowest in the low supplemented treatment at Pwllpeiran (1.08 µg/ml). Merrell, (1998) suggested that differences in plasma tocopherol concentration between farms may have been related to differences in vitamin E absorption owing to the rate of passage, with vitamin E absorption being lower at Pwllpeiran where ewes were fed on a silage based diet. Similarly, Daniels *et al.* (2000) supplemented twin pregnant ewes 32 days before lambing with α-tocopherol acetate (400 IU/head/day) or left them unsupplemented. Serum α-tocopherol within 4 hours post lambing was lower in non-supplemented ewes (1.24 vs 1.87 µg/ml) compared with the supplemented group. Likewise, Capper *et al.* (2005) fed twin-bearing ewes with either 50 or 500 mg/kg DM vitamin E 42 days *pre-partum*, plasma vitamin E was higher at both 14 days *pre-partum* and *post-partum*. Rooke *et al.* (2009) supplemented pregnant ewes with 50, 100, 150 and 250 IU/ewe/day vitamin E four weeks before lambing and reported that the lowest plasma α-tocopherol was 1.1 µg/ml in the 50 IU treatment, and the highest level was 2.3 in the 250 IU treatment. Likewise, Dønnem *et al.* (2015) supplemented either a placebo 0 or 360 IU/ewe/day with forages that contained 40 mg/kg DM of vitamin E in late pregnancy 6 to 7 weeks before lambing, and reported that ewes plasma α-tocopherol was higher in supplemented treatment (4.18 vs 3.21 mg/l) after 1-2 week of supplementation.

1.3.2.3. Inclusion rates in UK sheep diets

Both EBLEX (2014) and AHDB (2018) recommend that vitamin E requirements of sheep remain as ARC (1980) recommendation. However, they suggest that vitamin E is supplemented at higher levels than ARC (1980) recommended (100 mg/kg DM) when there is an increased demand, for example, during late pregnancy, early lactation, or when concentrates and conserved forages are fed (AHDB, 2016). The UK feed industry is currently following this recommendation. A brief internet survey of four feed company websites suggests that the vitamin E content of ewe concentrates is typically 100-150 IU/kg to obtain more vigorous lambs, higher live weight gain and protect lambs from the white muscle disease. These feeding levels are lower than NRC (2007) and higher than both ARC (1980) and NRC (1985) recommendations.

1.3.3. Deficiency and toxicity

1.3.3.1. Assessment of vitamin E status

The vitamin E status of an animal depends on the tocopherols concentration in the stores and its physiological activities (Rucker *et al.*, 2001). Plasma tocopherols concentration is the most common indicator of vitamin E status (Rucker *et al.*, 2001; Zempleni *et al.*, 2007; Liu *et al.*, 2014).

There is a high correlation between plasma vitamin E concentration and the vitamin concentration in many body tissues (Fry *et al.*, 1993; Hidiroglou *et al.*, 1994). When lambs were supplemented with an α-tocopherol deficient diet, concentrations in plasma fell at the same rate as those in skeletal muscles, adipose tissue, heart muscle and adrenal tissue; however, liver and erythrocyte α-tocopherol concentration reduced quicker than those in muscle (Fry *et al.*, 1993).

The clinical lesions linked to white muscle disease are associated with the plasma level of α-tocopherol of < 1.0 to 1.5 μg/ml. Therefore, plasma α-tocopherol levels less than 2.0 μg/ml are considered deficient (NRC, 2007). Rice *et al.* (1981) documented that blood linolenic acid concentrations rapidly rise to high levels, if protected from hydrogenation in the rumen, which is associated with an increase in plasma creatine phosphokinase which is an indicator of muscular myopathy.

1.3.3.2. Vitamin E and selenium deficiency

Vitamin E and selenium deficiency means that there is an association between a reduction of these nutrients and particular deficiency diseases in different animal species (McDowell *et al*., 1996; Van Metre and Callan, 2001). In ruminants, the clinical sign of vitamin E and selenium deficiency are nutritional muscular dystrophy or white muscle disease (McDowell *et al*., 1996; Mcdowell, 2000; CSIRO, 2007). Development of white muscle disease may take place in *utero* or *ex utero*, in newly born ruminants (McDowell *et al*., 1996). White muscle disease (WMD) is tissue degeneration of the striated muscle of the animal, which can occur in both skeletal and smooth muscle (McDowell *et al*., 1996; Mcdowell, 2000; Rucker *et al*., 2001). There are two clinical patterns of white muscle disease; the first one happens as a congenital type of muscular dystrophy in which neonatal animals are stillborn or die within three to four day of lambing after sudden physical efforts, such as running or nursing. The second pattern is delayed WMD that occurs from three to six weeks, and up to four months after birth (McDowell *et al*., 1996; Mcdowell, 2000; CSIRO, 2007). There is considerable variation in the clinical and sub-clinical symptoms of vitamin E and selenium deficiency, which depend on the animal species and disease severity (Table 1.4). White muscle disease often leads to sudden death in young animals as a result of cardiac failure, caused by lesions within the cardiac muscles, with no previous symptoms (Kennedy and Rice, 1992; Mcdowell, 2000; CSIRO, 2007). In moderately acute cases, symptoms start first with weakness and walking with a stiff gait, followed by an inability to stand with trembling muscles, difficulty in respiration and finally death due to muscle and cardiac myopathy (Steele *et al*., 1980). Cattle are less susceptible to selenium deficiency than sheep (Muth, 1963; NRC, 2007). Under extreme conditions, the mortality of lambs may be up to 65% (NRC, 2007).

The main cause of WMD is an imbalance between pro-oxidants (PUFA) and antioxidants (Se and vitamin E) in the animal’s diet (Lykkesfeldt and Svendsen, 2007). Supplementation of high levels of unsaturated fatty acids leads to increases in the production of hydroperoxides in the animal's body and the development of nutritional myopathy (Pavlata *et al*., 2001). White muscle disease is easily induced in calves fed polyunsaturated oils (Mcdowell, 2000). Hydrogenation or saturation of PUFA occurs before absorption in mature ruminants, and this may reduce oxidative stress and the requirement for vitamin E (Herdt and Stowe, 1991). However, Herdt and Stowe (1991) reported that ruminants consuming fresh grass which is high in PUFA increase the incidence of WMD in cases of selenium and vitamin E deficiency. At turn out to pasture, growing animals are more susceptible to WMD, especially after feeding selenium (<0.2 mg/kg) or vitamin E deficient diets during long

periods of indoor housing (Pehrson *et al.*, 1986; Hakkarainen *et al.*, 1987). Vitamin E and selenium deficiency also occur in young ruminant animals that have a limited ability to hydrogenate PUFA as a result of incomplete rumen development (Herdt and Stowe, 1991).

Thyroid metabolism malfunction may also occur as a result of selenium deficiency; this leads to a reduce growth rate, and a reduction in fertility and disease resistance (Mehdi and Dufrasne, 2016). Thyroid metabolism malfunction occurs particularly with selenium deficiency as selenium is required for the synthesis of the thyroid hormones (triiodothyronine (T_3) tetraiodothyronine (T_4)). A diet deficient in selenium causes a reduction in T_3 and an increase in T_4 hormones which reduces blood T_3/T_4 ratio (Beckett *et al.*, 1989; Thompson *et al.*, 1995). Triiodothyronine (T_3) is the active form of iodothyronine (T_4) which is associated with growth and can impact on growth rate (Mehdi and Dufrasne, 2016).

The amount of selenium in forages varies according to soil and region (Mehdi and Dufrasne, 2016). Thus, animal diets that are grown in selenium deficient areas may be another reason for WMD (Walsh *et al.*, 1993). According to NRC (2007) soils in regions that contain selenium at <0.5 mg/kg soil are considered deficient in selenium.

1.3.3.3. Vitamin E toxicity

Both chronic and acute studies have shown that supplementation of several animal species with high levels of vitamin E is non-toxic (Mcdowell, 2000; Rucker *et al.*, 2001; Zempleni *et al.*, 2007; Traber, 2013). Vitamin E toxicity is prevented by increasing hepatic vitamin E metabolism (Schubert *et al.*, 2018), as described previously in section 1.2.3.3 on vitamin E metabolism and excretion. In this case, high levels are excreted by the liver such that plasma vitamin E does not rise more than 2-4 fold (Fuller *et al.*, 1996; Bruno *et al.*, 2005; Imai *et al.*, 2011). However, diets containing an extremely high concentration of vitamin E may cause health problems in different animal species (Table 1.5).

1.3.3.4. Selenium toxicity

Selenium is a hazardous and toxic mineral. The maximum tolerable level of selenium in animal diets was increased from 2.0 (NRC, 1980) to 5.0 mg/kg DM (NRC, 2005). Therefore, the dietary recommendations should at least 5-10 times lower than the toxic levels (CSIRO, 2007).

Selenium poisoning or selenosis occurs as an acute or chronic toxicity (ARC, 1980). A sudden high level of Se intake is the cause of acute poising; this happens as a result of

ingestion of plants that accumulate high concentrations of selenium or poor mixing of mineral supplements that contain selenium (ARC, 1980; NRC, 1980; NRC, 1985). Signs of acute poisoning include, increased pulse rate, temperature rise, oedema, watery diarrhoea, haemorrhagia, collapse and death from respiratory failure (ARC, 1980; CSIRO, 2007).

Chronic selenium poisoning usually happens during consumption of plants growing in soil rich in selenium (>3 mg Se/kg diet), containing high quantities of organoselenium compounds for a long period (ARC, 1980; NRC, 1980; NRC, 1985). In addition, chronic selenium toxicity can be found with diets containing 5-40 mg/kg DM for long periods (ARC, 1980; NRC, 2005; CSIRO, 2007). Symptoms of chronic toxicity include, appetite loss, reduction in wool growth, ill thrift, loss or reduced vision, severe lameness, infertility, vomiting, diarrhoea and nausea, hoof fragility, liver damage, reduced triiodothyronine concentration, and loss of natural killer cell (NRC, 1980; NRC, 1985; Kieliszek and Błazejak, 2016).

Table 1. 4. Some clinical and subclinical symptoms of vitamin E and selenium deficiency in ruminants

Animal species	Symptoms of vitamin E deficiency	Reference
Sheep	Weakness, staggering walking, mouth and nostrils frothing, disability to stand.	(Steele *et al*., 1980)
Cattle, sheep	Weakness, newborn difficulty to stand, stiff gait, distress of respiration, severe diarrhoea	(ARC, 1980)
Sheep	Creatine kinase concentration increases in serum, α-tocopherol and glutathione peroxidase reduction.	(Rice and McMurray, 1982)
Sheep	Ewe infertility (subclinical), young animal growth problems.	(Sáez *et al*., 1996)
Sheep	Reduction in glutathione peroxidase activity, selenoprotein reduction, immunity decreased.	(Rock *et al*., 2001)
Lamb, calve, cow and sheep	Erythrocyte haemolysis, skeletal muscle degeneration, heart muscles microangiopathy, testes degeneration, cow retained placenta, impaired fertility (spermatozoa)	(Surai, 2002)
Sheep	Litter size reduced (subclinical), embryo survival reduction during implantation.	(Rooke *et al*., 2004)
Sheep	Decrease in the production of wool, growth rate reduction, ewe's infertility and embryo losses (subclinical).	(CSIRO, 2007)
Cattle	Immunity reduction, incidence of retained placenta.	(Spears and Weiss, 2008)

Table 1. 5. Symptoms of extremely high supplementation of vitamin E in some animal species.

Animal species	Vitamin E dose	Symptoms of vitamin E toxicity	Reference
Trout	α-tocopherol (5000 mg/kg diet)	Packed-cell volume reduction.	(NRC, 1993)
Chicken	vitamin E (16000 to 64000 IU/kg diet)	Pigmentation and waxy, mineralisation abnormality, abnormal clotting reduction.	(Rucker *et al*., 2001)
Pelicans	vitamin E (5000 IU/kg diet)	Vitamin K deficiency.	(Rucker *et al*., 2001)
Ruminants	Vitamin E (75 IU/ kg BW/ day)	No symptoms	(NRC, 2007)
Sheep	Vitamin E (2400 IU/ sheep/ day)	No symptoms	(Yue *et al*., 2010)
Male lambs	Vitamin E (2000 IU/ day)	No symptoms	(Qu *et al*., 2019)

1.3.4. Supra-nutritional vitamin E supplementation of sheep

Several studies have identified that the addition of vitamin E to ewe's diets in late pregnancy (within 8 weeks of parturition) above the requirements of both ARC (1980) and NRC (1985) which is between 15 to 30 (mg/kg DM) may have a benefit to both the ewe and lamb (Capper *et al.*, 2005; Capper *et al.*, 2007; Dønnem *et al.*, 2015). For example, reduction in stillborn rates (Dønnem *et al.*, 2015), an enhancement of vigour in lambs (Merrell, 1998), increase in lambs birth weight (Capper *et al.*, 2005), improved milk protein quality (Capper *et al.*, 2007), and an increase in neonatal immunity (Novoa *et al.*, 2014).

1.3.4.1. Effects on ewe performance

There is conflicting evidence in the literature regarding the effects of supra-nutritional levels of vitamin E on the performance of pregnant ewes. In a study on the impact of vitamin E supplementation during late pregnancy on ewe performance, Merrell, (1998) allocated 270 twin-bearing ewes eight weeks before lambing into two treatments on two upland and hill farms (Pwllpeiran or Redesdale). Ewes were fed on silage *ad libitum* at Pwllpeiran farm and restricted hay at Redesdale farm, concentrates were fed to supplement ewes with either 50 or 200 IU/ewe/day of vitamin E. Merrell *et al.* (1998) found that there was no effect of vitamin E supplementation on ewe weight and body condition score, and no impact on lamb birth weight, or lamb mortality. However, lambs born to ewes supplemented with high vitamin E (200 IU/ ewe/day) tended to be more vigorous directly after lambing. Favourable weather conditions could be the reason of these results as the animals were not subjected to severe environmental stress (Merrell *et al.*, 1998). In addition, the reason that the vitamin E supplemented lambs tended to be more vigorous could be due to the lambs being housed for a period directly after lambing (Merrell *et al.*, 1998).

In a 3 years experiment (430 ewes/year), Kott *et al.* (1998) allocated ewes randomly to 2 groups, 3 weeks prior to lambing. Ewes were fed on 2.3 kg of lucerne-grass hay and 0.23 kg barley pellets/day with or without vitamin E to supply either an additional 330 IU/ewe/day or 0 IU/ewe/day. Results indicated that there was no effect of vitamin E supplementation on ewe weight and body condition score. However, a reduction in lamb mortality (17% to 12%) was recorded in the high vitamin E supplementation treatment (Kott *et al.*, 1998). Kott *et al.* (1998) suggested that the lamb mortality rates may be increased as a result of the environmental stress (hypothermia), therefore, vitamin E supplementation might reduce lamb mortality by reduction of environmental stress.

Daniels *et al.* (2000) examined the effect of vitamin E supplementation of ewes during late pregnancy with a pathological infection challenge (infected or not infected with

parainfluenza type 3, PI_3) and the interaction effect between these two factors on ewe and lamb performance. Fifty-two pregnant ewes were supplemented with a gelatin capsule (0 or 400 IU /ewe/day) of vitamin E 32 days before their expected date of lambing with or without PI_3 vaccination. Daniels *et al.* (2000) documented that there was no effect of vitamin E supplementation on ewes live weight, body condition score, colostrum immunoglobulin (IgG) concentration, or ewe serum IgG status. Pathogenic or environmental stress were not sufficient to induce response to vitamin E supplementation (Daniels *et al.*, 2000).

Capper *et al.* (2005) fed 36 twin and 12 triplet bearing ewes with Megalac (saturated fat) or fish oil (long-chain PUFA) with either 50 or 500 mg/kg DM of vitamin E. High levels of vitamin E supplementation did not affect colostrum or milk yield, while both colostrum and milk vitamin E concentration increased with high vitamin E supplementation, although a reduction in colostrum vitamin E yield (1061 vs 2846 µg/h) was recorded with PUFA supplementation. The birth weight of lambs born to ewes fed on high levels of vitamin E was 0.31 kg higher than that of lambs born to ewes fed on the lower level of vitamin E.

In a trial on the effect of dietary long-chain PUFA and vitamin E supplementation during late pregnancy on lamb behaviour and colostrum production, Capper *et al.* (2006) supplemented 36 twin bearing and 12 triplet bearing ewes 103 days before their expected lambing date with Megalac or fish oil with either 50 or 500 mg/kg DM vitamin E. In this experiment, there was no effect of high levels of vitamin E supplementation on milk yield or composition, ewe live weight or body condition score, however, high vitamin E supplementation increased BCS loss *post-partum*. High vitamin E supplementation with fish oil also reduced the time from latency to stand of new-born lambs. High supplementation of vitamin E increased lamb birth weight (Capper *et al.* 2006).

In a clinical experiment, Kasimanickam *et al.* (2010) supplemented 18 pregnant ewes during late pregnancy (day 107 to 137) with 500 mg/day α-tocopherol (n=6), 1000 mg/day of gamma tocopherol (n=7) or a placebo (n=3). Blood samples were taken weekly, and ewes were euthanised at the end of the experiment for tissue collection. In this experiment, α-tocopherol or gamma tocopherol supplementation increased ($P<0.05$) vitamin E levels in ewe blood serum, placenta and uterus compared to the placebo (control). Alpha-tocopherol supplementation increased the expression of mRNA for placental growth factor (PIGF), endothelial nitric oxide synthase (eNOS) and hypoxia inducible factors (HIF-1α) in cotyledons. In supplemented ewes, an increase in angiogenesis was found by morphometry analysis. This suggests that vitamin E supplementation during late pregnancy increased the placental vascular network and angiogenesis which lead to an increase in the growth rate of the foetus by providing more nutrients (Kasimanickam *et al.*, 2010).

In two experiments Dafoe *et al.* (2008) investigated the effects of energy source and vitamin E supplementation on ewe and lamb performance. In the first experiment, 51 twin bearing ewes were individually housed and fed two energy sources (safflower seed or barley) with or without vitamin E (350 IU/ewe/day). In the second experiment 1182 twin and single bearing ewes were group housed and fed similar diets to the first experiment. In both experiments, there was no effect of vitamin E on ewe LW of BCS, although in experiment 1 ewes supplemented with vitamin E tended to have a higher BCS at weaning. There was no effect of vitamin E on lamb birth weight (Dafoe *et al.*, 2008). Dafoe *et al.* (2008) suggested that ewe supplementation with safflower seed (PUFA source) without additional supplementation of vitamin E could reduce the adaptation ability of neonatal lambs to cold environment conditions.

Rooke *et al.* (2009) conducted an experiment on the response of lambs to vitamin E supplementation above requirements during late pregnancy. In this experiment, 80 crossbred twin pregnant ewes were allocated to 4 diet treatments, 20 ewes in each diet treatment (2 genotype sires). Vitamin E was supplemented at 50, 100, 150 or 250 IU/ ewe/ day for seven weeks prior to their predicted lambing date. The results indicated that there was no effect of vitamin E supplementation on lamb birth weight, lamb vigour or lamb mortality (Rooke *et al.*, 2009). Rooke *et al.* (2009) suggested that the extent of the oxidative challenge and availability of vitamin E from the body tissue stores during late pregnancy could be important factors affecting the response of ewes to vitamin E supplementation.

In a study on the effect of vitamin E supplementation on pregnant ewes Dønnem *et al.* (2015), supplemented 2268 pregnant Norwegian white sheep in 19 flocks, 6-7 weeks prior the expected lambing date with either 360 IU/ ewe/day of vitamin E or placebo 0 IU/ ewe/day with forages containing vitamin E 40 mg/kg DM. In this study, a reduction in the percentage of stillborn lambs was recorded (P=0.0004) in ewes carrying triplets. However, there was no significant effect on the percentage of stillborn lambs in ewes carrying twins. Plasma α-tocopherol was significantly lower in ewes that produced stillborn lambs (Dønnem *et al.*, 2015). They suggested that the high oxidative challenge in ewes carrying large litters (triplets) than small litters (twins or single) might explain the different responses observed.

1.3.4.2. Effects on lamb performance

The effect of supra-nutritional supplementation vitamin E during late pregnancy and lactation on lamb performance has been measured in several experiments. Merrell *et al.* (1998) reported that lamb growth rate was higher in the vitamin E supplemented treatment, with lambs being significantly heavier at 30 day old. This could be due to higher ingestion of vitamin E that was available to suckled lambs from ewes colostrum contained a higher concentration of vitamin E. Kott *et al.* (1998) documented that lamb weaning weight was

higher in vitamin E supplemented lambs. Hatfield *et al.* (2000) suggested that vitamin E supplementation at 10 fold higher than NRC (1985) recommendations during late pregnancy could increase neonatal vigour, lamb immunity, lamb weight gain and ultimately lamb weaning weight. Daniels *et al.* (2000) found that there was no effect of supplementing ewe diets with vitamin E (0 or 400 IU /ewe/day) in a gelatin capsule 32 days before lambing on lamb serum IgG, birth weight, survival or weaning weight. Daniels *et al.* (2000) suggested that the level of pathogenic or environmental stress were not sufficient to elicit a response to vitamin E supplementation. Capper *et al.* (2007) reported that high vitamin E supplementation to ewes during late pregnancy and lactation did not affect lamb growth rate. In two experiments, Dafoe *et al.* (2008) recorded that vitamin E supplementation of pregnant and lactating ewes did not affect lamb survival. Rooke *et al.* (2009) found that there was no effect of vitamin E supplementation in late pregnancy and lactation on lamb weaning weight. Rooke *et al.* (2009) suggested that the oxidative challenge was low, and that this could explain the limited response to vitamin E supplementation.

Vitamin E supplementation of the diet of growing lambs after weaning may affect lamb performance and meat quality. In a study conducted to evaluate the effects of different levels and source of vitamin E on the vitamin E status and performance of lambs, Kasapidou *et al.* (2009) fed 56 Suffolk × Charollais wether lambs on seven treatment groups (five concentrates (C) and two grass silage (S)). Vitamin E supplementation in the concentrate treatments was 30 (C30), 60 (C60), 120 (C120), 250 (C250) and 500 (C500) mg/kg DM, in addition, the two silage based treatments were supplemented with either 60 (S60) or 500 (S500) mg/kg DM of vitamin E (Kasapidou *et al.*, 2009). Kasapidou *et al.* (2009) documented that there was no effect of vitamin E supplementation on lamb live weight, live weight gain, feed conversion, hot carcass weight, cold carcass weight and fat score. Kasapidou *et al.* (2012) used the same lambs and treatments of Kasapidou *et al.* (2009) to determine the effects of source and level of vitamin E supplementation on the colour and lipid oxidation stability of meat. The results indicated that both muscle colour saturation and redness were lower (P<0.001) in C30 treatment than the other groups, whilst muscles TBARS (lipid oxidation) after 3 and six days were higher (P<0.001) in C30 and C60 than the other treatments.

Berthelot *et al.* (2014) conducted an experiment to determine the effect of dietary vitamin E supplementation on lamb performance and muscle fatty acid composition from *extensior carpi radialis* of the forelimb. In this trial, 30 Romane male lambs were allocated to three dietary treatments containing DL-α-tocopherol acetate at 45, 286 and 551 mg/kg DM. Berthelot *et al.* (2014) found that there was no effect of vitamin E supplementation on growth rate, live weight at slaughter, cold carcass weight, killing out percentage, fatness score. Berthelot *et al.* (2014) suggested that the lower live weight of the lambs at the beginning of

the experiment could be the reason that there was no effect of vitamin E supplementation on lamb performance or slaughter parameters. To determine the relationship between different levels of vitamin E supplementation and stability of meat colour, Jose *et al.* (2016) used 70 mixed sex crossbred lambs aged between 6-8 months. Before supplementation, 10 lambs were slaughtered and the remaining 60 lambs were allocated to 5 treatments for 56 days. One of the treatments was allocated to pasture and the others were fed on a pelleted diet containing either 30, 150, 275 or 400 IU/kg vitamin E. Jose *et al.* (2016) indicated that there was no effect of vitamin E supplementation on growth rate, although the heaviest lambs at slaughter were in vitamin E treatments 30 and 275 IU. High vitamin E supplementation (275 IU/kg DM) increased meat colour stability by increasing the oxy/metmyoglobin ratio. Jose *et al.* (2016) suggested that increasing the level of dietary vitamin E supplementation can increase the meat colour stability during shelf life storage.

1.3.4.3. Ewe to lamb transfer

A vitamin E does not cross through the placenta in any appreciable amounts (Njeru *et al.* 1994a; McDowell *et al.*, 1996; Rooke *et al.*, 2008). Therefore, supplementation of pregnant ewes with vitamin E *pre-partum* is an inefficient method to supplement neonates with vitamin E before suckling (Kumagai and White, 1995; McDowell *et al.*, 1996; Rooke *et al.*, 2004). In an *in vitro* experiment to investigate the efficiency of transport of three antioxidants through the placenta, including vitamin E (various forms), Schenker *et al.* (1998), used isolated placental cotyledons from a normal human placenta. Vitamin E transport was slow through the cotyledons (10%), but the natural form of vitamin E had the best transportation. In a study to investigate the effect of minerals, vitamin E and retinol on productivity and vitamin status of pregnant Merino ewes, Kumagai and White (1995) supplemented 420 pregnant ewes 5 to 1 weeks before lambing in 4 treatment groups. Kumagai and White (1995) reported that vitamin E supplementation was 166-186 mg/ewe per day, lamb plasma vitamin E increased in the supplemented ewes treatment (0.38 vs 0.25 µg/ml), however, lamb plasma vitamin E was still <15% lower than their mothers plasma vitamin E. Capper *et al.* (2005) supplemented 36 twin and 12 triplet bearing ewes with Megalac (saturated fat) or fish oil (long-chain PUFA) with either 50 or 500 mg/kg DM of vitamin E, to evaluate the effect of pregnant and lactating ewe supplementation with vitamin E and fatty acids upon placental and mammary transfer to the foetal and suckling lambs. Capper *et al.* (2005) reported that the neonate's plasma vitamin E was undetectable in 9 of 12 lambs (< 0.043 µg/ml). However, supra-nutritional supplementation of vitamin E increased vitamin E in the neonatal brain ($P<0.05$) and *semimembranosus* muscles ($P<0.01$) tissue. These results indicate that vitamin E might be transferred through the placenta and rapidly assimilated into neonatal tissues. Njeru *et al.* (1994a) supplemented 26 pregnant ewe lambs 28 days *pre-partum* and 28 days *post-partum* with DL-alpha tocopherol acetate (0, 15, 30 and 60

IU/ewe/day) to investigate alpha-tocopherol transfer through the placenta and mammary gland. However, although there was a difference in ewe's serum alpha-tocopherol (0.93, 1.94, 2.63 and 4.07 µg/ml) at day 28 of supplementation, vitamin E supplementation to pregnant ewes had no effect on the neonate's serum alpha-tocopherol before suckling in the different treatment groups, which suggests inefficient transfer of alpha-tocopherol through the placenta. Sterndale *et al.* (2018) supplemented 55 Merino ewes orally with 4g of all-rac-α-tocopherol acetate at days 11, 125 and 140 of pregnancy and selenium (subcutaneous injection of 60 mg at day 111). In addition, 58 Merino ewes were not supplemented to evaluate the effect of vitamin E and selenium supplementation of pregnant ewes on ewe and lamb plasma α-tocopherol concentration and lamb immunity. Directly after lambing and before suckling, plasma α-tocopherol concentrations were lower in the non-supplemented treatment in both lambs (0.08 vs 0.14; $P<0.001$) and ewes (0.67 vs 1.13; $P<0.001$). Sterndale *et al.* (2018) reported that the lamb plasma α-tocopherol concentration was still <15% lower than their mother's plasma vitamin E. However, the lamb plasma α-tocopherol concentration was higher ($P<0.001$) in the supplemented treatment which is in line with Kumagai and White (1995) results.

Most of the evidence suggests that the level of vitamin E in neonatal plasma (or serum) is not increased by maternal supplementation during late pregnancy. The work of Capper *et al.* (2005) confirms this, but also indicated higher levels in lamb tissues, suggesting that vitamin is rapidly assimilated from plasma.

Vitamin E is easily transported into colostrum and milk via the mammary gland (Liu *et al.*, 2014). The best way to provide the neonate with α-tocopherol is supplementation of ewes with high levels of vitamin E during lactation (Pehrson *et al.*, 1990; Njeru *et al.*, 1994; Kumagai and White, 1995; McDowell *et al.*, 1996; Capper *et al.*, 2005). Njeru *et al.* (1994) reported that the quantity of α-tocopherol in colostrum was increased ($P<0.05$) by oral vitamin E supplementation (0, 15, 30 and 60 IU/ewe/day) using gelatine capsules. Colostrum α-tocopherol concentration was 3.30 and 9.55 µg/ml in 0 and 60 IU/ewe/day treatments respectively. At day 3 of suckling, lambs, serum α-tocopherol increased with a difference between supplemented treatments to 1.41, 1.84, 2.43 and 4.46 µg/ml. Merrell (1998) found that the addition of vitamin E to ewe diets in late pregnancy (50 or 200 IU/ewe/day) with *ad-libitum* silage increased the α-tocopherol concentration of colostrum. Merrell (1998) reported that the colostrum vitamin E was 11.60 vs 16.76 µg/ml in control and supplemented treatments respectively, while lamb plasma vitamin E was 2.77 vs 3.93 µg/ml in control and supplemented treatments respectively between 24 to 36 hours after lambing. Likewise, Capper *et al.* (2005) supplemented 48 pregnant ewes with vitamin E (50 or 500 mg/kg DM) with either Megalac or fish oil, 6 weeks prior to parturition and during the first 4 weeks of lactating period *Pre-partum* supra-nutritional supplementation of vitamin E

to ewes increased the colostrum α-tocopherol yield, especially when high levels of vitamin E were supplemented with Megalac, which was approximately three times higher than those supplemented with fish oil (2846 vs 1061 μg/h). A similar difference was recorded in the lamb's plasma α-tocopherol at 14 days of age (~ 0.86 vs 3.45 μg/ml). Similarly, Rooke *et al.* (2009) documented that *pre-partum* dietary supplementation of vitamin E significantly increased the α-tocopherol concentration of ewes colostrum. Rooke *et al.* (2009) reported that the colostrum α-tocopherol concentration was 4.9, 4.0, 4.6 and 11.4 μg/ml in Suffolk sired and 4.4, 13.4, 13.0 and 17.9 μg/ml in Texel sired lambs in supplemented with 50, 100, 150 or 250 IU of vitamin E/ewe/day respectively. These results indicate that suckling colostrum suckling is the most effective way to provide neonates with an adequate quantity of vitamin E.

1.3.4.4. Effects on immunity

Vitamin E may have an essential role in enhancing immunity and decreasing neonatal mortality in ruminants by increasing the production of lymphocytes when exposed to antigens (Hatfield *et al.*, 2000). Vitamin E is also involved in stimulating serum antibody synthesis, especially immunoglobulin (IgG) antibodies. In addition, during phagocytosis vitamin E protects macrophages and leukocytes (McDowell *et al.*, 1996). Neonates are born with a poorly developed immune system (Perino and Rupp, 1994). Ewe colostrum is required during the first 24 hours of life to confer passive immunity from the ewe to the lamb and protect it during the first few weeks of life (Liu *et al.,* 2014).

The results of studies that have been conducted on the effect of vitamin E on neonatal immunity are inconsistent. For example, in a study to evaluate the effect of vitamin E supplementation to pregnant ewes on both ewe and lamb immunity, Novoa-Garrido *et al.* (2014), supplemented 40 Norwegian white breed ewes with 4 treatments. The treatments were seaweed, natural vitamin E, synthetic vitamin E and control (no extra vitamin E or seaweed). Natural and synthetic vitamin E was added to supply 140 IU vitamin E/ ewe daily. Novoa-Garrido *et al.* (2014) found that vitamin E supplementation of pregnant ewes increased significantly the IgG concentration in ewe plasma at parturition as well as the lamb's plasma IgG one week after lambing. However, it did not affect colostrum IgG concentration (mg/ml). In contrast to the above, the results of a number of studies indicate no effect of vitamin E on colostrum or ewe and lamb plasma IgG. For example, Daniels *et al.* (2000) conducted research on the effect of vitamin E supplementation of ewes during late pregnancy with a pathological infection challenge (infected or not infected with parainfluenza type 3, PI_3). Fifty-two pregnant ewes were supplemented with a gelatin capsule (0 or 400 IU /ewe/day) of vitamin E, 32 days before the expected date of lambing

with or without PI_3 vaccination; ewes were offered *ad libitum* lucerne grass mix hay. There was no effect of vitamin E on the IgG in ewe colostrum, ewe serum or lamb serum.

1.4. Responses of ewes to supra-nutritional supplementation of vitamin E

There is conflicting evidence in the literature regarding the effects of supra-nutritional levels of vitamin E supplementation on ewe performance, lamb mortality, neonatal birth weight, and vigour. Merrell *et al.* (1998) documented that there was a limited effect of vitamin E supplementation on neonatal lamb vigour. Similarly, Daniels *et al.* (2000) reported that there was a limited response in both ewes and lambs in terms of immunity and production, to supra-nutritional levels of vitamin E supplementation. Capper *et al.* (2005) reported a significantly higher vitamin E content in the brain and SM of lambs from supplemented ewes, even though plasma levels were negligible. Capper *et al.* (2005) proposed that vitamin E may be transported across the placenta, but it is avidly assimilated by brain and muscle tissue. The response to vitamin E supplementation regarding lamb birth weight may have been due to enhanced health status in the ewe, facilitating more efficient transfer of nutrients from the ewe to the lamb (Kasimanickam *et al.*, 2010).

Dietary supplements may affect the response to supra-nutritional vitamin E supplementation. For example, Capper *et al.* (2006) found that lamb birth weight was increased and latency to stand reduced when vitamin E was supplemented with fish oil (long-chain PUFA), compared to Megalac (saturated fatty acids). Capper *et al.* (2006) suggested that supra-nutritional vitamin E supplementation was more effective when the oxidative challenge was higher. These benefits of supra-nutritional vitamin E supplementation might not be extended during lactation; for example, Capper *et al.* (2007) found that PUFA in lactation reduced colostrum and milk component yields. Therefore, PUFA and vitamin E might be beneficial during late pregnancy. Dafoe *et al.* (2008) documented that lambs born to ewes fed on sunflower seed (PUFA source) without supplemental vitamin E had a lower body temperature than those supplemented with vitamin E. Rooke *et al.* (2009) reported that vitamin E availability could be increased from body fat reserves during fat mobilisation. Dønnem *et al.* (2015) documented that the percentage of lambs stillborn reduced with vitamin E supplementation (360 IU vs placebo vitamin E) when the pregnant ewes had large litter sizes (carried ≥ 3 lambs). Therefore, pregnant ewe with large liiter sizes might have a greater response to supra-nutritional vitamin E supplementation.

1.4.1. Factors affecting responses to vitamin E

1.4.1.1. Body composition of the ewe

As it is fat-soluble, lipoprotein carries vitamin E and delivers it to body tissues through the same pathway as lipid delivery to body tissues (Debier and Larondelle, 2005). However, the adrenal glands can store the highest α-tocopherol per gram tissue in the body (Hornsby and Crivello, 1983; Bjørneboe *et al.*, 1990). Skeletal muscles, liver and adipose tissue, can accumulate 90% of total α-tocopherol in the body (Machlin and Gabriel, 1982; Bjorneboe *et al.*, 1986). Among many body tissues and organs, the liver is considered as the main vitamin E storage organ in the body (Hidiroglou *et al.*, 1970), that can mobilize hepatic reserves and provide plasma or serum with vitamin E short-term, when the quantity of vitamin E intake is inadequate (Hidiroglou *et al.*, 1970b; Karpinski and Hidiroglou, 1990; Fry *et al.*, 1993). In a survey study to measure vitamin E in the liver in market lambs and cull ewes in Ontario, Menzies *et al.* (2003) found that vitamin E was low to deficient in 10% of cull ewes liver and 90% of lambs market livers. In addition, Menzies *et al.* (2004) diagnosed a hepatic lipidosis and myopathy in weaning lambs; however, the lambs were fed diets containing vitamin E according to NRC (1985) requirements

The vitamin E concentration in body tissues, including adipose tissue is highly variable (2 - 30 µg/ g), and depends upon the vitamin E content of the previous diet (Ochoa *et al.*, 1992; Fry *et al.*, 1993; Kasapidou *et al.*, 2009). For example, Ochoa *et al.* (1992) orally supplemented 35 crossbred wether lambs in 7 treatment groups (*n*=5) with different synthetic vitamin E sources (dl-α-tocopherol) to determine tissue (liver, adipose, longissimus muscle, gluteus medius, heart kidney, pancreas and adrenal gland) uptake of α-tocopherol and retention of different sources of vitamin E. The experimental animals were offered 0.5 kg of a diet that contained vitamin E (1000 IU/kg diet) from 6 different vitamin E sources or a control diet containing no vitamin E. Tissue α-tocopherol content was higher ($P<0.05$) in all supplemented treatments than the control except heart and pancreas, with the highest tocopherol concentration recorded in the liver followed by the pancreas. Serum α-tocopherol concentration tended to be higher in the animals supplemented with an alcohol form of vitamin E than acetate. Regardless of vitamin E source, Kasapidou *et al.* (2009), documented that α-tocopherol concentration in liver, muscles and adipose tissues increased by factors of 20.6 (~0.9 to 19.0 µg/g), 5.1 (~1.0 to 5.0 µg/g) and 10.1 (~1.5 to 15.6 µg/g) respectively when lambs were fed on diets containing vitamin E 30 *vs* 500 mg/kg DM.

Machlin and Gabriel (1982) conducted an experiment to determine the effect of high levels of vitamin E supplementation over a long period on tissue vitamin E concentration. Mature rats were supplemented with either 1,000 or 10,000 mg/kg diet of *all-rac-* α-tocopherol

acetate, and α-tocopherol content of tissues (plasma, liver, skeletal muscle, adipose tissue, brain, platelets, red blood cells, heart, and lung) was measured. Tocopherol accumulation rate was very rapid in both liver and adipose tissue compared with the other tissues. Tocopherol reduced very rapidly in the liver and very slowly in adipose tissue after the animals were returned to a non-supplemented α-tocopherol diet, suggesting that the liver is a major storage organ for tocopherol, at least for a short period (Machlin and Gabriel, 1982).

The results of published work are inconsistent in regards to tocopherol mobilisation from body fat cells during weight loss or fasting in humans (Schaefer *et al.*, 1983; Janneke Brouwer *et al.*, 1998), guinea pigs (Machlin *et al.*, 1979) and sheep (Fry *et al.*, 1993). Traber and Kayden(1987) documented that 90% of total α-tocopherol in the body can be found in adipose tissue and 99% of this α-tocopherol is stored in bulk lipid where it is not available for exchange, and only slowly mobilised from this depot (Bjørneboe *et al.*, 1990). In a study to determine the effect of fasting on vitamin E availability from adipose tissue, Machlin *et al.* (1979) fed guinea pigs with a diet containing *dl*- α-tocopherol acetate (200 mg/kg diet) for 3 weeks followed by 8 weeks feeding on a diet without α-tocopherol acetate. To determine α-tocopherol in adipose tissue, five guinea pigs were killed in weeks 1,2,4,6 and 8, then 8 pigs were fasted for 2 days and 8 were fasted for 4 days and killed. In this experiment, α-tocopherol depletion rate from fat was barely detectable, myopathy (WMD) developed after 8 weeks feeding the diet without vitamin E, and α-tocopherol was not available from adipose tissue to prevent vitamin E deficiency signs or plasma α-tocopherol level. During fasting, there was no α-tocopherol mobilisation from adipose tissue (Machlin *et al.*, 1979). Schaefer *et al.* (1983) conducted a study to test the hypothesis of triglyceride, tocopherol or cholesterol mobilisation from human adipocytes during weight loss. In this study, 3 humans were subjected to weight reduction for 6 months; subcutaneous fat samples were collected by a needle at frequent intervals before and during weight loss (Schaefer *et al.*, 1983). Both tocopherol and cholesterol of adipocytes were not mobilised from fat cells. However, 40% of adipocyte cells size reduced as a result of triglyceride mobilisation (Schaefer *et al.*, 1983). To determine the effect of fasting on plasma α-tocopherol concentrations Janneke Brouwer *et al.* (1998) conducted an experiment on 6 healthy adults (3 males and 3 females). Adults were subjected to fasting for 17 hours at various days before, during and after α-tocopherol supplementation (455 mg/day) for 41 days; subcutaneous adipose tissue samples were taken by needle aspiration at regular intervals. Supplementation increased adipose tissue α-tocopherol while discontinuation did not return adipose tissue α-tocopherol to baseline (Janneke Brouwer *et al.*, 1998). Fasting increased plasma free fatty with small increases in plasma α-tocopherol (Janneke Brouwer *et al.*, 1998). To keep plasma α-tocopherol at high levels, Janneke Brouwer *et al.* (1998) suggested to supplement high levels of vitamin E regularly. In another study to investigate the relationship between tissue and plasma α-

tocopherol concentration during depletion of vitamin E, Fry *et al.* (1993) conducted an experiment on 34 Merino wethers, with animals allocated to 6 treatments, 8 animals in the control treatment were bled and slaughtered at day 0 and the remaining animals were fed on a deficient vitamin E diet and slaughtered at weeks 1,2,4,8 or 21. Vitamin E was measured in liver, three skeletal muscles (*tensor fascia lata, vastus lateralis* and *vastus intermedius*), perirenal adipose tissue, adrenal glands and interventricular septum of the heart. Fry *et al.* (1993) recorded that depletion rate of α-tocopherol was similar in plasma, adipose tissue, adrenal and all muscle tissues, while depletion rate was faster in the liver than both the plasma and muscles, but was identical with both adipose tissue and adrenal. As discussed above, vitamin E availability from tissue stores of pregnant ewes may be an important factor in explaining observed responses to additional supplementation of vitamin E.

1.4.1.2. Vitamin E source and absorption

The source of vitamin E (natural or synthetic) may affect the response to vitamin E in ruminants (Hidiroglou *et al.*, 1989; Kasapidou *et al.*, 2009). Natural α-tocopherol is the free form which occurs naturally in feedstuff and is easily absorbed, while, a synthetic form of vitamin E (α-tocopherol acetate) which is used as a supplement is more stable against oxidation, but biologically less active (Burton and Traber, 1990; Schneider, 2005). It needs to be hydrolysed in the gut to the phenol (free tocopherol) prior to absorption in the small intestine (Burton and Traber, 1990). In an *in vitro* study to determine free α-tocopherol disappearance and α-tocopherol acetate hydrolysis in the rumen, Han and Owens (1999), used rumen fluid collected from 6 beef steers fitted with rumen fistula and fed on a high roughage or concentrate. Both tocopherol and tocopherol acetate were added to incubation tubes to achieve concentrations of 5 or 10 µl/ml rumen fluid and incubated at 39 ^{o}C for 24 hours. After incubation for 24 hours, tocopherol acetate hydrolysed to free tocopherol was less than 10% (Han and Owens., 1999). The free α-tocopherol concentration was unchanged, which supports the theory that α-tocopherol is not degraded in the rumen. In terms of vitamin E bioavailability, these results indicate that there is little effect of the rumen environment on increasing or reducing responses to vitamin E supplementation because α-tocopherol acetate hydrolysis to α-tocopherol, which is important for intestinal absorption, is insufficient to increase the bioavailability of α-tocopherol acetate in the rumen, and there is no degradation of free α-tocopherol. In two experiments (*in vivo* and *in vitro*), Hymøller and Jensen (2010) used four ruminal cannulated Holstein cows in the first experiment and five cows in the second experiment. In experiment one, ruminal contents (10 to 15 kg) were taken through the cannulas from each cow, a sample was taken and the rest was mixed with *all-rac*-α-tocopherol acetate (4360 mg) or (4360 mg, 250 mg vitamin D_2 and 250 mg vitamin D_3), then the mixture returned to the cows rumen. Rumen fluid samples were taken

at 0,1,2,4,6,24 and 30 hours following vitamin supplementation. Blood samples were taken at 0, 6, 24 and 30 hours after the vitamins were introduced into the rumen (Hymøller and Jensen, 2010). In experiment 2 (*in vitro*), 6 subsamples of ruminal contents from each cow were taken from 1-hour (one hour after placing the vitamins in the rumen) and incubated at 37 ^{o}C and taken out at 2,4,6,12,24 and 30 hours. The results indicated that *all-rac*-α-tocopherol acetate was stable in the rumen and there were no hydrolyses of α-tocopherol acetate to free α-tocopherol (alcohol form) in the rumen (Hymøller and Jensen, 2010). In addition, plasma α-tocopherol did not increase after *all-rac*-α-tocopherol acetate supplementation into the rumen, which indicated that *all-rac*-α-tocopherol acetate did not hydrolyse in the rumen to the alcohol form (free α-tocopherol) and reduced intestinal ability for α-tocopherol absorption (Hymøller and Jensen, 2010).

In terms of vitamin E absorption, the source affects the absorption of vitamin E. Hidiroglou *et al*. (1989) conducted two experiments on the bioavailability of dl-α-tocopherol and dl-α-tocopherol acetate in sheep and cattle using a single oral dose. In experiment one, 10 crossbred wether lambs were housed in metabolism crates and allocated to two groups, which were supplemented intraruminally with either 100 mg/kg BW dl-α-tocopherol or dl-α-tocopherol acetate. In the second experiment, Hidiroglou *et al*. (1989) used 4 crossbred dairy heifers in an experiment consisting of two 20 days periods, with 3 weeks wash out between the periods prior to reversing the treatments. In each period the heifers were orally supplemented with either dl-α-tocopherol (n=2) or α-tocopherol acetate (n=2) at 50 mg/kg BW (Hidiroglou *et al*., 1989). In both experiments, the tolerance curve area of α-tocopherol in blood plasma was higher for dl-α-tocopherol than dl-α-tocopherol acetate, and α-tocopherol in the plasma reached a maximum concentration in a shorter period of time than dl-α-tocopherol acetate. These results indicate that the bioavailability of dl-α-tocopherol is higher than α-tocopherol acetate. Kasapidou *et al*. (2009) conducted an experiment to evaluate the effects of different levels and source of vitamin E on vitamin E status and lambs performance. In this experiment, 56 Suffolk × Charollais wether lambs were allocated to seven treatment groups (five concentrates (C) and 2 grass silage (S)). Vitamin E supplementation in the concentrate treatments was 30 (C30), 60 (C60), 120 (C120), 250 (C250) and 500 (C500) mg/kg DM. In addition, the two silage base diet treatments were supplemented with either 60 (S60) or 500 (S500) mg/kg DM of vitamin E (Kasapidou *et al*., 2009). By the end of the experiment, plasma vitamin E was higher in S500 than C500. In addition, plasma vitamin E was higher in S60 than treatments C30, C60, C120 or C250 (Kasapidou *et al*., 2009). Liver and muscle vitamin E concentration was higher in S500 than C500, while there was no difference in adipose vitamin E content in treatments S500 and C500 (Kasapidou *et al*., 2009). Tissue (liver, muscles and adipose) vitamin E in S60 was similar to the C250 and higher than in treatments C30, C60 or C120. Supra-nutritional α-

tocopherol acetate supplementation reduced α-tocopherol absorption which leads to low plasma α-tocopherol (Machlin and Gabriel, 1982; Bjørneboe *et al.*, 1990). The difference in plasma vitamin E concentration between silage and concentrate treatments as a natural or synthetic source of vitamin E at the same vitamin E supplementation level could be due to poor absorption efficiency of α-tocopherol acetate from the intestine (Burton and Traber, 1990; Kasapidou *et al.*, 2009) . Alpha-tocopherol acetate needs to be hydrolysed to free α-tocopherol before intestinal absorption (Burton and Traber, 1990), while vitamin E from natural sources (silage) is free α-tocopherol which is ready for absorption (Burton and Traber, 1990; Kasapidou *et al.*, 2009). Another reason for higher plasma and tissues vitamin E concentrations is α-tocopherol stereoisomers; vitamin E from natural sources has *RRR* isomers while the synthetic form of α-tocopherol (all-*rac*-α-tocopherol) consists of a racemic mixture of the eight stereoisomers (*RRR, RRS, RSR, RSS, SSS, SRS, SSR and SRR*) (Meglia *et al.*, 2006; Wilburn *et al.*, 2008 and Yang, 2003). Hepatic protein (α-TTP) which is responsible for the uptake of α-tocopherol from the hepatic cells into the very low-density lipoprotein (VLDL) prefer to bind the *RRR* isomer than the other isomers (Burton and Traber, 1990).

1.4.1.3. Rumen environment

The rumen environment is anaerobic (Belanche *et al.*, 2016; Belanche *et al.*, 2017). However, an amount of dissolved oxygen (maximum 3 mmol/L) can be found for at least 18 hours in a day which causes free radical accumulation from cell membrane oxidation (Naziroğlu *et al.*, 2002; Belanche *et al.*, 2017). The presence of oxygen in the rumen affects the growth of rumen microorganisms (Hillman *et al.*, 1989). Tocopherol is resistant to alkali and heat, up to 100 ºC in an anaerobic environment (Machlin, 1984). However it is easily oxidised in aerobic environments (Han and Owens., 1999). Rumen fibrolytic bacteria are particularly sensitive to oxygen (Belanche *et al.*, 2017). Therefore, vitamin E supplementation might reduce the negative effects of free radicals on microbial activity and potentially increase feed digestibility by improving the rumen microbiome (Belanche *et al.*, 2016; Belanche *et al.*, 2017).

In a study on the effect of vitamin E supplementation and method of forage conservation on rumen function using the rumen simulation technique, Belanche *et al.* (2016) found that tocopherol supplementation of either ryegrass or hay based diets containing 20% concentrates *in vitro* had a possible effect on rumen fermentation, increasing rumen gas production, total VFA, protozoal activity and resulted in a small increase in diet digestibility. The higher protozoa and bacterial concentrations was possibly due to the antioxidant activity of vitamin E (Belanche *et al.*, 2016). Increases in rumen microbial activity resulting from vitamin E supplementation suggest that microorganisms actively metabolise vitamin

E. In an *in vitro* experiment to determine the effect of vitamin E supplementation (50 IU/day) on microbial colonisation of ryegrass hay *vs* ryegrass in vitro, Belanche *et al*. (2017) documented that colonisation was slightly accelerated by vitamin E supplementation, which may have been responsible for the small increase in dry matter degradation. Source of fat supplementation with supra-nutritional vitamin E may affect rumen feed digestibility. Chikunya *et al*. (2004) conducted research to investigate the ruminal bio-hydrogenation of dietary n-3 PUFA sources. In this study, six cannulated sheep were offered three fat sources (Megalac, linseed oil or a combination of linseed and fish oil) with either a low or high level of vitamin E (50 or 500 mg/kg DM) in a Latin square design. The high level of vitamin E supplementation increased cellulose whole tract digestibility (or degradability) in the Megalac, but not the Linseed or linseed fish diets (Chikunya *et al*., 2004).

Schäfers *et al*. (2018) conducted an *in vivo* experiment to investigate the effect of vitamin E supplementation on rumen microbial activity. In this study, eight rumen and duodenal cannulated German Holstein cows were supplemented with diets containing DL-α-tocopherol acetate at either 23.1 or 138.6 IU/kg DM. The results indicated that there was no effect of a vitamin E supplementation on organic matter fermentation, or microbial protein synthesis, no effect on rumen short-chain fatty acids (VFAs) concentration (Schäfers *et al*., 2018). The level of vitamin E (138.6 IU/kg DM) used in this study may have been insufficient to make a difference in rumen activity (Schäfers *et al*., 2018). In an *in vitro* experiment to determine the effects of vitamin E supplementation on rumen fermentation, Naziroğlu *et al*. (2002) used 100 ml of rumen fluid from crossbreed beef steers fed on 55% barley straw with 50% concentrate. Rumen fluid was not supplemented (control) or supplemented with vitamin E at either 0.4 or 0.8 mg and incubated for 24 hours at 39 ^{o}C. Vitamin E supplementation increased acetate and propionate concentration, rumen ammonia concentration and total protozoa counts (Naziroğlu *et al*., 2002).

1.5. Conclusions

Adipose tissue can assimilate vitamin E more rapidly and can accumulate 90% of the total quantity of α-tocopherol. There is conflicting evidence whether α-tocopherol is mobilised from adipose tissue or not during nutritional restriction and body fat mobilisation. The body fat content may effect on the response to vitamin E supplementation. Further work is required to investigate the effect of ewe body composition on responses to supra-nutritional vitamin E supplementation.

Vitamin E from natural sources is biologically more available from small intestine than synthetic vitamin E supplements. Evidence suggests that the availability of vitamin E depends on diet type, with vitamin E being more available from forage than concentrate-based diets. Research on growing lambs in term of α-tocopherol deposition in meat and

meat quality suggests that the response to vitamin E supplementation is greater in forage than concentrate fed animals. However, there is limited data on the effect of diet type (forage vs concentrate) on the response of ewes and lambs to supra-nutritional vitamin E supplementation. Therefore, further investigation is required to determine the effect of nutritional status and diet type on the response of ewes to supra-nutritional levels of vitamin E supplementation.

The objectives of the current study were;

1-Determine the effect of nutritional restriction in mid-pregnancy to achieve different body condition scores at late pregnancy on the response of ewes to supra-nutritional levels of vitamin E in late pregnancy and early lactation.

2-Investigate the effect of source and level of vitamin supplementation on ewe and lamb performance during late pregnancy and early lactation.

3-Investigate the effect of source and level of vitamin E supply on vitamin E apparent absorption and availability in growing lambs.

Chapter Two

General Materials and Methods

2.1. Samples proximate analysis

In accordance with Association of Official Analytical Chemists (AOAC, 2016), all of the experimental samples (feed samples, faecal samples from experiments one, two and three, and colostrum samples from experiments one and two were analysed in duplicate in the Harper Adams University laboratories.

2.1.1. Dry matter

Feed and faecal samples dry matter (DM) content was measured according to the Association of Official Analytical Chemists (AOAC, 2016; 930.15). Approximately 100 g of feedstuff was accurately weighed into an aluminium tray (pre-weighed), and oven dried overnight at 105°C (Binder, Cole-Palmers, UK) or freeze dried (Edwards Modulyo freeze dryer, Sussex, UK) until a stable weight was obtained. After being taken from the oven, samples were cooled in a desiccator and reweighed. The DM was then calculated:

$$\text{DM (g/kg DM)} = \left[\frac{\text{Weight of dried sample (g)}}{\text{Weight of fresh sample (g)}}\right] \times 1000 \qquad \text{Eq. 2.1.1}$$

Dried samples were ground through a 1 mm screen for subsequent chemical analysis using a cyclone mill (Cyclotec, FOSS, Warrington, UK).

2.1.2. Ash and organic matter

Ash and organic matter (OM) were determined according to method 942.05 (AOAC, 2016). Approximately 2 g of dried and ground feed and faecal samples were weighed accurately into a pre-weighed labelled porcelain crucible and ashed in a muffle furnace (Gallenkamp Muffle Furnace, Size 3, GAFSE 620, Gallenkamp, Loughborough, UK) for 4 h at 550°C. The ash in the crucible was cooled in a desiccator to room temperature before being reweighed. Ash content was determined according to the following equation:

$$\text{Ash (g/kg DM)} = \left[\frac{\text{Weight of ash (g)}}{\text{Weight of dry sample before ashing (g)}}\right] \times 1000 \qquad \text{Eq. 2.1.2a}$$

$$\text{Organic matter (g/kg DM)} = 1000 - \text{ash content (g/kg DM).} \qquad \text{Eq. 2.1.2b}$$

2.1.3. Crude protein (CP)

Feed and faecal samples crude protein (CP) was measured according to method 968.06 (AOAC, 2016) using an auto analyser (LECO FP528, Corp., St. Joseph, MI, USA) operating

the Dumas method with EDTA as the standard (Sweeney, 1989). Approximately 0.15 g of dried milled sample was accurately weighed into an aluminium foil square, which was then placed into the auto analyser. The sample was heated to 950 C in oxygen for rapid combustion. The nitrous oxide released was then reduced to N_2 using copper filings. CP content of samples were calculated as:

$$\text{CP (g/kg DM)} = \text{Nitrogen content (g/kg DM)} \times 6.25 \qquad \text{Eq. 2.1.3}$$

2.1.4. Ether extract (EE)

Feed and faecal samples ether extract content was measured according to the solvent method of (FOSS, 1987) using Soxtec apparatus (HT 1043 extraction apparatus, FOSS, Warrington, UK). Approximately 2-3 g of dried milled sample was weighed accurately into a cellulose extraction thimble (Whatman Plc, Maidstone, UK). The thimble top was plugged with cotton wool and put in the extraction unit. Samples were boiled in 25 ml of petroleum ether (Fisher Scientific, UK) for 1 h. They were then rinsed for an additional 15 min after removing from the solvent, and the solvent removed by evaporated for 5 minutes. The total ether extract was collected in the extraction cups which were then removed from the apparatus and placed in a fume cupboard. The extraction cups were cooled, and the ether extract determined according to the following equation:

$$\text{EE (g/kg DM)} = \left[\frac{\text{Weight of fat (g)}}{\text{Weight of dry sample (g)}}\right] \times 1000 \qquad \text{Eq. 2.1.4}$$

2.1.5. Neutral detergent fibre

Neutral detergent fibre (NDF) content of dried, feed, and faeces samples was determined using the method of Van Soest *et al.* (1991) using the Fibertec apparatus (Tecator Fibertec 1020 Hot extractor, FOSS, UK Ltd, Warrington, UK). Firstly, NDF solution was prepared by dissolving 93 g of disodium ethylene diamine tetra-acetate dihydrate and 34 g of sodium borate using gentle heat, in deionised water (3L), then 150 g sodium dodecyl sulphate (SDS), 50 ml tri-ethylene glycol were added to this solution. In a different beaker, 22.8 g of anhydrous disodium hydrogen phosphate was dissolved in approximately 500 ml of deionised water. The two solutions were then mixed, and deionised water added to make 5 L of NDF reagent. Sodium hydroxide (NaOH) 0.1 M or HCl 0.1 M was used to adjust the solution pH to be between pH 6.9 and 7. Then, a α-amylase solution was prepared by dissolving 2.8 g of stable alpha-amylase (E.C.3.2.1.1. from Bacillus subtilis 73 Sigma, Gillingham, UK) in a mixture of 10 ml of 2-ethylene glycol and 90 ml of distilled water.

Approximately 0.4-0.6 g of dried milled sample was accurately weighed into a pre-weighed glass crucible (porosity 1, Soham Scientific, Ely, UK). Crucibles were placed into the

apparatus making sure that the valves were in the closed position. Neutral detergent fibre (NDF) reagent (25 ml) was added to each sample. To reduce the foam, a few drops of octanol, reagent grade (Sigma, Aldrich, Dorset, UK) was added to each sample. After being boiled for 30 minutes, an additional 25 ml of NDF reagent and 2 ml of α-amylase solution were added to the samples. The samples were then boiled for a further 30 minutes. To remove the NDF reagent, samples were filtered under vacuum, washed with 3×3 ml hot (80°C) distilled water. An extra 2 ml of α-amylase solution were added to the samples followed by 25 ml hot distilled water (80°C) for further 15 minutes. Samples were then filtered, washed with 3×3 ml hot (80°C) distilled water. Crucibles were then removed from the apparatus and oven dried at 105°C overnight. After cooling in a desiccator, crucibles were weighed and then placed in a muffle furnace at 550°C for 4 h. Crucibles were reweighed after being allowed to cool in a desiccator to room temperature.

NDF (g) = (crucible + dry fibre weight) – (crucible + ash weight) Eq. 2.1.5a

$$\text{NDF (g/kg DM)} = \left[\frac{\text{NDF weight (g)}}{\text{Dry sample weight (g)}}\right] \times 1000 \qquad \text{Eq. 2.1.5b}$$

2.1.6 Acid detergent fibre (ADF)

The acid detergent fibre (ADF) content of dried, feed and faeces samples was determined using the method of Van Soest *et al.* (1991) using the Fibertec apparatus (1020, FOSS, Warrington, UK). Acid detergent fibre solution was prepared by dissolving 20 g of solid cetyl trimethylammonium bromide (CTAB) in 1 L of 1 M sulphuric acid and boiled for 60 minutes. Approximately 1 g of dried milled sample was accurately weighed into a pre-weighed glass crucible (porosity 1, Soham Scientific, Ely, UK). Crucibles were then placed into the apparatus making sure that the valves were in the closed position. Acid detergent fibre reagent (100 ml) was added to each sample. After being boiled for 60 minutes in the ADF reagent, samples were filtered under vacuum, washed with 3×3 ml hot (80°C) distilled water. The crucibles were then removed from the apparatus and oven dried at 105°C overnight. After cooling in a desiccator, the crucibles were weighed and then placed in a muffle furnace at 550°C for 4 h. Crucibles reweighed after being allowed to cool in a desiccator to room temperature.

ADF (g) = (crucible + dry fibre weight) – (crucible + ash weight) Eq. 2.1.6a

$$\text{ADF (g/kg DM)} = \left[\frac{\text{ADF weight (g)}}{\text{Dry sample weight (g)}}\right] \times 1000 \qquad \text{Eq. 2.1.6b}$$

2.1.7. Gross energy determination (GE)

Gross energy of the feed and faeces samples was determined using an adiabatic bomb calorimeter (Parr 6200 Instrument Company, Moline, IL, 61265, USA) with benzoic acid as the standard. A 2811 Parr pellet press (Parr instrument Co., Moline. USA) was used to pellet approximately 1 g of dried sample. The pellet was weighed accurately and placed into a crucible. A fuse wire (10cm) was carefully inserted through the bomb's holes to avoid contacting the sample. The crucible was then placed in the bomb and the apparatus was then filled with the O_2 (pressure 450 psi for 1 minute). The bomb was then placed in a bucket containing 2 l of water and the wires connected. The sample was then electrically ignited and the heat of combustion (GE, MJ/kg DM) measured by the bomb calorimeter.

2.1.8. Whole tract digestibility determination by acid insoluble ash method

During weeks -3 and -2 faecal grab samples (approximately 50 g) were collected from six representative ewes on each treatment over a five day period at 11:00 a.m. to indirectly estimate diet digestibility using acid insoluble ash as an internal marker (Block *et al.*, 1981). Feed samples were collected from the same animals and freeze dried together with faecal samples. Dried feed and faecal samples (20 g) for each ewe over each five-day collection period were then bulked and milled through a 1 mm screen.

Acid insoluble ash was estimated indirectly in sheep by using the method of Van Keulen and Young (1977). Approximately 5 g (in duplicate) of dried milled feed or faeces samples was accurately weighed into a pre-weighed ceramic crucible. Samples were oven dried (135°C for 2 h) and re-weighed after being cooled in desiccator. Samples were then ashed at 550°C for 4 h in a muffle furnace (Gallenkamp muffle furnace, Size 3, GAFSE 620, Gallenkamp, Loughborough, UK), and re-weighed after being cooled in a desiccator. The residual ash was then quantitatively transferred from the crucible into a labelled Kjeldahl boiling tube (Foss Tecator Digestor Unit, Hilleroed, Denmark), and the crucibles washed into the tube using two 5ml aliquots of 2M hydrochloric acid HCl (Fisher Scientific Ltd, Leicestershire, UK). Another 90 ml of 2M HCl was added to each tube. The tubes were then placed on a heating block (at approximately 175°C) in a fume cupboard and allowed to boil for 10 minutes. The hot digest was then filtered through ash free filter paper (Whatman No. 41 Fisher Scientific Ltd, Leicestershire, UK). All of the ash was washed from the tube into the filter paper using hot water (85- 100°C). The filter paper containing the ash was then folded and replaced into a crucible and ashed in a muffle furnace at 550°C for 4 hours. The crucibles were reweighed after being cooled in desiccator and the acid insoluble ash (AIA) calculated as:

$$\%AIA = \left[\frac{\text{weight of crucible(g)+ash} - \text{weight of crucible(g)}}{\text{weight of dry sample (g)}}\right] \times 100 \qquad \text{Eq. 2.1.8.a}$$

Digestibility of feed DM was calculated as:

$$\text{DM Digestibility (kg/kg)} = 1 - 1 \times \frac{\text{(g/kg DM AIA in feed)}}{\text{(g/kg DM AIA in faecal)}} \qquad \text{Eq. 2.1.8b}$$

The faecal DM output (g d^{-1}) for each ewe was also calculated as:

Faecal DM output (g/d) = DMI (kg) – (DMI (kg) x digestibility (kg/kg))   Eq. 2.1.8c

The organic matter, CP and NDF digestibility were also calculated from the DMI, faecal output, diet and faecal analysis for OM, CP and NDF as bellow:

$$\text{Nutrient digestibility (g/d)} = \left[\frac{\text{Nutrient intake (g/d)} - \text{Nutrient output (g/d)}}{\text{Nutrient intake (g/d)}}\right] \qquad \text{Eq. 2.1.8d}$$

2.2. Blood sampling

Blood samples were obtained by venepuncture using a 21G x 1″ (0.8 x 25 mm) needles into Vacutainers (Becton Dickinson Vacutainer Systems, Plymouth, UK). The blood samples were collected into two different types of tube (6 ml) containing sodium heparin (for determination of vitamin E, total protein, albumin, beta hydroxybuterate (BHB), non-esterified fatty acid (NEFA), and urea), and (6 ml) containing potassium oxalate (for determination of glucose concentration). The tubes were gently shaken to avoid clotting and transferred to the laboratory. Blood plasma was obtained from blood collected in the sodium heparin and potassium oxalate vacutainers by spinning at 1000 RCF (relative centrifugal force) for 15 minutes at 4°C in Rotina 46R, Hettich Zentrifugen centrifuge (Anderson Hettich GmbH & Co, Tuttlingen, Germany). The plasma was removed and stored in 1.0 ml eppendorf tubes at -20 °C prior to further analysis.

2.3. Ewes and lambs weighting

All ewes and lambs were weighed weekly using a digital scale following calibration with metric standard weights (F.J. Thornton and Co. Ltd., Wolverhampton, UK). The lambs in experiments one and two were weighed 12 hours after lambing as a birth weight and thereafter weekly. Lambs in experiment three were weighed weekly.

2.4. Ewe body condition scoring (C)

All ewes were body condition scored weekly by the same (competent) person at a fixed time relative to feeding according to the method of Russel (1984). Ewes were condition scored by palpation of the lumbar region and around the backbone in the loin area, directly behind the last rib and above the kidney to assess the level of roundness or sharpness. The scoring system was based on a scale of 0 to 5 (Table 2.1).

Table 2. 1. Sheep body condition scoring system.

Grade	Description	Illustration
Score 1	The spinous processes are prominent and sharp. The transverse processes are also sharp, with fingers passing easily under the end of this process. The eye muscle areas are shallow with little to no fat cover.	
Score 2	The spinous processes are smooth but still prominent. The individual processes can still be felt but only as fine corrugations. The transverse processes are smooth and rounded. However, it is still possible to pass the fingers under the ends of the processes with some pressure. The eye muscle areas are of moderate depth, but have sparse fat cover.	
Score 3	The spinous processes are smooth and rounded, and individual bones can only be felt with some pressure applied. The transverse processes are also smooth and are well covered. Firm pressure is required to feel over the ends. Eye muscle area is full and covered by a moderate degree of fat.	
Score 4	With pressure applied, the spinous processes can just be detected, although the ends of the transverse processes cannot. Eye muscle areas are full with a thick covering of fat.	
Score 5	Even with firm pressure applied, the spinous processes cannot be detected. Due to a high level of fat adjacent to the spinous process, a depression directly above where the spinous processes would normally be felt may be present. It is not possible to detect the transverse processes. The eye muscle areas are very full with very thick fat cover. It is possible to have significant deposits of fat cover over the rump and tail.	

Source: Adapted from Russel (1984) and Kenyon *et al.* (2014).

2.5. Ewe back fat and eye muscle depth scanning

All ewes were back fat and Longissimus lumborum (LL) muscle thickness scanned according to the procedure of Davis (2010) using an ultrasound device (DP-6900Vet, Mindray Ltd.). At 90 degrees to the backbone on the third lumbar vertebrate the wool was parted. Liquid paraffin oil was used to give a contact after being applied on the third lumbar vertebrate. The transducer was placed on the selected site and adjusted to obtain a clear image of the eye muscle and fat layers. Back fat and eye muscle depth were measured after the picture was frozen. Fat depth was measured in three places at 1 cm intervals, with the mean fat depth being reported. A single muscle depth measurement was taken at the deepest point (Figure 2.1).

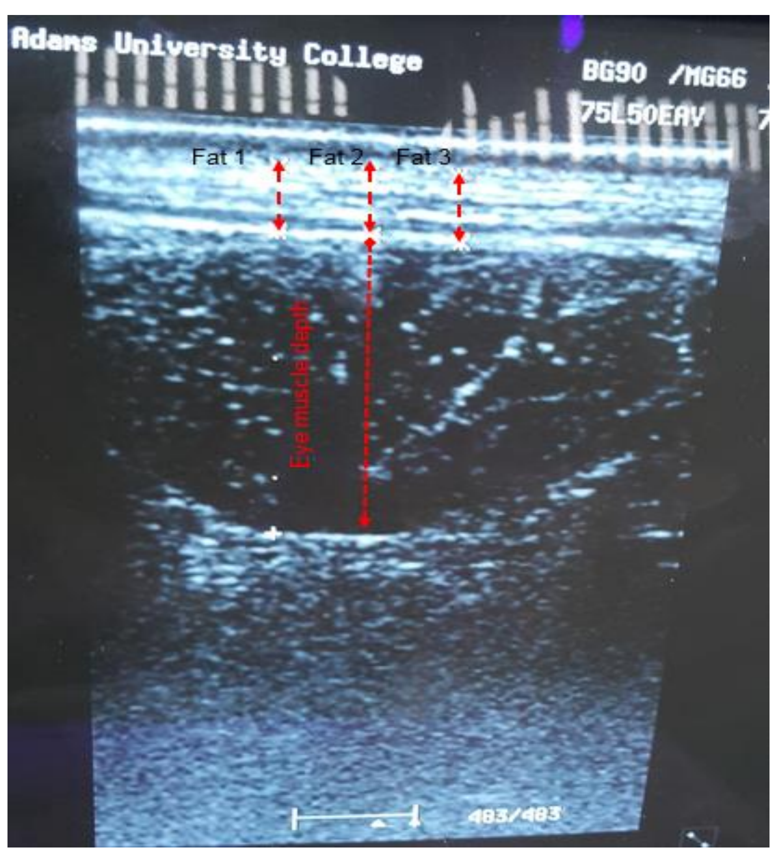


Figure 2. 1. Eye muscle area and fat layer of lamb.

2.6. Ewe milking

Both colostrum and milk yield were estimated from all ewes at 16 hours and 28 days *post-partum* using a method adapted from Doney *et al.* (1979). At +12 hours *post-partum* lambs were confined behind a wire mesh barrier, and the ewe was injected with 1.0 ml of oxytocin (Oxytocin-S, solution for injection, Intervet UK Let, Walton Manor, UK) to stimulate milk let down. After 2.0 minutes, ewes were milked out using an automatic milking machine (Bronzoni, Motori Eletirici Ramuseto- Italy) until the udder was empty. At 16 hours (4 hours after the initial milking), ewes were injected with a further 1.0 ml of oxytocin and the process repeated, prior to lambs being returned to the ewe. Colostrum secretion rate was then calculated over the four hour period and extrapolated to 24 hours. The same procedure was repeated at 28 days. Samples of colostrum and milk were collected and stored at -20 °C prior to further analysis.

2.7. Colostrum and milk analysis

Colostrum samples were analysed to measure total solid, protein, fat, ash and lactose. All of the samples were first defrosted in the fridge and mixed well.

Total solids were determined by oven drying 10 ml of colostrum at 45 °C and reweighed daily until there was no change in weight. Total solids were calculated using the following equation:

$$\text{Total Solid (g/kg)} = \left[\frac{\text{Final sample weight (g)}}{\text{Initial sample weight (g)}}\right] \times 1000 \qquad \text{Eq. 2.7.a}$$

Following analysis for total solids, the crucibles were weighed and ashed in a muffle furnace for 4 hours at 500 °C to determine total ash content. After that, the crucibles were weighed, and the ash was calculated as follows:

$$\text{Ash (g/kg)} = \left[\frac{\text{Final sample weight (g)}}{\text{Initial sample weight (g)}}\right] \times 1000 \qquad \text{Eq. 2.7.b}$$

The fat content of colostrum was measured using the Gerber method described by MAFF (1986). The samples were first diluted1:3 with deionised water and thoroughly mixed. Using a pipette, 10.0 ml of sulphuric acid (89.5- 91.0 %, Fisher, S9360/PB17) was placed into a butyrometer tube, then 10.94 ml of diluted colostrum and 1 ml of amyl alcohol were added to the butyrometer. A stopper was put in the neck of the butyrometer after being cleaned. The solution was mixed well until the white particles were dissolved using the inversion stand shaker, and then the butyrometers were centrifuged at 1100 g for 5 minutes. The butyrometer tubes were placed in a water bath at 65°C for 10 min after being removed from the centrifuge. Following removal from the water bath, the stopper was used to move the fat onto the bottom of the scale and the reading was recorded. To calculate the final fat percentage, the results were multiplied by four as a dilution factor.

Colostrum crude protein (6.25x N) was determined using the Dumas method (LECO FP-528). Colostrum samples (0.6 g) were weighed into a small aluminium capsule and dried in an oven for 24 hours at 45°C and reweighed daily until there was no change in the weight. After that, the protein content of the dried samples (approximately 0.15 g) was determined as described previously (Section 2.1.3) Sample protein content was calculated by multiplying nitrogen by 6.25.

Colostrum lactose content was calculated by subtracting all of the analysed colostrum components from the colostrum total solid.

$$\text{Lactose (g/kg)} = \text{TS} - \text{Ash} - \text{CP} - \text{fat} \qquad \text{Eq. 2.7.c}$$

Milk samples were analysed using a MilkoScan Minor (Foss UK Ltd., Warrington, UK) calibrated by the methods of AOAC (2000), to identify total solid, fat, crude protein and lactose.

2.8. Vitamin E analysis

2.8.1. Vitamin E analysis in the plasma and colostrum

Plasma and colostrum samples were analysed according to the method of Burton *et al.* (1985). The samples were thawed and mixed well. After that 0.5 ml of plasma pipetted into a 15 ml tube in duplicate, then 2 ml of ethanol was added and mixed using a vortex mixer. One ml 0.1M sodium dodecyl sulphate (SDS) was added and the sample vortex mixed again. After that 15µl of the internal standard, 5,7-dimethyl tocol (DMT) (Universal Biological Ltd, Stroud, UK) and 1 ml of hexane including 0.05% butylated hydroxytoluene (BHT) was added to the samples and shaken vigorously. After that, the samples were centrifuged in a bench centrifuge for 2 min. Next, the top layer was decanted into a clean tube taking care to avoid transferring the bottom aqueous layer. Then 1ml of 0.05% BHT in hexane was added and the process repeated twice more. The solvent was then evaporated under oxygen free nitrogen (OFN) at 60°C and 1 ml hexane was immediately added. The extract was then filtered into a 2 ml amber HPLC vial and stored at -20°C prior to vitamin E determination by high performance liquid chromatography (HPLC). The samples were run on an HPLC with DMT standard (15 µl in 1ml n-hexane) and vitamin E standard (Sigma® Chemical Co. St. Louis, MO. USA) (approx. 4-5 µg/ml).

Colostrum samples were thawed and mixed well. After that 1 g of colostrum pipetted into a 15 ml tube in duplicate, then 2 ml of ethanol were added and mixed using avortex mixer. One ml of 0.12M sodium dodecyl sulphate (SDS) was added and the sample vortex mixed again. After that 50 µl of the internal standard, 5,7-dimethyl tocol, and 1 ml hexane (fraction from petroleum) including 0.05% BHT were added to the samples and shaken vigorously. The samples were centrifuged in a bench centrifuge for 2 min. Next, the top layer was decanted into a clean tube taking care to avoid transferring the bottom aqueous layer. Then 1 ml 0.05% BHT in hexane was added and the process repeated twice more. The solvent was then evaporated under oxygen free nitrogen (OFN) at 60°C and 3 ml hexane immediately added. The extract was filtered into an amber HPLC vial and stored at -20°C prior to determination of vitamin using high performance liquid chromatography (HPLC). The samples were then run on the HPLC with DMT std (50 µl in 3ml n-hexane) and vitamin E standard (Sigma® Chemical Co. St. Louis, MO. USA) (approx. 4-5 µg/ml).

2.8.2. Vitamin E analysis in Feedstuff

Feedstuff samples were analysed by following the method of Hidiroglou *et al.* (1988). All of the diet samples were freeze dried as described in section 2.1.1. Then 0.3g to 1g ± 0.001g of dry milled sample was weighed into a 30 ml soveril tube, then 0.05g α-amylase 2.8 g of stable alpha amylase from *Bacillus Subtilis* (Sigma, Gillingham, UK) and 8 ml deionised water was added. The mixture was then vortex mixed and placed in a water bath at 37°C overnight (minimum 16 hours).

The following day, the samples were removed from the water bath and vortex mixed for a few seconds. After that 0.1g ascorbic acid (dry), 10 ml ethanol (100%) and 2 ml potassium hydroxide 50% were added to the samples. The internal standard (DMT) was added at a quantity appropriate to take into account any final dilution factors. Then the samples were boiled for 30 minutes in a boiling water bath. After that, the samples were removed from the water bath and cooled by standing in ice. The liquid samples were transferred via a funnel into a 50 ml volumetric flask (ambered) using 10 ml of hexane and then 10 ml of deionised water. After that 2 ml of ethanol were added and shaken vigorously for 30 sec. Then the samples were allowed to separate, and the top layer pipetted off into a separating funnel containing 10 ml of deionised water.

A further 10 ml of hexane was added to the sample with repeated shaking and separated, this process was repeated until hexane have been collected in the separating funnel. Then the collected sample in the separating funnel was gently shaken in order to wash the sample, allowed to separate and run bottom layer (deionised water) into a waste beaker. Then 10 ml of deionised water was added to the separating funnel and the washing process repeated. This process was repeated to make three washes in total. The extract was transferred to a 50 ml beaker and transferred to a 30 ml soveril leaving any water phase behind, and was then was evaporated to dryness in a water bath at 60°C under oxygen free nitrogen. After evaporation of the extract, 5-6 ml of 97% of n-hexane was immediately added to solubilize. Then the samples were transferred to a 10 ml volumetric with hexane and mixed well. After that, it was filtered through a 0.2 μm filter into a HPLC vial and stored -20°C until run on the HPLC (Gilson, France for experiment one and Agilent 1100, Germany for experiment two).

2.8.3. HPLC conditions and quantification

A (40 μl) of sample was injected onto an HPLC (Gilson, France for experiment one and Agilent 1100, Germany for experiments two and three) silica column 5 μm (250 x 4.6mm, Phenomenex Hyperclone) at 40°C with a mobile phase of 4% 1, 4-Dioxine 97% n-hexane at a 1.6 ml/min flow rate. The detection of α-tocopherol was by fluorescence detectors. The

peak area of samples and standards were measured, and the following equations were used to determine the α-tocopherol concentration in samples:

α- tocopherol μg /sample weight(μg/g) or size(μg/ml)

$$= \frac{\left(\frac{area\ of\ \alpha-tocopherol\ in\ sample}{area\ of\ pure\ \alpha-tocopherol\ in\ std}\right) * conc.\ of\ pure\ \alpha-tocopherol\ in\ std}{\frac{area\ of\ DMT\ in\ samples}{area\ of\ DMT\ in\ std}} \qquad \text{Equation 2.8.3.a}$$

$$\text{Plasma (μg /ml) or colostrum (μg /g) α- tocopherol} = \frac{\alpha-\ tocopherol\ \mu g}{sample\ size\ (ml)\text{or weight (g)}} \qquad \text{Equation 2.8.3.b}$$

$$\text{Feedstuff α- tocopherol μg /g DM} = \frac{\alpha-\ tocopherol\ \mu g}{sample\ weight\ (g)} \qquad \text{Equation 2.8.3.c}$$

2. 9. Plasma immunoglobulin (IgG)

Lamb plasma IgG content was measured based on the method of Mancini *et al.* (1965). Briefly, 16 ml of the agar (2% molten in TRIS buffer, pH 7) was added to rabbit-anti-sheep IgG (1 ml of 2 mg/ml diluted in 25 ml of PBS) (Thermo Fisher scientific, USA). A 5 μl of diluted sample (1:5 in PBS) and standard (125, 500 and 1000 μg/ml, purified sheep IgG; Thermo Fisher scientific, USA) were applied in 20 wells (prepared using a 4 mm cork borer) in 10x10cm petri dishes. The plates were incubated at 37°C for 24 hours. Using digital callipers, the diameter of rings around the wells was measured. The standards Log^{10} concentration was calculated from the standard concentrations (125, 500 and 1000 μg/ml). A standard curve was constructed by plotting the measured standards diameters against standards Log^{10} concentration. Immunoglobulin concentration as mg/ml in lamb's plasma was measured by using the following equations:

Y = (11.041X - 18.211)

$$Log^{10}\ \text{plasma IgG} = \left[\frac{\text{Sample ring diameter (mm)} + 18.211}{11.041}\right] \qquad \text{Equation 2.9.a}$$

Diluted IgG (mg/ml) = power (10, Log^{10} plasma IgG) Equation 2.9.b

IgG concentration (mg/ml) = Diluted IgG *10 Equation 2.9.c

Where Y is diameter of rings around the wells, X is the unknown concentration of plasma IgG (mg/ml) which is obtained from standard curve (figure 2.2), 10 is dilution factor.

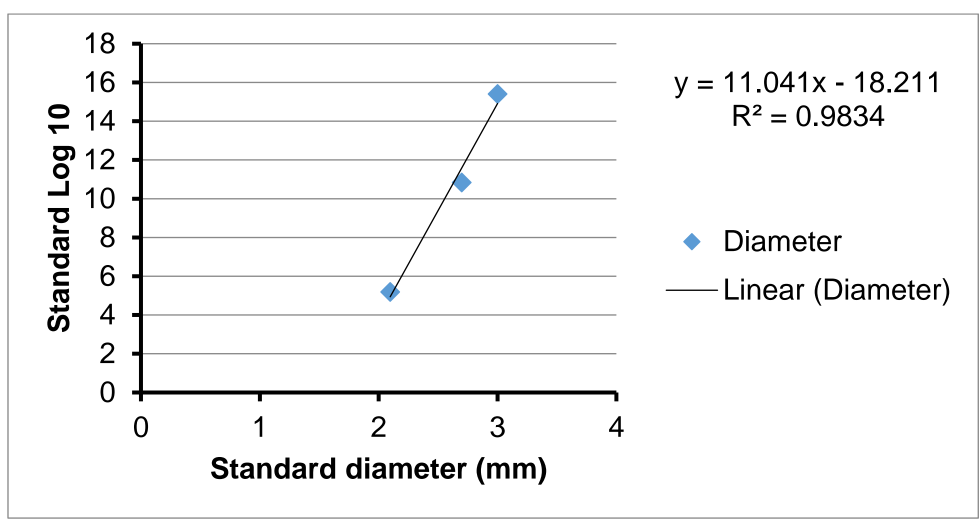


Figure 2. 2. Standard calibration curve of rabbit-anti-sheep IgG for lamb's plasma IgG determination

Chapter 3

Effect of nutritional restriction during mid pregnancy on the response of ewes to vitamin E supplementation during late pregnancy and early lactation

3.1. Introduction

Food is the main source of nutrients, and nutrients in excess can be stored in body tissues, such as intramuscular fat and adipose tissue, body tissues are often mobilised when demands are high (Kenyon *et al.,* 2014). During nutritional restriction of ewes, and when there is a high demand, nutrients are mobilised from body reserve to meet these demands (Kenyon *et al.,* 2014). Sheep body condition score (C) is a practical technique used to assess nutritional status (Russel, 1984; Kenyon *et al*., 2014). The C of sheep is reduced when nutrients are mobilised from body reserves (Russel, 1984; Kenyon *et al*., 2014).

Skeletal muscle, liver and adipose tissue have the ability to accumulate around 90% of the total quantity of vitamin E in the body (Machlin and Gabriel, 1982; Bjorneboe *et al.,* 1986; Bjørneboe *et al*., 1990). As a fat-soluble vitamin, the majority of vitamin E is stored in adipose tissue (Bjørneboe *et al*., 1990; Bramley *et al.,* 2000). In addition, vitamin E is assimilated into adipose tissue more avidly but is depleted at a similar rate to muscle (Hidiroglou *et al.,* 2003). The vitamin E concentration in adipose tissue is highly variable and depends upon the previous diet (Ochoa *et al.,* 1992). For example, Ochoa *et al.* (1992) and Njeru *et al.* (1994) reported that α-tocopherol concentration in sheep tissues was increased with increasing α-tocopherol acetate concentration in the diet.

Inconsistent results have been published as to whether tocopherol is mobilised from adipose tissue during nutritional restriction (Machlin *et al.,* 1979; Schaefer *et al.,* 1983; Fry *et al.,* 1993). The mobilisation of α-tocopherol was very slow from adipose tissue when vitamin E was deficient (Bjørneboe *et al.,* 1990). Alpha-tocopherol mobilisation from adipose tissue has been documented in sheep (Fry *et al.,* 1993), while, limited mobilisation has been recorded in humans (Schaefer *et al.,* 1983; Janneke Brouwer *et al.,*1998) and guinea pigs (Machlin *et al.,*1979).

The response of pregnant and lactating ewes to supra-nutritional levels of vitamin E supplementation may be dependent on body tissue reserves (Fry *et al.,* 1993). Ewes with low body condition score may have less adipose tissue (Kenyon *et al.,* 2014), and lower vitamin E reserves than those that have not experienced nutritional restriction. This may influence their response to supra-nutritional levels of vitamin E supplementation during late pregnancy. Availability of vitamin E from body reserves may be an important factor influencing responses to vitamin E supplementation. In both the studies of Merrell (1998)

and Capper *et al.* (2005) where responses to supra-nutrition supplementation were observed, plasma vitamin E levels declined in late pregnancy. Whereas, in other studies (Rooke *et al.,* 2009), where no responses were observed, plasma vitamin E levels remained unchanged. It this case vitamin E reserves in adipose tissue and their rate of mobilisation may have been sufficient to support lamb responses. Therefore, the objective of the current trial was to determine the effect of nutritional restriction in mid-pregnancy on the response of ewes to the supra-nutritional levels of vitamin E supplementation in the late pregnancy and early lactation.

Hypothesis

The response of pregnant and lactating ewes to supra-nutritional levels of vitamin E supplementation in late pregnancy and early lactation may be dependent on body tissue reserves.

3.2. Materials and methods

3.2.1. Experimental design

The experiment was conducted at Harper Adams University between December 2015 and April 2016 in the Animal Production, Welfare and Veterinary Sciences APWVS project buildings. Local ethical approval was obtained from the Animal Welfare and Ethical Review Board (AWERB). Initially, at day 70 (week -10) of pregnancy, 48 twin-pregnant ewes (Suffolk x Mule) from the Harper Adams University flock with a live weight (LW) and condition score (C) of 81.7 kg ±SD=5.65 and 3.15 ±SD=0.29 respectively, were allocated by parity, live-weight and condition C to one of two groups. The two groups were then fed to either maintain or lose 0.5 units of C between days 70 and days 103. This was achieved by offering either wheat straw *ad-libitum* plus concentrate (0.45-0.62 kg DM/d) to maintain the condition score (HC) as the unrestricted group or wheat straw *ad-libitum* to lose approximately 5 kg LW (0.15 kg/day) as the restriction group over a 33 day period, which equated to a condition score loss of 0.5 unit (LC) (MLC, 1988). From day 105 of gestation (week -6) to week +4 *post-lambing* all ewes were housed individually in randomized blocks on sawdust and fed straw *ad-libitum*, together with one of two concentrates formulated to supply either 50 or 500 mg/kg DM vitamin E, to provide four experimental treatments in a 2 x 2 factorial design.

No restriction (HC): Low vitamin E (LV) (HCLV)

No restriction (HC): High vitamin E (HV) (HCHV)

Restriction (LC): Low vitamin E (LV) (LCLV)

Restriction (LC): High vitamin E (HV) (LCHV)

From week +4 ewes, were group housed within each treatment group and maintained on the experimental treatments until weaning at week +8.

3.2.2. Diet formulation and treatments

All ewes were offered chopped wheat straw and water *ad-libitum*. Both concentrates were manufactured by HJ Lea Oaks Ltd, Nantwich, Cheshire. The two concentrates were formulated to provide the same raw material and chemical composition, but different levels of vitamin E (either 50 or 500 mg/kg) (Table 3.1). Concentrates were fed to meet the metabolisable energy (ME) and metabolisable protein (MP) requirement of twin bearing ewes during late pregnancy and producing 3.0 litres of milk during early lactation (from lambing to week +4) (AFRC, 1993) (Table 3.2). Concentrates were fed twice daily from

week -6 as two equal feeds until feeding levels exceeded 1.0 kg/day when an additional third feed was introduced. Between weeks +4 to +8, the ewes were group housed with their lambs and the quantity of concentrates was reduced to meet the requirements of 2.2 litres of milk production. Lambs were offered creep *ad libitum* from weeks +4 to +8. Feed samples were taken weekly for further analysis.

Table 3.1. Raw material and predicted chemical composition of the experimental concentrate diets (g/kg)

	LV	HV
Raw material composition		
Barley	282	278
Wheat	282	278
Sugar beet pulp	141	140
Soya hulls	77.0	77.0
Soya bean meal	90.0	90.0
Megalac©	40.0	40.0
Urea	13.0	13.0
Molasses	50.0	50.0
Vitamin E premix (5%)[1]	-	9.00
Minerals and vitamins[2]	25.0	25.0
Total	**1000**	**1000**
Predicted[3] chemical composition (g/kg DM)		
Dry matter (g/kg)	871	871
Crude protein	188	188
ERDP (0.05)	136	136
DUP (0.05)	28.0	28.0
Ether extract	49.0	49.0
Ash	76.0	76.0
NDF	205	205
Starch	348	348
Vitamin E (mg/kg DM)	57.0	574
ME (MJ/kg DM)	13.4	13.4
FME (MJ/kg DM)	11.4	11.4
ERDP/FME	12.0	12.0

[1] Vitamin E Top supplement (BESTMIX™) containing Vit. E 50,000.00 IU/kg, calcium 35.57 %.
[2] Mineral/ vitamins supplement, Mineral premix (Rumenco LTD., Burton upon Trent, UK) supplied per kg of diet: Vit. A 7500.00 IU/kg, Vit. D3 1500.00 IU/kg Vit. B12 600.00 mcg/kg, calcium 1.45%, magnesium 0.50%, sodium 0.27%, selenium 1.0 mg/kg, copper 8.45mg/kg, iodine 2.94 mg/kg, iron 694.48 mg/kg, manganese 109.27 mg/kg, salt 0.84%, and zinc 128.54 mg/kg.
[3] Diet chemical composition predicted according to AFRC (1993).

Table 3. 2. Concentrate feeding levels (kg/day) for twin bearing ewes offered straw ad-libitum during late pregnancy and early lactation.

Ewe Wt. (kg)	Milk yield (L)	Weeks							
		-6 to -5	**-5 to -4**	**4 to -3**	**-3 to -2**	**-2 to -1**	**-1 to 0**	**0 to +4**	**+4 to +8**
65	3.00	0.70	0.75	0.80	0.90	1.00	1.15	1.90	1.35
70	3.00	0.75	0.80	0.90	1.00	1.10	1.20	1.95	1.40
75	3.00	0.80	0.85	0.95	1.05	1.15	1.25	2.00	1.45
80	3.00	0.85	0.95	1.00	1.15	1.25	1.35	2.05	1.50
85	3.00	0.90	1.00	1.05	1.20	1.30	1.40	2.10	1.55
90	3.00	0.95	1.05	1.15	1.25	1.35	1.45	2.15	1.60
95	3.00	1.00	1.10	1.20	1.30	1.40	1.50	2.20	1.65
Mean		0.85	0.95	1.00	1.15	1.25	1.35	2.05	1.50

Based on AFRC (1993).

3.2.3. Experimental Routine

All ewes and lambs were weighed, and condition scored as described in Chapter 2 (sections 2.4 and 2.5), and ultra-sound scanned for back-fat and eye-muscle depth in weeks -10, -6, -3, -1, +2, +4, +6 and +8 as described in Chapter 2 (section 2.6). Both colostrum and milk yield were estimated from all ewes at 16 hours and 28 days *post-partum* using the method adapted from Doney *et al.* (1979) as described in chapter 2 (section 2.5). Concentrates and straw samples were taken weekly and placed in a freezer at -20ºC, prior to further analysis.

Blood samples were obtained by jugular venepuncture at 11:00 a.m. as described in Chapter two (section 2.2) from six representative ewes on each treatment at day 70 of pregnancy before the nutritional restriction, and in weeks -6, -3, -1, +2, +4 and +8. Blood samples were taken from all lambs at 12 hours *post-lambing* and week +2. The blood samples were collected into two types of tubes (potassium oxalate) for glucose analysis and (sodium heparin) for vitamin E, total protein, albumin, beta-hydroxybutyrate (BHB), non-esterified fatty acid (NEFA) and urea. The plasma was removed and stored in 1.0 ml eppendorf tubes at -20 ºC before further analysis.

3.2.4. Chemical analysis

The weekly feed samples (concentrates and straw) were bulked together *pre-partum* and *post-partum,* and analysed in duplicate according to method of A.O.A.C (2016) as described in Chapter 2 for dry matter (DM, AOAC, 2016; 930.15) section (2.1.1), crude protein (section 2.1.3) CP (CP, AOAC, 2016; 968.06) (6.25x N) by the Dumas method using a Leco automatic analyser (FP-528 N; Leco Corp., St. Joseph, MI, USA) with EDTA as a standard, ash (ash, AOAC, 2016; 942.05). Ether extract (EE) was determined according to MAFF (1986) using a Soxtec apparatus (FOSS, Warrington, UK (Section 2.1.4). Neutral detergent fibre (NDF) was determined according to the method of Van Soest *et al.* (1991) (section 2.1.5).

Colostrum samples were analysed for total solids, protein, fat, ash and lactose. All of the samples were first defrosted and mixed well and then analysed as described in chapter 2 (section 2.8).

Milk samples were analysed for total solids, protein, fat, and lactose using an infra-red milk analyser (Foss MilkoScan) as described in chapter 2 (Section 2.6). High performance liquid chromatography (HPLC) was used to determine the vitamin E concentration as described in chapter 2 for plasma and colostrum (Section 2.9.1) and feedstuff (Section 2.9.2). Blood plasma was analysed for glucose (Randox Laboratories kit, GL1611), beta-hydroxybutyrate (BHB) (Randox Laboratories kit, RB1008), non-esterified fatty acid (NEFA) (Randox

Laboratories kit, FA115), urea (Randox Laboratories kit, UR 221), albumin (Randox Laboratories kit, AB 362), and total protein (Randox Laboratories kit, Tp 245), by using Cobas-Mira (Mira plus ABX Diagnostics) blood analyser. Lambs plasma IgG content was measured at week +2 based on the method of (Mancini *et al.,* 1965) described in chapter two (section 2.10).

3.2.5. Statistical analysis

The experiment was analysed by ANOVA as a 2 X 2 factorial design using GenStat 17th edition (VSN Int. Ltd., Hempstead. UK), with the main effects being nutritional restriction and vitamin E level. Repeated measure analysis of variance for ewe plasma vitamin E and blood plasma metabolites components and their interactions were carried out using GenStat 17th edition (VSN Int. Ltd., Hempstead. UK). Week -10 has set as a covariate for blood plasma metabolites. Differences between means were determined using the protected least significant difference (LSD) (Snedecor and Cochran, 1989).

3.3. Results

3.3.1. Animal health

In general, all of the ewes and their lambs were in good health except five ewes that were removed with their lambs from the trial. Two ewes were excluded from treatment (HCHV), one of them aborted with two dead lambs approximately one week before the expected lambing time, and the other had mastitis. In addition, one ewe from treatment (LCHV) lambed with two lambs, but one of them was dead with a low weight (0.3 kg). Two other ewes were excluded from the trial from treatments (HCLV) and (LCLV), one of them had mastitis, and the other was removed from the experiment because one of her lambs was dead.

3.3.2. Diet composition

The chemical composition of the experimental diets is shown in Table 3.3. The results indicate that the straw had a low content of crude protein (CP) (52.9 g/kg DM) and high NDF (843 g/kg DM). The composition of the two concentrates was similar to that expected from the formulation diet (Table 3.1). However, in both concentrates, the fat content was slightly lower, and both the ash and NDF were slightly higher. The vitamin E content of the concentrates was similar to that predicted.

Table 3. 3. The chemical composition (g/kg DM) of experimental feeds.

Chemical composition	Straw	LV	HV
Dry Matter (g/kg)	887	883	880
Fat	10.3	31.3	31.3
Ash	38.0	103	101
NDF	834	226	235
Protein	52.9	202	202
GE (MJ/kg DM)	17.8	17.8	17.8
Vitamin E (mg/kg DM)	19.9	76.9	523
Selenium (mg/kg)	0.02	0.69	1.28

LV= Concentrate+vitamin E (50 mg/kg dry matter); HV= Concentrate+vitamin E (500 mg/kg dry matter)

3.3.3. Ewe and lamb performance

3.3.3.1. Ewes live weight and weight gain (LW)

The initial weight of the ewes was similar at week -10 of pregnancy without any difference (P>0.05) between the treatment groups (Table 3.4). The ewes that were nutritionally restricted from week-10 to week -6 lost more weight compared with the others (-9.22 vs -1.05 kg; P<0.001). At week -6 *pre-partum*, ewes that were nutritionally restricted had a lower live weight than non-restricted (73.0 vs 80.3 kg; P<0.001). There was no C×V interaction (P>0.05) on ewe live weight. As expected, all ewes gained live weight *pre-partum*

with the mean weight gain being 16.0 kg (Figure 3.1). However, ewes offered LCHV tended to have the highest weight gain (18.4 kg, P= 0.094). Likewise, *post-partum*, all the ewes lost live weight with the mean weight loss being -8.08 kg and ewes offered LCHV tended to have the lowest live weight loss (-0.03 kg, P=0.055) from 0 to 4 weeks.

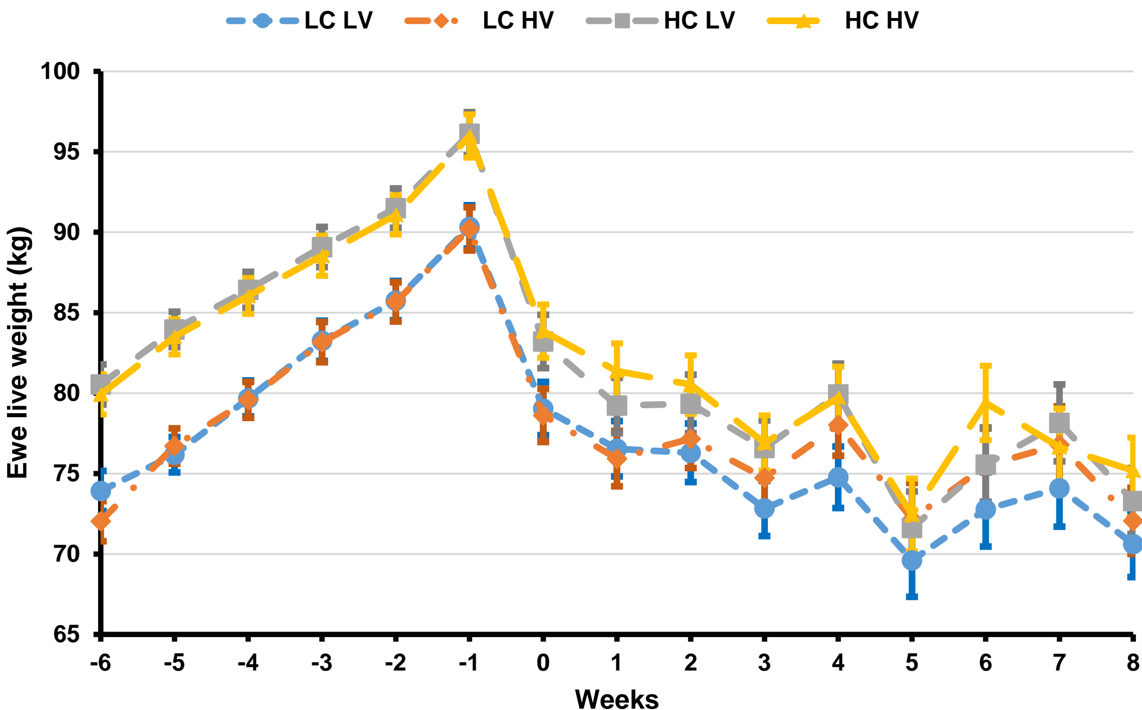


Figure 3. 1. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on the ewe live weight (kg) and live weight change (kg) during the experiment. Error bars indicate SED.

Table 3. 4. Effect of nutritional restriction and vitamin E supplementation on ewe live weight (kg) and live weight changes (%)

Weeks	LC		HC		SED	*P-value*		
	LV	HV	LV	HV		C	V	CxV
LW (kg)								
-10	82.0	81.4	81.5	81.8	0.811	0.902	0.757	0.443
-6	73.9	72.0	80.5	79.9	1.248	<0.001	0.166	0.484
-1	90.3	90.2	96.1	96.0	1.344	<0.001	0.888	0.992
0	79.0	78.6	83.2	83.9	1.657	<0.001	0.923	0.654
4	74.8	78.0	79.9	79.7	1.913	0.017	0.269	0.214
8	70.6	72.1	73.3	75.2	2.055	0.056	0.262	0.878
LW change (kg)								
-10 to -6	-8.11 (-10%)	-10.3 (-12%)	-0.68 (-1%)	-1.41 (-2%)	1.189	<0.001	0.093	0.385
-6 to -1	15.8 (22%)	18.4 (25%)	15.7 (19%)	15.1 (20%)	1.262	0.065	0.271	0.094
0 to 4	-3.44^{b} (-5%)	-0.03^{a} (-1%)	-3.36^{b} (-4%)	-4.24^{b} (-5%)	1.537	0.064	0.244	0.055
0 to 8	-7.59 (-11%)	-5.93 (-8%)	-9.99 (-12%)	-8.79 (-10%)	1.618	0.029	0.220	0.824

LC: low body condition score (2.5 units); HC: high body condition score (3 units); LV: Concentrate supplemented with vitamin E (50 mg/kg dry matter); HV: Concentrate supplemented with vitamin E (500 mg/kg dry matter).
^{a,b} Means with the same superscript are not significantly different (*P*<0.05).

3.3.3.2. Ewe body condition score (C)

The initial condition score (C) of the ewes was similar at week -10 of pregnancy with no difference (P>0.05) between the treatment groups. However, the ewes that were nutritionally restricted from the week -10 to week -6 lost more C compared with the others (-0.74 vs -0.28 unit; P<0.001) (Table 3.5). At week -6 pre-lambing, the ewes subjected to nutritional restriction had a lower C than the other group (2.57 vs 2.98 unit; P<0.001). There was no C×V interaction (P>0.05) on the ewes C and C change. *Pre-partum* (week -6 to -1), all of the ewes lost C with a mean C loss of -0.11 (Figure 3.2). However, the C loss of ewes that were nutritionally restricted was lower than that of the other group (0.06 vs 0.20 unit; P<0.025). *Post-partum* ewes on all treatments lost C with a mean C loss of -0.36. There was no interaction between treatment groups (P>0.05). However, there was an effect (P<0.05) of vitamin E supplementation on ewe C in the period between week+1 to +4, with ewes supplemented with high vitamin E losing less C than those offered low vitamin E.

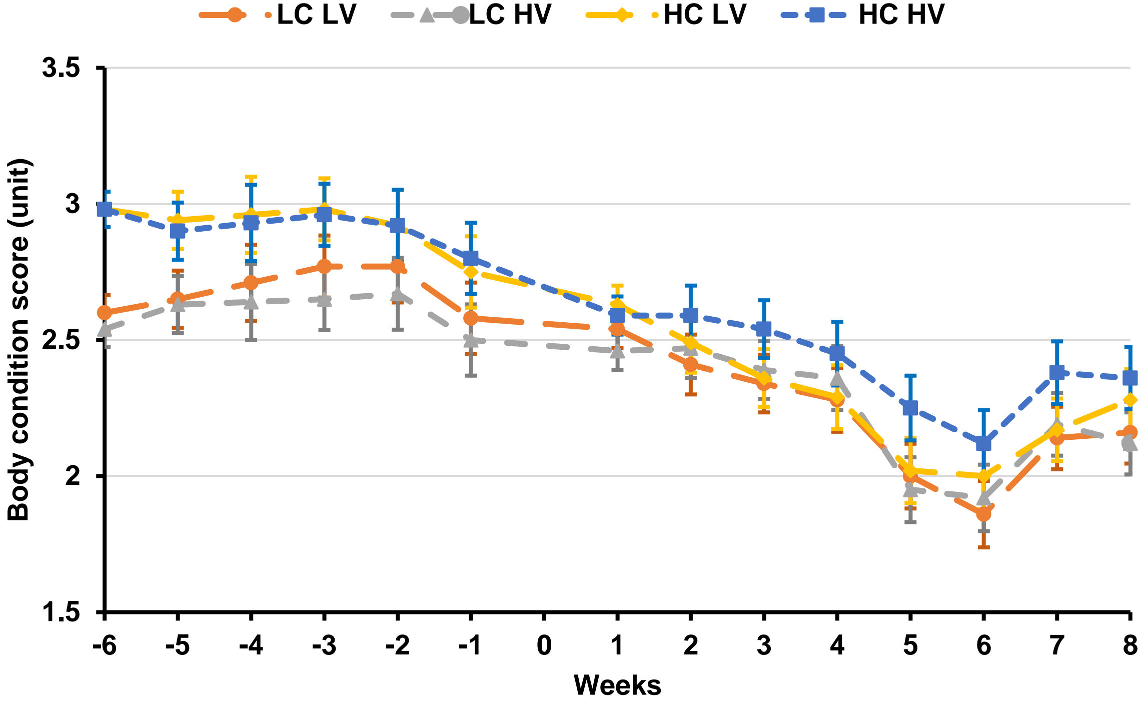


Figure 3. 2. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on the ewe condition score (unit) and condition score changes (unit) during the experiment.
Error bars indicate SED.

Table 3. 5. Effect of nutritional restriction and vitamin E supplementation on ewe condition score (unit) and condition score changes (%)

Weeks	LC		HC		SED	*P-value*		
	LV	HV	LV	HV		C	V	CxV
-10	3.19	3.00	3.19	3.21	0.122	0.238	0.343	0.238
-6	2.60	2.54	2.98	2.98	0.065	<0.001	0.633	0.633
-1	2.58	2.50	2.75	2.80	0.131	0.017	0.860	0.474
1	2.54	2.46	2.63	2.59	0.070	0.134	0.414	0.725
4	2.28	2.36	2.29	2.45	0.117	0.576	0.143	0.641
8	2.16	2.12	2.28	2.36	0.114	0.032	0.836	0.456
C changes (unit)								
-10 to -6	-0.74 (-19%)	-0.73 (-15%)	-0.26 (-7%)	-0.30 (-7%)	0.100	<0.001	0.848	0.721
-6 to -1	-0.01 (-0.8%)	-0.04 (-2%)	-0.21 (-8%)	-0.18 (-6%)	0.101	0.025	0.980	0.654
1 to 4	-0.20 (-10%)	-0.11 (-4%)	-0.33 (-13%)	-0.08 (-5%)	0.093	0.449	0.015	0.240
1 to 8	-0.56 (-15%)	-0.38 (-14%)	-0.32 (-13%)	-0.19 (-9%)	0.219	0.173	0.319	0.878

LC: low body condition score (2.5 units); HC: high body condition score (3 units); LV: Concentrate supplemented with vitamin E (50 mg/kg dry matter); HV: Concentrate supplemented with vitamin E (500 mg/kg dry matter).

3.3.3.3. Ewe back fat thickness

The initial back fat of the ewes was similar (*P*>0.05) at week -10 of pregnancy with no difference (*P*>0.05) between the treatment groups (Table 3.6). Ewes that were nutritionally restricted tended to lose more back fat from week -10 to week -6 than those that were unrestricted (-0.73 *vs* 0.17 mm; P<0.068). At week -6 *pre-partum*, the ewes subjected to nutritional restriction had a lower back fat cover than the other group (4.62 vs 5.59 mm; *P*=0.017). There was no C×V interaction (P>0.05) on the ewes back fat and back fat changes throughout the experiment. There was however an effect (*P*<0.05) of nutritional restriction on ewes back fat. Ewes that were nutritionally restricted lost less back fat *pre-partum* (-0.56 *vs* -1.17 mm; P<0.006) (Figure 3.3). There was also an effect of vitamin E supplementation on ewe back fat cover. Ewes that were supplemented with high vitamin E lost less back fat (-1.16 *vs* -0.57 mm; P=0.007) *pre-partum*.

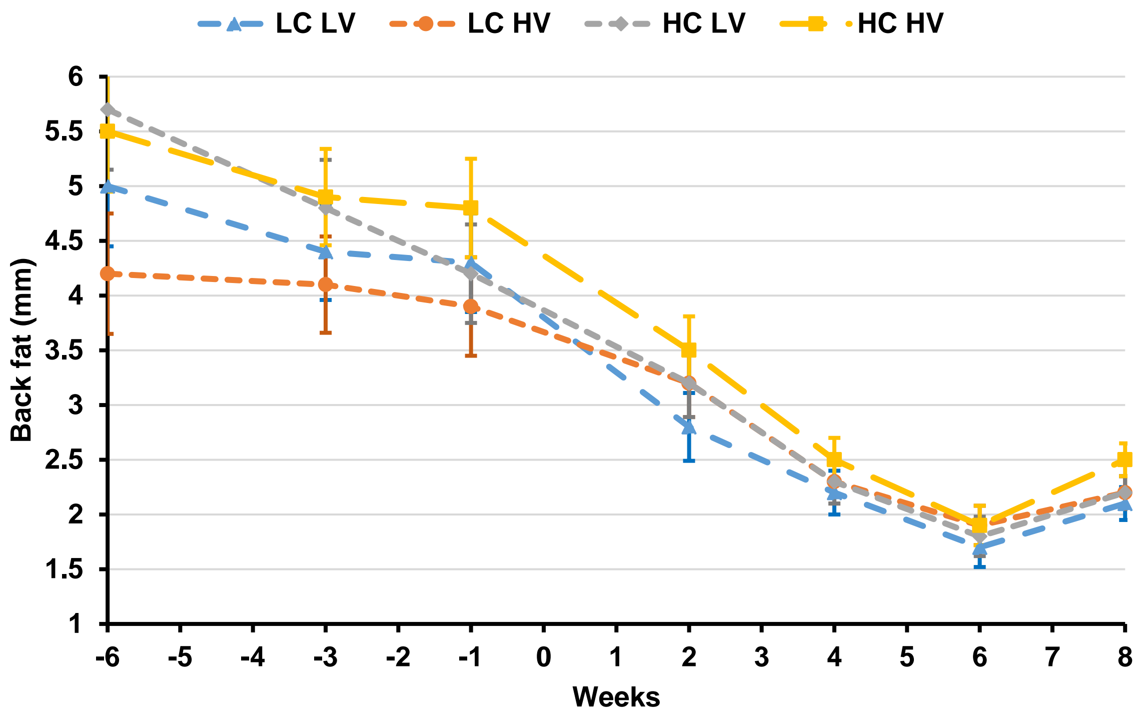


Figure 3. 3. Effect of nutritional restriction and vitamin E supplementation on ewes back fat (mm)
Error bars indicate SED.

Table 3. 6. Effect of nutritional restriction and vitamin E supplementation on ewes back fat (BF) and back fat changes (mm)

Weeks	LC		HC		SED	*P-value*		
	LV	HV	LV	HV		C	V	CxV
BF								
-10	5.75	5.00	5.71	5.85	0.565	0.316	0.448	0.270
-6	5.00	4.25	5.73	5.46	0.550	0.017	0.199	0.529
-1	4.30	3.92	4.24	4.82	0.453	0.185	0.737	0.155
2	2.85	3.19	3.22	3.49	0.307	0.129	0.165	0.866
4	2.24	2.30	2.31	2.53	0.195	0.289	0.299	0.568
8	2.10	2.17	2.21	2.50	0.159	0.100	0.168	0.458
BF changes								
-10 to -6	-0.63	-0.82	0.01	-0.34	0.417	0.068	0.366	0.791
-6 to -1	-0.77	-0.35	-1.55	-0.78	0.290	0.006	0.007	0.389
2 to 4	-0.61	-0.89	-0.92	-0.96	0.204	0.198	0.268	0.425
2 to 8	-0.73	-1.15	-1.17	-1.15	0.292	0.309	0.340	0.297

LC: low body condition score (2.5 units); HC: high body condition score (3 units); LV: Concentrate supplemented with vitamin E (50 mg/kg dry matter); HV: Concentrate supplemented with vitamin E (500 mg/kg dry matter).

3.3.3.4. Ewe muscle depth

The initial muscle depth of the ewes was similar (*P*>0.05) at week -10 of pregnancy with no significant difference between the treatment groups (Table 3.7). Ewes that were nutritionally restricted lost more muscle depth from week -10 to week -6 (-1.78 *vs* 0.82 mm; P<0.001). At week -6 *pre-partum*, the ewes subjected to nutritional restriction had a lower muscle depth than the other group (25.1 vs 27.3 mm; P<0.001). There was no C×V interaction (P>0.05) on ewe muscle depth and muscle depth changes.

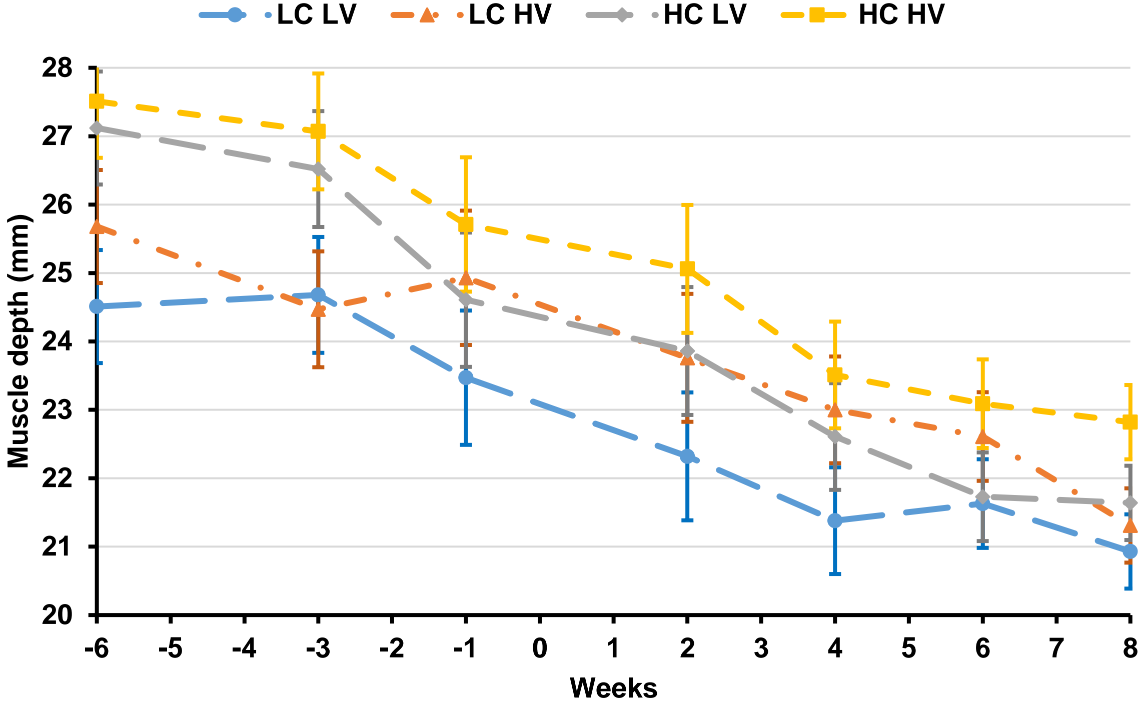


Figure 3. 4. Effect of nutritional restriction and vitamin E supplementation on ewe muscle depth
Error bars indicate SED.

Table 3. 7. Effect of nutritional restriction and vitamin E supplementation on ewes muscle depth (MD) and muscle depth changes (mm).

Weeks	LC		HC		SED	*P*-value		
	LV	HV	LV	HV		C	V	CxV
MD								
-10	27.1	26.2	25.8	26.7	0.895	0.507	0.938	0.172
-6	24.5	25.7	27.1	27.5	0.826	<0.001	0.191	0.512
-1	23.5	24.9	24.6	25.7	0.982	0.059	0.209	0.758
2	22.3	23.8	23.9	25.1	0.935	0.041	0.055	0.854
4	21.4	23.0	22.6	23.5	0.780	0.126	0.030	0.511
8	20.9	21.3	21.6	22.8	0.543	0.008	0.052	0.309
MD changes								
-10 to -6	-2.40	-1.15	1.33	0.31	0.883	<0.001	0.848	0.078
-6 to -1	-1.30	-1.07	-2.78	-1.58	0.914	0.136	0.279	0.458
2 to 4	-0.94	-0.76	-1.25	-1.55	0.975	0.432	0.928	0.725
2 to 8	-1.39	-2.45	-2.22	-2.24	0.949	0.645	0.427	0.444

LC: low body condition score (2.5 units); HC: high body condition score (3 units); LV: Concentrate supplemented with vitamin E (50 mg/kg dry matter); HV: Concentrate supplemented with vitamin E (500 mg/kg dry matter).

There was no effect (*P*>0.05) of nutritional restriction on muscle depth change *pre-partum* and *post-partum* (Figure 3.4). There was no effect (*P*>0.05) of vitamin E supplementation on ewe muscle depth *pre-partum* while *post-partum* ewe supplemented with high vitamin E had a higher muscle depth in week +4 (23.3 *vs* 22.0 mm; P=0.030). In addition, *post-partum*

ewe supplemented with high vitamin E tended to have a higher muscle depth in week +2 (24.4 *vs* 23.1 mm; P=0.055) and week +8 (22.07 *vs* 21.29 mm; P=0.052).

3.3.3.5. Colostrum yield and composition

There was no C×V interaction (P>0.05) on colostrum secretion rate, colostrum composition or colostrum component yields (Table 3.8). There was no effect (P>0.05) of nutritional restriction on colostrum secretion rate, colostrum composition and colostrum component yield. There was no effect (P>0.05) of vitamin E on colostrum secretion rate. There was an effect (P<0.05) of vitamin E level on colostrum composition and colostrum component yield. -Ewes supplemented with HV had a higher colostrum lactose (58.3 vs 39.5 g/kg), and higher colostrum vitamin E content (16.7 vs 5.94 µg/g; P<0.001) and vitamin E yield (2.20 vs 0.80 mg/h; P<0.001).

3.3.3.6. Milk yield and composition

There was no C×V interaction (P>0.05) on milk secretion rate, milk composition and milk component yield (Table 3.9). There was no effect (P>0.05) of nutritional restriction on milk secretion rate, milk composition and milk component yield. Similarly, there was no effect (P>0.05) of vitamin E supplementation on milk secretion rate, milk composition and milk component yield. However, ewe receiving HV tended to have a lower milk fat (60.5 vs 70.9 g/kg; P=0.098), and higher milk lactose yield (8.28 vs 7.25 g/h; P=0.072).

3.3.3.7. Litter weight performance

There was no C×V interaction (P>0.05) on litter birth weight and litter weight gain (Table 3.10). There was no effect (P>0.05) of nutritional restriction in mid pregnancy on litter birth weight. Lambs from nutritionally restricted ewes had a lower weight in weeks +1 (14.3 vs 15.5 kg; P<0.023) and week +7 (39.5 vs 42.3 kg; P<0.015). They also tended to have a lower weight in weeks +2 (19.3 vs 20.5 kg; P=0.058) and +3 (23.6 vs 24.9 kg; P=0.071). Nutritional restriction had no effect (P>0.05) on live weight gain during the period between week 0 to week 4 and week 0 to week 8. There was no effect (P>0.05) of vitamin E supplementation on litter birth weight and litter weight gain throughout the experiment.

Table 3. 8. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on colostrum yield, composition and components yield.

Colostrum	LC		HC		SED	*P-value*		
	LV	HV	LV	HV		C	V	CxV
Secretion rate (ml/h)	131	136	124	124	20.16	0.507	0.834	0.858
Composition (g/kg)								
Total Solid	228	278	265	303	31.87	0.185	0.071	0.797
Fat	113	117	123	138	16.48	0.197	0.447	0.668
Total Protein	85.9	103	92.4	108	15.63	0.603	0.168	0.960
Lactose	29.4	58.6	49.6	57.9	10.72	0.219	0.026	0.190
Vitamin E (μg/g)	5.41	16.6	6.46	16.9	1.624	0.569	<0.001	0.745
Component yield (g/h)								
Total Solid	30.6	40.0	32.7	37.7	8.000	0.986	0.226	0.695
Fat	15.0	16.6	15.5	17.4	3.930	0.820	0.546	0.947
Total Protein	11.4	15.0	10.9	13.1	3.030	0.584	0.206	0.752
Lactose	4.13	8.49	6.35	7.19	2.085	0.763	0.099	0.252
Vitamin E (mg/h)	0.80	2.40	0.80	2.00	0.370	0.566	<0.001	0.448

LC: low body condition score (2.5 units); HC: high body condition score (3 units); LV: Concentrate supplemented with vitamin E (50 mg/kg dry matter); HV: Concentrate supplemented with vitamin E (500 mg/kg dry matter).

Table 3. 9. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on milk yield, composition and components yield.

	LC		HC		SED	*P-value*		
	LV	HV	LV	HV		C	V	CxV
Secretion rate (ml/h)	129	151	144	155	14.20	0.356	0.111	0.584
Milk Composition (g/kg)								
Total Solid	221	198	219	214	13.26	0.471	0.144	0.365
Fat	71.0	54.0	70.8	67.1	8.580	0.296	0.098	0.283
Total Protein	45.6	45.4	44.8	44.4	1.585	0.455	0.783	0.951
Lactose	53.4	55.0	52.9	53.2	1.039	0.119	0.219	0.400
Component yield (g/h)								
Total Solid	28.3	30.1	31.1	32.2	3.000	0.253	0.491	0.868
Fat	9.01	8.26	9.94	9.85	1.271	0.173	0.641	0.716
Total Protein	5.46	6.14	5.88	5.73	1.031	0.993	0.715	0.574
Lactose	6.89	8.31	7.61	8.24	0.775	0.561	0.072	0.474

LC: low body condition score (2.5 units); HC: high body condition score (3 units); LV: Concentrate supplemented with vitamin E (50 mg/kg dry matter); HV: Concentrate supplemented with vitamin E (500 mg/kg dry matter).

Table 3.10. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on the litter weight gain during post-lambing.

Weeks	LC		HC		SED	*P-value*		
	LV	HV	LV	HV		C	V	CxV
0	9.78	9.16	10.4	9.90	0.701	0.166	0.255	0.925
1	14.1	14.5	15.8	15.2	0.692	0.023	0.876	0.346
2	19.3	19.3	20.8	20.2	0.875	0.058	0.658	0.647
3	24.0	23.3	25.0	24.8	0.999	0.071	0.529	0.800
4	27.8	28.0	28.7	28.5	1.124	0.393	0.956	0.769
5	32.6	32.6	33.2	32.6	1.565	0.803	0.800	0.772
6	35.7	37.3	38.0	38.0	1.647	0.212	0.524	0.512
7	37.4	41.6	43.1	42.5	2.110	0.035	0.229	0.118
8	44.1	45.4	49.2	45.0	2.204	0.138	0.351	0.085
Live weight change (kg)								
0 to 4	18.1	18.4	17.7	18.6	0.985	0.906	0.432	0.703
0 to 8	34.3	36.1	38.2	35.1	2.134	0.349	0.636	0.416
4 to 8	16.2	17.7	20.5	16.5	1.554	0.177	0.255	0.020
Daily live weight gain (kg)								
0 to 4	0.65	0.66	0.63	0.66	0.035	0.906	0.432	0.703
0 to 8	0.61	0.64	0.68	0.63	0.038	0.349	0.636	0.116
4 to 8	0.58^{a}	0.63^{ab}	0.73^{b}	0.59^{a}	0.055	0.177	0.255	0.020

LC: low body condition score (2.5 units); HC: high body condition score (3 units); LV: Concentrate supplemented with vitamin E (50 mg/kg dry matter); HV: Concentrate supplemented with vitamin E (500 mg/kg dry matter).

3.3.4. Ewe plasma Vitamin E

There was an effect (P<0.05) of time on ewe plasma vitamin E concentration (Table 3.11). Ewe mean plasma vitamin E declined from 5.66 µg/ml at week -10 to 1.04 µg/ml at week -6 then increased to 1.44 µg/ml at week -1 and declined to 1.22 µg/ml at week 2. There was an effect (P<0.05) of nutritional restriction in mid-pregnancy on ewe plasma vitamin E concentration. At week -6 ewes that were nutritionally restricted had a lower plasma vitamin E concentration (0.93 vs 1.16 µg/ml; P=0.013). There was an effect (P<0.05) of vitamin E supplementation on ewe plasma vitamin E concentration. High vitamin E supplementation increased (P<0.05) ewe plasma vitamin E at week -1 (0.85 vs 2.06 µg/ml; P<.001) and week+2 (0.71 vs 1.72 µg/ml; P<.001). There was no C×V interaction (P>0.05) on the level of vitamin E in ewe plasma. There was no time × C interaction (P>0.05) on the level of vitamin E in ewe plasma. There was time × V interaction (P<0.05) effect on the level of vitamin E in ewe plasma. Ewe that were supplemented with high vitamin E had higher plasma vitamin E from 1.07 µg at week -6 to 2.03 µg at week -1 and then reduced to 1.72 µg at week 2. There was no time × C × V interaction (P>0.05) on the level of vitamin E in ewe plasma.

3.3.5. Lamb plasma vitamin E and immunoglobulin (IgG)

There was no C×V interaction (P>0.05) on lamb plasma vitamin E and IgG concentration (Table 3.12). However, lamb from ewes on treatment LCHV trended to have a higher plasma vitamin E concentration at hour 12 hours *post-partum* (1.16 µg/ml; P=0.064). There was no effect (P>0.05) of nutritional restriction on the lamb plasma vitamin E or IgG concentration. However, lamb from restricted ewe tended to have a higher plasma IgG concentration (6.87 vs 6.44 mg/ml; P=0.072). There was an effect of vitamin E supplementation on lamb plasma vitamin E concentration. Lamb from ewes offered HV had a higher (P<0.05) plasma vitamin E concentration at both 12 h and week +2 than those of ewes offered LV.

Table 3. 11. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on ewe plasma vitamin E (µg/ml).

Weeks	**LC**		**HC**		**SED**	***P-value***		
	LV	**HV**	**LV**	**HV**		**C**	**V**	**CxV**
-10	5.68	5.84	5.82	5.46	0.212	0.456	0.523	0.107
-6	0.94	0.91	1.09	1.23	0.118	0.013	0.507	0.336
-1	0.84	2.10	0.86	2.02	0.211	0.856	<0.001	0.724
+2	0.68	1.92	0.74	1.52	0.572	0.387	<0.001	0.239

LC: low body condition score (2.5 units); HC: high body condition score (3 units); LV: Concentrate supplemented with vitamin E (50 mg/kg dry matter); HV: Concentrate supplemented with vitamin E (500 mg/kg dry matter).
SED values: C= 0.083, V=0.083, C×V=0.117, Time= 0.101, Time×C=0.149, Time×V=0.149, Time×C×V=0.210.
P-values: C= 0.898, V=<0.001, C×V=0.225, Time= <0.001, Time×C=0.246, Time×V=<0.001, Time×C×V=0.376

Table 3. 12. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on lamb plasma vitamin E (µg/ml) *and IgG (mg/ml).*

	LC		HC		SED	*P-value*		
	LV	HV	LV	HV		C	V	CxV
Lambs Plasma								
Vitamin E (12 hours)	0.30	1.16	0.49	0.55	0.280	0.298	0.036	0.064
Vitamin E (wk+2)	1.04	1.76	0.99	1.91	0.306	0.809	0.002	0.655
Plasma IgG (wk+2)	6.45	7.28	6.19	6.69	1.399	0.072	0.411	0.115

LC: low body condition score (2.5 units); HC: high body condition score (3 units); LV: Concentrate supplemented with vitamin E (50 mg/kg dry matter); HV: Concentrate supplemented with vitamin E (500 mg/kg dry matter).

3.3.6. Blood plasma metabolites

3.3.6.1. Plasma glucose

There was an effect (P<0.05) of time on ewe plasma glucose concentration (Table 3.13). Plasma glucose concentration increased from 2.52 mmol/l at week -10 to 3.31 mmol/l at week +8. Nutritional restriction had no effect (*P*>0.05) on plasma glucose concentration. However, ewes that were nutritionally restricted trended to have higher plasma glucose concentration at weeks +2 (3.47 vs 3.32 mmol/l; P=0.065) and week +4 (3.52 vs 3.28 mmol/l; P=0.092). High vitamin E supplementation increased ewe plasma glucose concentration at week -3 (3.15 vs 2.87 mmol/l; P=0.045) and tended to increase plasma glucose concentration at week -1 (3.14 vs 2.95 mmol/l; P=0.061). There was a C× V interaction (P<0.05) on ewe plasma glucose concentration, ewes with fed treatment HCLV had the lowest plasma glucose concentration (3.12 mmol/l; P=0.003). There was no time × C or time × V interaction (P>0.05) on plasma glucose concentration. There was a trend time × C × V interaction on ewe plasma glucose concentration, ewes receiving treatment LCHV had the highest plasma glucose concentration.

Table 3. 13. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on ewe plasma glucose concentration (mmol/l)

Weeks	LC		HC		SED	*P-value*		
	LV	HV	LV	HV		C	V	CxV
-6	2.78	2.28	2.61	2.53	0.211	0.605	0.111	0.151
-3	2.86	3.17	2.88	3.12	0.161	0.649	0.045	0.752
-1	2.99	3.13	2.92	3.16	0.117	0.704	0.061	0.505
2	3.56^{b}	3.39^{ab}	3.12^{a}	3.53^{b}	0.110	0.065	0.238	0.003
4	3.51	3.53	3.29	3.26	0.198	0.092	0.968	0.865
6	3.43	3.47	3.23	3.51	0.210	0.537	0.341	0.404
8	3.42	3.37	3.22	3.29	0.173	0.257	0.919	0.626

LC: low body condition score (2.5 units); HC: high body condition score (3 units); LV: Concentrate supplemented with vitamin E (50 mg/kg dry matter); HV: Concentrate supplemented with vitamin E (500 mg/kg dry matter).
^{a,b} Means with the same superscript are not significantly different (P<0.05).
SED values: C= 0.066, V=0.078, C×V=0.099, Time= 0.071, Time×C=0.113, Time×V=0.115, Time×C×V=0.162.
P-values: C= 0.108, V=0.397, C×V=0.266, Time= <0.001, Time×C=<0.334, Time×V=0.351, Time×C×V=0.078

3.3.6.2. Plasma BHB

There was an effect (P<0.05) of time on ewe plasma BHB concentration (Table 3.14). Plasma BHB concentration increased from 0.33 mmol/l at week -6 to 0.56 mmol/l at week -1 and 0.69 mmol/l at week +4 and declined to 0.53 mmol/l at week +8. There was an effect (P<0.05) of nutritional restriction on ewe plasma BHB concentration with ewe that were nutritionally restricted having a higher plasma BHB concentration at week -6 (0.37 *vs* 0.30 mmol/l; *P*=0.008). Vitamin E supplementation had an effect (P<0.05) on plasma BHB concentration with ewes that were supplemented with high vitamin E having a lower plasma BHB concentration at week -3 (0.46 *vs* 0.53 mmol/l; *P*=0.043). There was a C × V interaction (P<0.05) on ewe plasma BHB, with ewes fed treatment LCHV having the highest plasma BHB concentration at week -1 (0.65 mmol/l; P=0.023). There was no time × C interaction P>0.05 effect on ewe plasma BHB. Similarly, there was no time × V interaction (P>0.05) on plasma BHB. There was no time × C × V interaction (P>0.05) on ewe plasma BHB concentration.

Table 3. 14. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on ewe plasma BHB (mmol/l).

Weeks	LC		HC		SED	*P-value*		
	LV	HV	LV	HV		C	V	CxV
-6	0.38	0.36	0.27	0.32	0.034	0.008	0.525	0.171
-3	0.51	0.44	0.54	0.47	0.045	0.378	0.043	0.898
-1	0.50^{a}	0.65^{b}	0.58^{ab}	0.52^{a}	0.060	0.577	0.351	0.023
2	0.76	0.80	0.88	0.76	0.119	0.645	0.645	0.350
4	0.72	0.73	0.65	0.69	0.115	0.500	0.719	0.874
6	0.72	0.71	0.67	0.60	0.094	0.236	0.612	0.635
8	0.53	0.58	0.51	0.48	0.068	0.210	0.797	0.445

LC: low body condition score (2.5 units); HC: high body condition score (3 units); LV: Concentrate supplemented with vitamin E (50 mg/kg dry matter); HV: Concentrate supplemented with vitamin E (500 mg/kg dry matter).
^{a,b} Means with the same superscript are not significantly different (P<0.05).
SED values: C= 0.025, V=0.025, C×V=0.036, Time= 0.037, Time×C=0.055, Time×V=0.055, Time×C×V=0.078.
P-values: C= 0.406, V=0.856, C×V=0.279, Time= <0.001, Time×C=<0.338, Time×V=0.699, Time×C×V=0.524

3.3.6.3. Plasma NEFA

There was an effect (P<0.05) of time on ewe plasma NEFA concentration (Table 3.15). *Pre-partum*, ewe plasma NEFA concentration reduced from 0.53 mmol/l at week -6 to 0.29 mmol/l at week -1. *Post-partum*, ewe plasma NEFA concentration increased to 0.67 mmol/l at week +2 and 0.77 mmol/l at week +4, then reduced to 0.28 mmol/l at week +8. There was an effect (P<0.05) of nutritional restriction on ewe plasma NEFA concentration with ewe that were nutritionally restricted having a higher plasma NEFA concentration at week -6 (0.77 *vs* 0.16 mmol/l; *P*=0.015). Vitamin E supplementation had effect (P<0.05) on plasma NEFA concentration with ewe that were supplemented with high vitamin E having a lower

plasma NEFA concentration at week 4 (0.50 *vs* 0.91 mmol/l; *P*=0.046). There was no C× V interaction (P>0.05) on ewe plasma NEFA concentration. However, ewe receiving treatment LCLV tended to have the highest plasma NEFA concentration at week 4 (1.10 mmol/l; P=0.069). There was no time × C interaction (P>0.05) on ewe plasma NEFA; however, a greater reduction in plasma NEFA concentration from the week -6 to -1 occurred in ewe that were nutritionally restricted, while from week 2 to 8 the greater reduction was in non-restricted ewes. There was no time × V or time × C × V interaction (P>0.05) on ewe plasma NEFA concentration.

Table 3. 15. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on ewe plasma NEFA (mmol/l).

Weeks	**LC**		**HC**		**SED**	***P-value***		
	LV	**HV**	**LV**	**HV**		**C**	**V**	**CxV**
-6	0.75	0.79	0.14	0.18	0.245	0.015	0.882	0.590
-3	0.35	0.41	0.36	0.52	0.102	0.495	0.153	0.495
-1	0.21	0.25	0.35	0.37	0.105	0.168	0.653	0.938
2	0.58	0.58	0.89	0.62	0.320	0.510	0.545	0.528
4	0.71	0.68	1.10	0.32	0.255	0.984	0.046	0.069
6	0.23	0.25	0.29	0.12	0.095	0.457	0.270	0.162
8	0.31	0.46	0.19	0.19	0.124	0.079	0.405	0.398

LC: low body condition score (2.5 units); HC: high body condition score (3 units); LV: Concentrate supplemented with vitamin E (50 mg/kg dry matter); HV: Concentrate supplemented with vitamin E (500 mg/kg dry matter).
SED values: C= 0.080, V=0.068, C×V=0.102, Time= 0.081, Time×C=0.127, Time×V=0.126, Time×C×V=0.179.
P-values: C= 0.391, V=0.355, C×V=0.171, Time= 0.003, Time×C= 0.054, Time×V=0.290, Time×C×V=0.203

3.3.6.4. Plasma total protein

There was an effect (P<0.05) of time on ewe plasma protein concentration (Table 3.16). *Pre-partum*, ewe plasma total protein concentration reduced from 61.69 g/l at week -6 to 54.13 g/l at week -1. *Post-partum*, ewe plasma total protein concentration increased to 59.26 g/l at week 2 and 62.04 g/l at week 4, then reduced to 51.21 g/l at week 8. There was an effect (P<0.05) of nutritional restriction in mid pregnancy on ewe plasma total protein concentration, at both weeks -6 and -1; ewe that were nutritionally restricted had a lower plasma total protein concentration (57.47 *vs* 66.15 g/l; *P*=0.031) and (51.45 *vs* 56.46 g/l; *P*=0.023) and tended to have a lower plasma total protein at week 8 (49.12 *vs* 53.50 g/l; *P*=0.076). There was no effect (*P*>0.05) of vitamin E supplementation on ewes plasma protein concentration.

There was C × V interaction (P<0.05) effect on ewe plasma protein. Nutritionally restricted ewes supplemented with low vitamin E had the lowest plasma total protein at week -3 (49.07 g/l; P=0.039). There was time × C interaction (P<0.05) on plasma protein concentration; ewes that were nutritionally restricted had a lower reduction in plasma total protein from

week -6 to -1, while plasma total protein reduction was higher in non-restricted ewes from week 2 to 8. There was no time × V or time × C × V interaction (P>0.05) on ewe plasma total protein concentration.

Table 3. 16. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on ewe plasma total protein concentration (g/l).

Weeks	LC		HC		SED	*P-value*		
	LV	HV	LV	HV		C	V	CxV
-6	55.8	59.2	66.3	66.0	4.598	0.031	0.576	0.579
-3	49.1^{b}	52.4^{ab}	55.7^{a}	51.0^{ab}	2.518	0.408	0.804	0.039
-1	51.4	51.6	57.3	55.6	2.404	0.023	0.663	0.575
2	57.8	59.0	63.1	57.8	2.468	0.544	0.245	0.080
4	63.2	59.9	61.7	62.9	3.502	0.668	0.617	0.362
6	54.3	53.2	50.6	53.4	2.222	0.615	0.691	0.236
8	52.2	46.0	52.9	54.1	3.438	0.076	0.232	0.145

LC: low body condition score (2.5 units); HC: high body condition score (3 units); LV: Concentrate supplemented with vitamin E (50 mg/kg dry matter); HV: Concentrate supplemented with vitamin E (500 mg/kg dry matter).
a,b Means with the same superscript are not significantly different (P<0.05).
SED values: C= 1.244, V=1.034, C×V=1.599, Time= 1.366, Time×C=2.097, Time×V=2.060, Time×C×V=2.948.
P-values: C= 0.037, V=0.542, C×V=0.621, Time= <0.001, Time×C= 0.035, Time×V=0.734, Time×C×V=0.179

3.3.6.5. Plasma albumin

There was an effect (P<0.05) of time on ewe plasma albumin concentration (Table 3.17). Plasma albumin concentration increased from 24.53 g/l at week 2 to 29.11 g/l at week +4 and then declined to 25.31 g/l at week +8. There was an effect (P<0.05) of nutritional restriction in mid pregnancy on ewe plasma albumin concentration; at week -6 ewes that were nutritionally restricted had a lower plasma albumin concentration (26.21 *vs* 28.74 g/l; *P*=0.003) and tended to have lower plasma albumin concentration at week -1 (26.95 *vs* 27.95 g/l; *P*=0.076). Vitamin E supplementation had no effect (*P*>0.05) on plasma albumin concentration. There was a trend towards C × V interaction on ewe plasma albumin concentration. At week 2 and 8 ewe fed treatment HCHV trended to have the highest plasma albumin concentration. There was a trend towards a time × C interaction on ewes plasma albumin concentration; ewes that were non-restricted in mid pregnancy trended to have a lower plasma albumin concentration from week -6 to -1 and increase plasma albumin concentration from week 2 to 8. There was no time × V interaction (P>0.05) on plasma albumin concentration. There was a time × C × V interaction (P<0.05) on ewe plasma albumin concentration; from week -6 to -1 ewes receiving treatment LCHV maintained plasma albumin concentration, while albumin concentration declined in ewes receiving treatment HCLV or HCHV. In addition, from week 2 to 8 plasma albumin concentration increased only in ewes fed treatment HCLV.

Table 3. 17. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on ewe plasma albumin (g/l)

Weeks	LC		HC		SED	*P-value*		
	LV	HV	LV	HV		C	V	CxV
-6	25.7	26.8	28.3	29.2	1.055	0.003	0.155	0.937
-3	23.5	25.8	26.1	25.3	1.461	0.364	0.405	0.210
-1	27.1	26.8	27.4	28.5	0.866	0.076	0.606	0.334
2	25.5	24.1	22.2	26.9	2.104	0.950	0.293	0.098
4	28.6	29.8	30.0	28.2	1.029	0.752	0.762	0.091
6	26.1	26.3	25.9	26.7	0.663	0.782	0.269	0.546
8	25.7	24.1	24.7	26.6	1.048	0.181	0.925	0.060

LC: low body condition score (2.5 units); HC: high body condition score (3 units); LV: Concentrate supplemented with vitamin E (50 mg/kg dry matter); HV: Concentrate supplemented with vitamin E (500 mg/kg dry matter).
SED values: C= 0.506, V=0.499, C×V=0.767, Time= 0.513, Time×C=0.835, Time×V=0.834, Time×C×V=1.192.
P-values: C= 0.138, V=0.345, C×V=0.577, Time= <0.001, Time×C= 0.086, Time×V=0.657, Time×C×V=0.017

3.3.6.6. Plasma urea

There was an effect (P<0.05) of time on ewe plasma urea concentration (Table 3.18). *Pre-partum*, ewe plasma urea concentration increased from 3.56 mmol/l at week -6 to 3.87 mmol/l at week -1. *Post-partum*, ewe plasma urea concentration increased to 4.96 mmol/l at week 2 and 5.01 mmol/l at week 4 then reduced to 3.53 mmol/l at week 8. There was an effect (*P*<0.05) of nutritional restriction on ewe plasma urea concentration; ewes that were nutritionally restricted in mid-pregnancy had lower plasma urea concentration at week -6 (2.65 *vs* 4.47 mmol/l; *P*=0.049). There was no effect (*P*>0.05) of vitamin E supplementation on ewe plasma urea concentration. However, ewes that were supplemented with high vitamin E trended to have a higher plasma urea concentration at week 2 (5.27 *vs* 4.64 mmol/l; *P*=0.050). There was no C×V interaction (P>0.05) on ewe plasma urea concentration. However, at week 6 ewes fed treatment HCHV trended to have the highest plasma urea concentration (5.24 mmol/l; P=0.053). There was no time × C interaction on ewe plasma urea concentration; however, ewes receiving LC treatment had trended to increase plasma urea concentration from week -6 to -1 while ewes receiving HC treatment had trended to decrease plasma urea concentration from week -6 to -1. From week 2 to 8 ewes fed LC treatment reduced higher plasma urea concentration than fed HC treatment. There was no time × V or time × C × V interaction (P<0.05) on ewe plasma urea concentration.

Table 3. 18. The effect of nutritional restriction and vitamin E supplementation in late pregnancy on ewe plasma urea (mmol/l).

Weeks	LC		HC		SED	*P-value*		
	LV	HV	LV	HV		C	V	CxV
-6	2.79	2.52	3.92	5.03	1.263	0.049	0.647	0.492
-3	4.31	3.81	4.39	4.31	0.489	0.389	0.376	0.590
-1	3.77	3.92	4.03	3.60	0.508	0.896	0.689	0.473
2	4.23	5.33	5.04	5.22	0.431	0.278	0.050	0.186
4	4.81	4.81	4.57	5.95	0.782	0.378	0.210	0.271
6	4.73^{ab}	4.41^{ab}	3.69^{a}	5.24^{b}	0.568	0.886	0.134	0.053
8	3.47	3.07	3.70	3.88	0.837	0.361	0.848	0.658

LC: low body condition score (2.5 units); HC: high body condition score (3 units); LV: Concentrate supplemented with vitamin E (50 mg/kg dry matter); HV: Concentrate supplemented with vitamin E (500 mg/kg dry matter).
a,b Means with the same superscript are not significantly different (P<0.05).
SED values: C= 0.325, V=0.327, C×V=0.487, Time= 0.283, Time×C=0.493, Time×V=0.493, Time×C×V=0.702.
P-values: C= 0.164, V=0.457, C×V=0.453, Time= <0.001, Time×C= 0.082, Time×V=0.386, Time×C×V=0.393

3.4. Discussion

To achieve differences in body condition score (C) between high and low C treatments, ewes in the current experiment were nutritionally restricted from day 70 to day 105 of pregnancy by offering either straw *ad-libitum* plus concentrate (0.45-0.62 kg/d DM) to maintain their condition score in the high condition score treatment (HC), or straw *ad-libitum* to reduce their LW by approximately 5 kg over a 33 day period (0.15 kg/day) which is equal to a condition score loss of approximately 0.5 units (AHDB, 2016) in the low condition score treatment (LC). The changes in ewe LW on all treatments reflected the nutrient intake, with ewes offered straw only losing more C than those offered straw plus concentrates. Therefore, the imposition of a nutritional restriction at day 70 of pregnancy was effective at reducing the LW, C, back fat and eye muscle depth in the LC treatment, so that by day 103, ewes offered LC had a lower C than those offered HC (2.57 vs 2.98). Predictors of energy status in the body include blood plasma glucose, glucagon, insulin, NEFA, BHB, triglycerides and total lipids (Caldeira *et al.,* 2007). Among them, insulin, glucose and NEFA can provide substantial information on the energy status of an animal (Caldeira *et al.,* 2007). Blood NEFA concentration reflects energy balance change (Pedernera *et al.,* 2008; Giuliodori *et al.,* 2011) and mobilisation of lipid (Butler, 2014). High plasma NEFA concentration is a clear indicator of negative energy balance (Caldeira *et al.,* 2007). In addition, predictors of protein status in the body include blood plasma total protein, albumin, globulin, creatinine and urea (Caldeira *et al.,* 2007). A good indicator of protein status and nitrogen intake are both plasma urea and albumin (Caldeira *et al.,* 2007; Keogh *et al.,* 2015). High intake of protein increased blood plasma urea (Keogh *et al.,* 2015). In the current experiment, the relatively low ewe plasma concentration of metabolic parameters (urea, total protein and albumin), and high plasma concentration of metabolic parameters (NEFA and BHB) in week -6 *pre-partum* indicate that ewes had experienced nutritional restriction. These parameters rapidly returned to normal levels when feeding levels were increased from day 103 onwards.

One unit of condition score is equal to 10 to 13% of mature ewe body weight (AHDB, 2016). For example, for an 80 kg ewe, this equates to about 8 to 10.4 kg. The target condition score for lowland ewes at mating is 3.5, with a loss of 0.5 units during mid-pregnancy being considered acceptable (AHDB, 2016). Therefore, a target C of 3.0 by week -6 is acceptable (EBLEX, 2008; EBLEX, 2014a; AHDB, 2018). In the current experiment, two concentrates were formulated to meet the metabolisable energy (ME) and metabolisable protein (MP) requirement of twin bearing ewes during late pregnancy and producing 3.0 litres of milk during early lactation (AFRC, 1993), but provided different levels of vitamin E (50 or 500 mg/kg DM). The high level of vitamin E was above both the (ARC, 1980) (10 -15 mg/kg DM)

and (NRC, 1985) (20 mg/ kg DM) requirement. The analysed chemical composition of the concentrates was similar to predicted throughout the experiment.
During the *pre-partum* period, all ewes gained weight due to foetal growth. During the *post-partum period,* all ewes lost LW, C, back-fat and eye muscle depth despite being fed to meet the requirements for milk production according to AFRC (1993). Increases in the relatively ewe plasma concentration of metabolic parameters (NEFA and BHB) indicated that the ewes were mobilising body tissue (Caldeira *et al.,* 2007). The recent Agri Food and Biosciences Institute report (AFBI, 2018) suggests that AFRC (1993) underestimates the metabolisable energy requirements of sheep for maintenance. Ewe live weight loss during early lactation could be due to an under estimate of metabolisable energy requirements for maintenance calculated as AFRC (1993). Blood plasma urea concentration is an indicator of protein turnover and of dietary nitrogen intake (Keogh *et al.,* 2015). Increasing ewe plasma total protein, albumin and urea concentration in the current study during early lactation could reflect to higher dietary protein intake compared to late pregnancy (Keogh *et al.,* 2015).

In the current experiment, the lambs from nutritionally restricted ewes had a slightly lower birth weight and lower weight during the first few weeks of life. However, there was no effect of a nutritional restriction on both colostrum or milk component yields. Condition score loss of up to 0.5 unit in late pregnancy has been shown to have no effect on lamb birth and weaning weight (Kenyon *et al.,* 2012). However, a reduction of 0.8 unit of C has been shown to reduce ewe milk yield (Gibb and Treacher, 1980). Consequently, a C loss more than 0.5 unit is probably detrimental. MLC (1988) guidelines suggest that up to 0.5 units of C loss in mid-pregnancy have no effect of placental development and subsequent lamb performance in early lactation. In the current experiment, C loss in mid pregnancy was greater than 0.5 units (0.74 unit). This could be the reason for the reduction in lamb birth and weaning weight.

Vitamin E concentration in the body tissues, including adipose tissue is highly variable (2-30 μg/g) and depends upon the vitamin E content of previous diet (Ochoa *et al*., 1992; Fry *et al*., 1993; Kasapidou *et al*., 2009) Inconsistent results have been documented in previously published works in regards to tocopherol mobilisation from adipose tissue ((Machlin *et al*., 1979; Fry *et al*., 1993; Janneke Brouwer *et al*., 1998). Fry *et al.* (1993) documented α-tocopherol depletion from adipose tissue in sheep from approximately 8.0 to 0.5 μg/ g over 14 weeks. Machlin *et al.* (1979) reported that there was extremely slow depletion (not significant) of vitamin E from adipose tissue during nutritional restriction or vitamin E deficiency in the guinea pig. Schaefer *et al.* (1983) documented that both tocopherol and cholesterol of adipocytes were not mobilised from fat cells in human. However, 40% of adipocyte cells size reduced as a result of triglyceride mobilisation.

Janneke Brouwer *et al.* (1998) reported that there was no vitamin E mobilisation from adipose tissue in human. Traber and Kayden(1987) documented that 90% of total α-tocopherol in the body can be found in adipose tissue and 99% of this α-tocopherol is stored in bulk lipid where it is not available for exchange, and only slowly mobilised from this depot (Bjørneboe *et al.*, 1990). The current results are in agreement with previously published work (Machlin *et al.,* 1979; Schaefer *et al.,* 1983; Janneke Brouwer *et al.,* 1998). In the current experiment, ewe plasma vitamin E reduced in mid pregnancy, suggesting no vitamin E mobilisation from the adipose tissue in both nutritionally restricted, and non-restricted ewes. In addition, the similar ewe plasma vitamin E concentration in LC or HC treatments during both late pregnancy and lactation suggests that there was no vitamin E mobilisation from body tissue reserves especially from adipose tissue. Ewe condition score LC or HC in the current experiment was 2.5 or 3.0 units respectively. The fact that there was no response to vitamin E supplementation between treatments LC or HC could be due to that the difference in tissue reserves (condition score) was not sufficient to make difference. This means that pregnant and lactating ewe require vitamin E supplementation with either low or high body condition score. The imposition of a nutritional restriction at day 70 of pregnancy was effective at reducing the back fat in the LC treatment, so that by week -6, ewes offered LC had a lower back fat than those offered HC (4.62 vs 5.59 mm; P=0.017). From week -6 onwards ewes in all treatments lost back fat, so that by week -1 and during lactation there was no difference between LC and HC treatments. The similar back fat thickness at week -1 and during lactation could be another reason of no response to vitamin E in LC or HC treatments.

Before the experiment, all ewes were grazed. Forages are an important natural source of vitamin E (Elgersma *et al.,* 2012). The naturally occurring *RRR*-α-tocopherol is considered to be the most bioactive form of tocopherol that can be found in grains and forages (Azzi, 2018), and is easier for absorption (Burton and Traber, 1990). This could be the reason for the relatively high vitamin E concentration in ewe plasma at week -10.

The slightly higher plasma vitamin E content of the non restricted ewes at week -6 probably reflects the fact that they were fed 0.5 kg/day of a standard ewe concentrate containing 100 mg/kg vitamin E. Ewes that were fed concentrates with a high vitamin E content during late pregnancy and lactation had a higher colostrum, and plasma vitamin E content. Their lambs also had a higher plasma vitamin E content. In previously published experiments, high levels of vitamin E supplementation to ewes in late pregnancy and lactation have been shown to increase ewe plasma α-tocopherol concentration (Njeru *et al.,* 1994; Rooke *et al.,* 2009; Dønnem *et al.,* 2015). These findings are in line with the present experimental results. From week -6, plasma vitamin E was 2-3 times higher in ewes offered the concentrate containing a high compared to a low level of vitamin E, even though concentrate levels were

approximately ten times higher. Ewes offered the high vitamin E concentrate had plasma vitamin E levels of approximately 2.10 and 1.92 µg/ml during the *pre-partum* and *post-partum* periods respectively, which is considered adequate by NRC (2007) (≥2 µg/ml). However, those offered the low vitamin E concentrate had lower plasma levels (0.85 and 0.71 µg/ml) *pre-partum* and *post-partum* respectively. However, no clinical signs of deficiency were observed. During lactation, the plasma vitamin E content of all ewes declined and was lower than the 2.0 µg/ml recommended by NRC (2007). This decline could be due to the additional vitamin E demand for colostrum and milk production (Liu *et al.,* 2014). The NRC (2007) recommendations currently make no allowance for an additional vitamin E requirement for milk production and assume that the vitamin E requirement of both pregnant and lactating ewes is similar. The reduction in vitamin E status of all ewes during lactation suggests that the vitamin E requirement of lactating ewes is higher than that of pregnant ewes and that this should be taken into account by feeding standards. To keep plasma α-tocopherol concentration at ≥ 2.0 µg/ml in pregnant and lactating ewes, the NRC (2007) recommendation is to supply more than 5.6 IU/kg live weight. Therefore, a 85 kg sheep requires at least 476 mg vitamin E per day; whereas, supplementation of approximately 700 mg/ day might be required during early lactation.

The effect of vitamin E supplementation on ewe performance is inconsistent in previously published works. Capper *et al.* (2007) found that a high level vitamin E supplementation increased C loss, plasma NEFA and BHB during lactation as a result of body fat mobilisation, while Merrell *et al.* (1998) reported that there was no effect of vitamin E on ewe C change. Nieto *et al.* (2016) reported that vitamin E supplementation tended to reduce weight loss in undernourished ewes during late pregnancy and lactation. In the current experiment, ewes that lost C in mid pregnancy appeared to respond to vitamin E supplementation, they lost less back fat during late pregnancy and C back fat. The lower blood plasma NEFA concentration in high vitamin E supplemented treatments during early lactation indicated that the ewes lost less C (Caldeira *et al.,* 2007). The current results are in agreement with those of Nieto *et al.* (2016) that vitamin E supplementation may have a positive effect on the performance of ewes that have experienced nutritional restriction. Vitamin E supplementation during late pregnancy improved reproductive performance, prevented pathology of the reproductive system or foetus, and reduced myocardial necrosis (Buchanan-Smith *et al.,* 1969). In addition, vitamin E supplementation has benefit to prevent reproductive problems like retained placenta and endometritis (Hemingway, 2003). Protection of ewes from reproduction problems resulting from oxidative stress and tissues damage may help to improve performance especially in ewes that have experienced nutritional restriction. A number of vitamin E functions has been documented. As an antioxidant, vitamin E prevents the oxidation of polyunsaturated fatty acids (PUFA) and

lipoproteins in cell membranes to hydroperoxides and protects animal cell membrane from the oxidative damage (Bramley *et al.,* 2000; Mcdowell, 2000). It has also been suggested that vitamin E improves rumen fermentation. Belanche *et al.* (2016) documented that supplementation of vitamin E increased rumen gas production, total VFA, protozoal activity and resulted in a small increase in feed digestibility (+8%). High vitamin E supplementation improved feed conversion efficiency according to (Macit *et al.,* 2003a) in Morkaraman sheep and (Macit *et al.,* 2003b) awassi sheep. The above functions might be the reasons that high vitamin E supplementation had an effect on ewes performance in the current experiment.

In previously published experiments, lamb plasma vitamin E concentration at birth was less than 15% of ewe plasma concentration (Kumagai and White, 1995; Schenker *et al.,* 1998 and Sterndale *et al.,* 2018). In the current experiment, lamb plasma vitamin E concentration was 39 and 42% of ewe plasma vitamin E concentration, for ewes on treatment LV and HV respectively, with the highest percentage being recorded for treatment LCHV (55%). These higher percentages may reflect the fact that lamb plasma vitamin E was measured at 12 hours *post-partum*, after ingestion of colostrum, which is the main route by which vitamin E is transferred from the ewe to the lamb.

In the current experiment, vitamin E supplementation did not affect lamb birth weight or weaning weight. These results are in agreement with the results of Merrell *et al.* (1998), Daniels *et al.* (2000), Dafoe *et al.* (2008) and Rooke *et al.* (2009), who reported that vitamin E supplementation of pregnant ewes had no effect on lamb birth weight. On the other hand, the current results do not agree with those of Capper *et al.* (2005) who documented that lamb birth weight was significantly increased by vitamin E supplementation of ewes in late pregnancy. In the current experiment supra-nutritional vitamin E supplementation was enough to maintain plasma vitamin E at NRC (2007) recommended levels (2.0 µg/ml) in late pregnancy, but not to elicit a supra-nutritional response. In contrast to Capper *et al.* (2006), no effect of vitamin E supplementation on lamb birth weight was observed. This might be because in the work of Capper *et al.* (2006), ewes were fed diets containing a higher concentration of PUFA and were probably under more oxidative stress. Consequently, they were perhaps more likely to response to vitamin supplementation.

Colostrum and milk are the main sources of nutrients for the growing lamb during the *pre-weaning* period. In the current study, supra-nutritional vitamin E supplementation had no effect on colostrum and milk component yield. This might be another reason for the lack of effect of high vitamin E supplementation on lamb weight gain. Previous studies have documented no effect of vitamin E supplementation on colostrum and milk yield and composition in ewes (Capper *et al.,* 2005; Capper *et al.,* 2007), and milk production in Holstein dairy cows (Al-Mabruk *et al.,* 2004; Politis *et al.,* 2004). Colostrum composition

results in the current experiment contradict those of Rosales *et al.* (2015) who found that vitamin E increased colostrum lactose percentage and milk fat percentage. The reason for colostrum fat increases by vitamin E supplementation might be the antioxidant function of vitamin E which protect the fat from the peroxidation (Vagni, 2011).

3.5 Conclusion

Supra-nutritional vitamin E supplementation had an effect on ewe performance characteristics. The live weight gain of the ewes that experience nutritional restriction during mid-pregnancy and fed high vitamin E during late pregnancy was slightly higher than that of ewes in other groups. Ewes fed a high level of vitamin E lost less C during early lactation than those fed low vitamin E. Lamb birth weight, colostrum and milk yield and composition were not affected by vitamin E supplementation. Litter weights were reduced by nutritional restriction in mid-pregnancy compared with ewes not subjected to nutritional restriction. The vitamin E concentration of ewes and lamb plasma or colostrum was higher in groups fed high vitamin E supplementation. High vitamin E supplementation increased plasma α-tocopherol concentration to 2.10 and 1.92 µg/ml during late pregnancy and lactation respectively. High dietary vitamin E was required to maintain plasma levels ≥2.0 µg/ml (NRC, 2007). This level of supply equates to 476 mg/kg DM (5.6 mg/kg LW) for an 85 kg sheep, which is similar to the NRC recommendation of 5.6 mg/kg LW and significantly higher than fed in most UK ewe rations.

Chapter 4

Effect of source and level of vitamin E supplementation on ewe and lamb performance during late pregnancy and early lactation.

4.1. Introduction

Vitamin E (tocochromanols) consists of eight biologically active isomers; four are tocopherols and the remaining four are tocotrienols, which occur naturally as free alcohols in the lipid fraction of green leaves and seeds (DellaPenna, 2005; Fritsche *et al.*, 2017). Alpha tocopherol is considered as the most important biologically active isomer in animal tissues (Scherf *et al.*, 1996). The naturally occurring α-tocopherol is considered the most bioactive among the four available tocopherols that can be found in grains and forages (Azzi, 2018). Whereas, the synthetic form of α-tocopherol that is used in feed supplementation consists of a racemic mixture of various stereoisomers (Meglia *et al.*, 2006; Wilburn *et al.*, 2008 and Yang, 2003). This consists of the chemical esterification of α-tocopherol with acetate to produce α-tocopherol acetate (NRC, 2007). The bioavailability of the naturally occurring form of vitamin E from forages may be considerably higher than that from concentrates in growing lambs (Kasapidou *et al.*, 2009). However, the mechanism is poorly understood. The form of vitamin E and rumen environment may be important factors in explaining responses to additional vitamin E supplementation.

In the UK, EBLEX (2014) recommended that vitamin E requirements of pregnant and lactating ewes are typically 100-150 mg/kg DM to obtain more vigorous lambs, higher live weight gain and protect lambs from the white muscle disease. These feeding levels are lower than NRC (2007) and higher than previous recommendations (ARC, 1980 and NRC, 1985). Whereas responses in terms of lamb birth weight and neonatal lamb vigour have mainly been reported using concentrates containing >500 mg/kg vitamin E (Merrell *et al.*, 1998; Capper *et al.*, 2005). According to NRC (2007), ewes require 5.6 mg/kg LW vitamin E to maintain plasma levels above the critical value of 2.0 µg/ml. For an 85 kg ewe, this equates to approximately 500 mg/day of vitamin E. Supra-nutritional levels of 9.0-10 mg/kg LW of vitamin E (765 mg/day) may be required to enhance immune responses or extend shelf life in meat (NRC, 2007). Ewes supplementation with supra-nutritional levels of 9.0-10 mg/kg LW (765 mg/day) of vitamin E during late pregnancy may also enhance lamb birth weight (NRC, 2007). In the previous experiment (chapter three), concentrates containing either 50 or 500 mg/kg DM vitamin E were fed to ewes offered straw based diets with high levels of concentrate feed. No effect on lamb birthweight were observed. In addition, the higher level (500 mg/kg DM) was required to maintain plasma vitamin E levels above critical values. This suggests that NRC (2007) estimates may well be correct. As a consequence, the vitamin E levels in typical UK commercial ewe concentrates may be underestimated,

and considerably higher concentrations (1000 mg/kg) may be required to elicit supra-nutritional responses. Therefore, the objective of the current experiment was to investigate the effect of source and level of vitamin supplementation on ewe and lamb performance during late pregnancy and early lactation.

4.2. Materials and methods

4.2.1. Experimental design

The experiment was conducted at Harper Adams University between January and April 2017 in the Animal Production, Welfare and Veterinary Sciences (APWVS) project buildings. Local ethical approval was obtained from the Animal Welfare and Ethical Review Board (AWERB). Forty-four twin-bearing Suffolk x Mule ewes from the Harper Adams University flock were housed individually on sawdust from week -6 to week +4 of lactation, with a mean lambing date of 17th February, and allocated by parity, live weight (LW) and body condition score (C) to one of four experimental treatments in a 2 x 2 factorial design:

Grass silage (GS): Low vitamin E (LV)	(GSLV)
Grass silage (GS): High vitamin E (HV)	(GSHV)
Straw (S): Low vitamin E (LV)	(SLV)
Straw (S): High vitamin E (HV)	(SHV)

From week +4, ewes and lambs were group housed within their respective treatment groups and maintained on the experimental diets prior to weaning at week +8.

4.2.2. Grass silage, diet formulation and treatments

Grass silage was made from a first cut perennial ryegrass (*Lolium perenne*) sward. The sward was mown on the 25th May 2016 and wilted for 24 h. A precision chop self-propelled forage harvester (John Deere 7840i, Nottinghamshire UK) was used to chop the harvested ryegrass to a chop length of 10 mm. An additive (Axphast Gold, Biotal, Worcestershire, UK) was then applied at 2.0 litres/tonne prior to ensiling in a roofed concrete clamp.

The two concentrates were manufactured by HJ Lea Oaks Ltd, Nantwich, Cheshire and had the same raw material and chemical composition, but differed in their vitamin E content, with concentrates low (LV) and high (HV) being formulated to supply 150 and 1000 mg/kg fresh respectively (Table 4.1). Forages were offered *ad-libitum* with concentrates being fed to provide a rising plane of nutrition to meet the metabolisable energy (ME) and metabolisable protein (MP) requirements of twin bearing ewes during late pregnancy and producing 3.0 litres/day of milk during early lactation (AFRC, 1993) (Table 4.2), (Table 4.3), (Table 4.4) and (Table 4.5). The grass silage and straw were analysed to contain 144 and 30 mg/kg DM vitamin respectively (Table 4.2). For ewes offered grass silage, it was

expected that the low and high vitamin E concentrates would provide approximately 240 mg/day (2.8 mg/kg LW) or 750 mg/day (9.0 mg/kg LW) vitamin E respectively during the last 3 weeks of gestation (Table 4.7). For ewes offered straw, the two concentrates were blended in appropriate proportions to provide the same levels of vitamin E supply (Table 4.6). Concentrates were fed twice daily as two equal feeds until concentrates feeding levels exceeded 1.0 kg/day when an additional third feed was introduced. Between weeks +4 to +8, the ewes were group housed with their lambs and the quantity of concentrates was reduced to meet the requirements of 2.2 litres/day of milk production. Feed samples were taken weekly for further analysis.

Table 4. 1. Raw material and predicted chemical composition of the experimental diets

	LV	HV
Raw material composition (g/kg)		
Wheat	280	280
Wheat feed	200	200
Maize	100	100
Sugar beet pulp	88.0	88.0
Rapeseed	80.5	80.0
Molasses	70.0	70.0
Distillers dark grains	65.0	65.5
Hipro Soya	50.0	50.0
Sunflower	30.0	30.0
Limestone flour bulk	20.3	3.60
Sodium chloride	7.50	7.50
Magnesite	4.50	4.50
Minerals and vitamins[1]	2.50	2.50
Vitamin E Top[2]	1.00	18.0
Flake oat feed	0.50	-
Total	**1000**	**1000**
***Predicted[3] Chemical composition* (g/kg DM)**		
Dry matter (g/kg)	871	871
Crude protein	180	180
ERDP	113	113
DUP	41.6	41.6
Ether extract	36.5	36.4
Ash	77.4	77.0
NDF	192	191
Starch	228	228
Vitamin E (IU/kg)	150	1000
ME (MJ/kg)	10.8	10.8

[1] Mineral/ vitamins supplement supplied per kg of diet: Vit. A 8000.00 IU/kg, Vit. D3 2000.00 IU/kg, Vit. B12 70.00 mcg/kg, calcium 1.18%, magnesium 0.50%, sodium 0.40%, selenium 0.56 mg/kg, copper 11.18 mg/kg, iodine 5.37 mg/kg, iron 354.49 mg/kg, manganese 123.70 mg/kg, salt 1.11%, and zinc 117.15 mg/kg.
[2] Vitamin E Top supplement (BESTMIX[TM]) containing Vit. E 50,000.00 IU/kg, calcium 35.57 %.
[3] Diet chemical composition predicted according to AFRC (1993).

Table 4. 2. Chemical composition (g/kg DM) of forages

	Grass silage	Straw
Dry matter (g/kg)[1]	231	850
Crude protein[1]	121	42.0
NDF[1]	495	717
Vitamin E (mg/kg DM)[1]	144	30.0
ME (MJ/kg DM)[2]	10.8	6.40
FME (MJ/kg DM)[2]	7.80	5.80

[1] The chemical composition was analysed prior diet formulation
[2] Chemical composition predicted according to AFRC (1993).

Table 4. 3. Predicted forage dry matter intake for 75 kg twin-bearing ewes producing 3.0 litres/ day of milk during early lactation according to (AFRC, 1993)

Forages	Pregnancy		Lactation	
	DM	Fresh	DM	Fresh
Silage (kg/day)	1.00	4.30	1.50	6.50
Straw (kg/day)	0.80	1.00	1.40	1.70

Table 4. 4. Concentrate allocation for 75 kg twin-bearing ewes producing 3.0 litres/ day of milk during early lactation or 2.0 litres/ day of milk during late lactation

Treatment base diet	Late pregnancy weeks						Lactating weeks	
	-6	-5	-4	-3	-2	-1	1 to 4	4 to 8
Silage (kg/ day)	0.20	0.30	0.40	0.50	0.70	0.85	1.40	0.90
Straw (kg/ day)	0.70	0.80	0.90	1.00	1.15	1.30	2.00	1.50

Table 4. 5. Total dry matter intake (TDMI) and nutrient supply for 75 kg twin-bearing ewes producing 3.0 litres/ day of milk during early lactation

Treatment base diet	Late pregnancy weeks						lactating weeks
	-6	-5	-4	-3	-2	-1	1 to 4
Straw base diet							
TDMI (kg/ day)	1.50	1.60	1.70	1.80	1.95	2.10	3.40
ME (MJ/ day)	13.3	14.4	15.6	16.7	18.4	20.1	31.7
CP (g/ day)	162	180	198	216	243	270	421
Silage base diet							
TDMI	1.20	1.30	1.40	1.50	1.70	1.85	2.90
ME (MJ/ day)	13.4	14.4	15.5	16.7	18.4	20.1	32.1
CP (g/ day)	157	175	193	211	238	265	430

Table 4. 6. Relative proportions of the low and high vitamin concentrates required by ewes offered the straw based diets to provide similar levels of vitamin E intake to those offered the grass silage based diets.

Concentrates	Late pregnancy weeks						Lactating weeks	
	-6	**-5**	**-4**	**-3**	**-2**	**-1**	**1 to 4**	**4 to 8**
SHV								
High Vitamin E	0.37	0.45	0.51	0.56	0.67	0.71	0.76	0.68
Low Vitamin E	0.63	0.55	0.49	0.44	0.33	0.29	0.24	0.32
SLV								
High Vitamin E	0.09	0.08	0.07	0.06	0.06	0.05	0.06	0.08
Low Vitamin E	0.91	0.92	0.93	0.94	0.94	0.95	0.94	0.92

4.2.3. Experimental Routine

All ewes and lambs were weighed and condition scored weekly as described in Chapter 2 (Section 2.4 and 2.5). In addition, they were ultra-sound scanned for back-fat and eye-muscle depth in weeks -6, -3, -1, +2, +4, +6 and +8 as described in Chapter 2 (Section 2.6). During weeks -3 and -2 faecal grab samples were obtained from 6 representative animals on each treatment over five days to estimate diet digestibility using acid insoluble ash as an internal marker (Block *et al*., 1981) as described in Chapter 2 (Section 2.1.8.). Following parturition, the placenta was collected, washed and weighted, and the number and size of placentomes recorded as described by Dwyer *et al*. (2005). Both colostrum and milk yield were estimated from all ewes at 16 hours and 28 days *post-partum* using the method adapted from Doney *et al*. (1979) as described in Chapter 2 (Section 2.5). Concentrates and straw samples were taken weekly and placed in a freezer at -20ºC for analysis. Blood samples were obtained by jugular venepuncture at 11:00 h as described in Chapter 2 (Section 2.2) from six representative ewes on each treatment at weeks -6, -3, -1, +2, +4 and +8. In addition, blood samples were taken from the lambs at week+2 *post-partum*. The blood samples were collected into two types of tubes. Potassium oxalate for glucose analysis and sodium heparin for vitamin E, total protein, albumin, beta-hydroxybutyrate (BHB), non-esterified fatty acid (NEFA) and urea analysis. The plasma was removed and stored in 1.0 ml Eppendorf tubes at -20 C prior to further analysis as described in Chapter 2 (Section 2.2).

Table 4. 7. Predicted vitamin E supply (mg/day) for 75 kg twin-bearing ewes producing 3.0 litres/ day of milk during late pregnancy early lactation

Diets	Late pregnancy weeks						Lactating weeks	
	-6	-5	-4	-3	-2	-1	1 to 4	4 to 8
GSLV								
Concentrate	30.0	45.0	60.0	75.0	105	127	210	135
Forage	145	145	145	145	145	145	217	217
Total	175	190	205	220	250	272	427	352
GSHV								
Concentrate	200	300	400	500	700	850	1300	900
Forage	145	145	145	145	145	145	217	217
Total	345	445	545	645	845	995	1517	1117
SLV								
Concentrate	158	173	188	203	233	255	398	323
Forage	17.0	17.0	17.0	17.0	17.0	17.0	29.0	29.0
Total	175	190	205	220	250	272	427	352
SHV								
Concentrate	328	428	528	628	828	978	1488	1088
Forage	17.0	17.0	17.0	17.0	17.0	17.0	29.0	29.0
Total	345	445	545	645	845	995	1517	1117

4.2.4. Chemical analysis

Weekly feed samples (concentrates and forages) were bulked together *pre-partum* and *post-partum,* and analysed in duplicate according to the methods of A.O.A.C (2016) as described in Chapter 2 for DM (Section 2.1.1), CP (Section 2.1.3), ash (Section 2.1.2), EE (Section 2.1.4) and NDF (Section 2.1.5).

Colostrum samples were analysed for total solids, protein, fat, ash and lactose. All of the samples were first defrosted and mixed well prior to analysis as described in Chapter 2 (Section 2.8). Milk samples were analysed by measuring total solids, protein, fat, and lactose using a Foss MilkoScan machine as described in Chapter 2 (Section 2.6). High performance liquid chromatography (HPLC) was used to determine the vitamin E concentration as described in Chapter 2 for plasma and colostrum (Section 2.9.1) and feedstuffs (Section 2.9.2). Blood plasma was analysed for glucose, beta-hydroxybutyrate (BHB), non-esterified fatty acid (NEFA), urea, albumin, and total protein, using a Cobas-Mira (Mira plus ABX Diagnostics) blood analyser. Lamb plasma IgG content was measured based on the method of Mancini *et al.* (1965), Chapter two (Section 2.10).

4.2.5. Statistical analysis

The experiment was analysed by ANOVA as a 2 X 2 factorial design using GenStat 17th, edition (VSN Int. Ltd., Hempstead. UK), with the main effects being forage type and vitamin

E level. Repeated measure analysis of variance for ewes dry matter intake (forage and total dry matter intake), plasma vitamin E and blood plasma metabolites components were carried out using GenStat 17th edition (VSN Int. Ltd., Hempstead. UK). Differences between means were determined using the protected least significant difference (LSD) (Snedecor and Cochran, 1989).

4.3. Results

4.3.1. Animal's health

Generally, ewes and lambs were healthy. However, eight ewes and their lambs were excluded from the experiment for different reasons. One ewe from treatment SLV died suddenly at week +2. Three ewes were excluded from treatment SHV. The first one, both her lambs died as a result of dystocia, the second one had a stillborn lamb and one lamb from the third ewe died during week+3. In treatment GSLV, four ewes were removed from the trial, one of the ewes aborted at week -1 and three of them had one dead lamb at week+1.

4.3.2. Diet composition

The analysed chemical composition of the experimental diets is shown in the Table 4.8. The results indicated that the straw had a higher dry matter, and protein content than expected (Table 4.2). Grass silage had a lower dry matter than expected, while NDF was slightly higher than expected (Table 4.2). The composition of both concentrates was similar to that expected from the formulated diet (Table 4.1). However, in both concentrates, dry matter, fat and organic matter content were slightly lower, while NDF was slightly higher than expected. Vitamin E content in the concentrates was similar to the predicted.

Table 4. 8. The chemical composition of experimental feedstuff (g/kg DM)

Chemical composition	Straw	Silage	LV	HV
Dry matter (g/kg)	891	186	856	857
Organic matter	952	911	920	916
Protein	73.2	115	205	202
NDF	756	567	260	276
ADF	473	347	118	120
EE	7.27	21.4	26.0	25.6
GE (MJ/kg DM)	16.9	17.7	18.8	18.1
Vitamin E (mg/kg DM)	30.4	146	156	1067
Selenium (mg/kg DM)	0.02	0.02	0.31	0.36

HV= high vitamin E (150 mg/kg DM), LV= low vitamin E (1000 mg/kg DM)

4.3.3. Food intake

4.3.3.1 Forage and total dry matter intake (DMI)

There was an effect ($P<0.05$) of time on forage DMI (Figure 4.1a). Forage DMI increased from 0.80 kg at week -6 to 0.89 at week -1, then increased to 1.35 at week +3. There was an effect ($P<0.05$) of forage type on forage DMI. Silage DMI was higher ($P<0.05$) than straw DMI throughout the experiment. There was no effect ($P>0.05$) of vitamin E supplementation

on forage DMI. There was no FT×V interaction (P>0.05) effect on forage DMI. There was a time × FT interaction (*P*<0.05) effect on forage DMI. From week -6 to -1 straw intake reduced from 0.72 to 0.58 kg DM, while, grass silage intake increased from 0.88 to 1.21 kg DM. There was an increase in both straw and grass silage intake from week 0 to week 3. There was no time ×V or time ×FT×V interaction (*P*>0.05) on forage DMI.

There was an effect (P<0.05) of time on total dry matter intake (TDMI) (Figure 4.1b). Total dry matter intake increased from 1.21 kg at week -6 to 1.84 at week -1, then increased to 2.85 kg at week +3. There was an effect (P<0.05) of forage type on TDMI. Total dry matter intake was higher (P<0.05) on straw than grass silage based diet treatment at week -6, while at weeks -4, -3 and -1 TDMI was higher in grass silage than straw based diet based. There was no effect (P>0.05) of vitamin E supplementation on TDMI. There was no FT×V interaction (P>0.05) effect on TDMI. There was a time × FT interaction (*P*<0.05) effect on TDMI. There was an increase in TDMI in both straw and grass silage based diet treatments from week 0 to week 3. From week -6 to -1 TDMI in straw based diet treatment increased from 1.36 to 1.74 kg then increased to 2.81 kg at week 3, while, TDMI in grass silage based diet increased from 1.06 to 1.94 kg from week -6 to -1, then increased to 2.84 kg at week 3. There was no time ×V or time ×FT×V interaction (*P*>0.05) on TDMI.

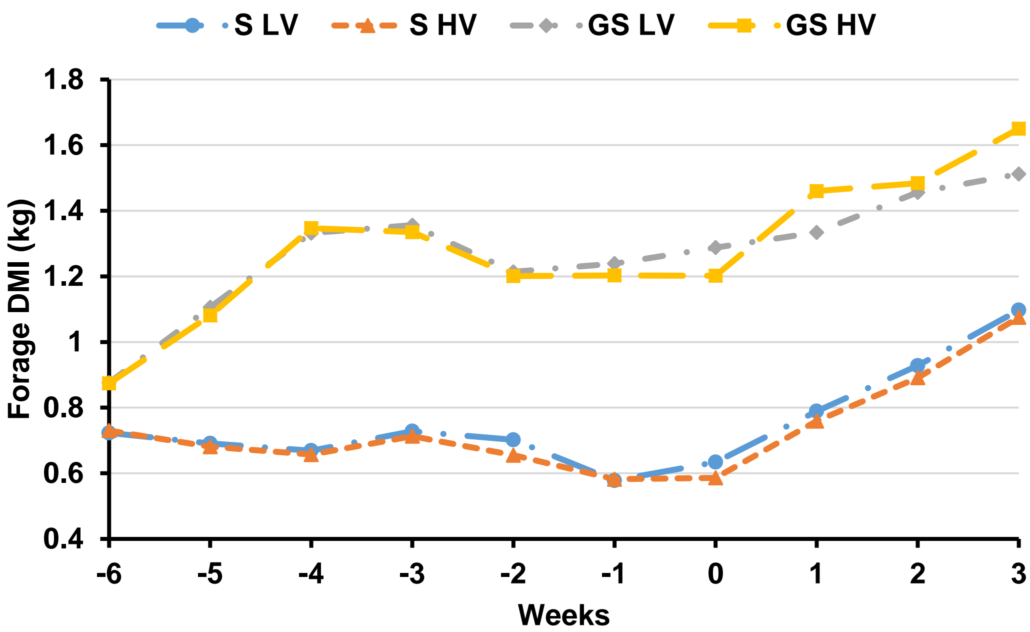


Figure 4. 1a. Ewes forage dry matter intake (DMI) in late pregnancy and early lactation

SLV= Straw low vitamin E supplementation (2.8 mg/kg LW), SHV= Straw high vitamin E supplementation (9.0 mg/kg LW), GSLV= Grass silage low vitamin E supplementation (2.8 mg/kg LW), GSHV= Grass silage high vitamin E supplementation (9.0 mg/kg LW).
SED values: FT= 0.065, V=0.065, FT×V=0.091, Time= 0.030, TimexFT=0.076, Time×V=0.076, TimexFT×V=0.108
P-values: FT= <0.001, V=0.844, FT×V=0.798, Time= <0.001, TimexFT=<0.001, Time×V=0.840, TimexFT×V=0.705

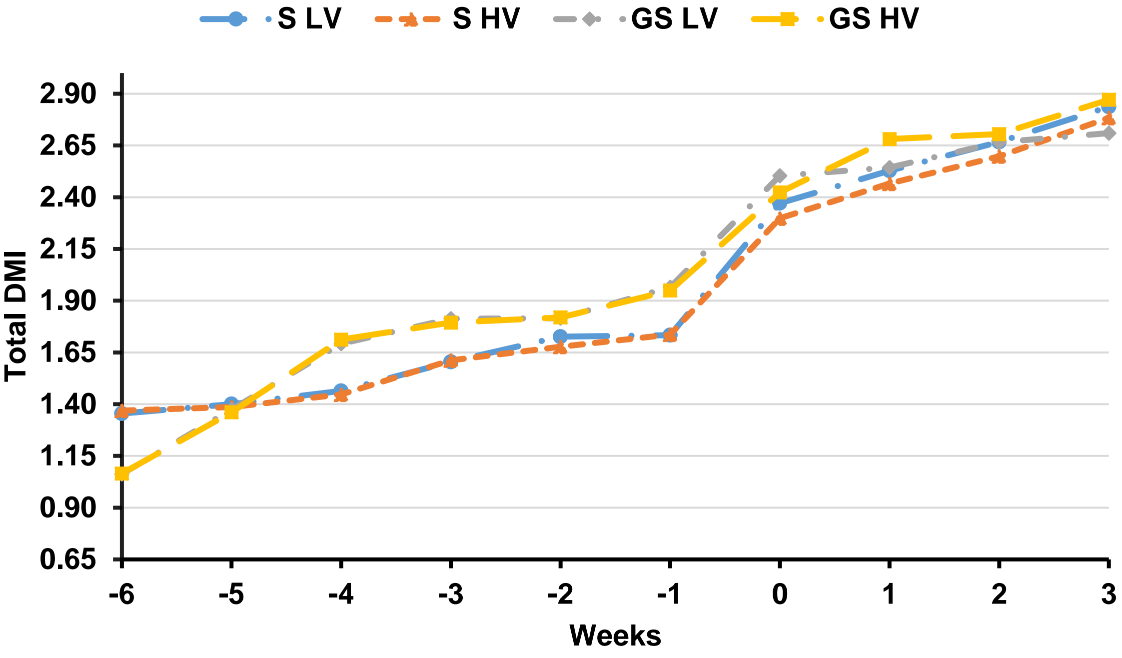


Figure 4. 1b. Ewes total dry matter intake (TDMI) in late pregnancy and early lactation

SLV= Straw low vitamin E supplementation (2.8 mg/kg LW), SHV= Straw high vitamin E supplementation (9.0 mg/kg LW), GSLV= Grass silage low vitamin E supplementation (2.8 mg/kg LW), GSHV= Grass silage high vitamin E supplementation (9.0 mg/kg LW).
SED values: FT= 0.068, V=0.068, FT×V=0.095, Time= 0.032, Time×FT=0.080, Time×V=0.080, Time×FT×V=0.113
P-values: FT= 0.219, V=0.825, FT×V=0.746, Time= <0.001, Time×FT=<0.001, Time×V=0.802, Time×FT×V=0.699

4.3.4. Ewes performance

4.3.4.1 Ewe live weight and weight gain (LW)

The initial weight of the ewes was similar at week -6 of pregnancy (*pre-partum*) (Figure 4.2). There was no FT×V interaction (P>0.05) on ewe live weight. All ewes gained live weight *pre-partum* (Table 4.9). There was an effect of forage type (P<0.05) on ewe live weight. Ewes offered the grass silage based treatments gained more LW (P<0.01) *pre-partum* (13.08 vs 10.39 kg) and lost less LW (P<0.05) *post-partum* (-1.13 vs -3.25 kg) than those offered the straw based treatments. There was no effect (P>0.05) of vitamin E supplementation on ewe live weight or live weight change.

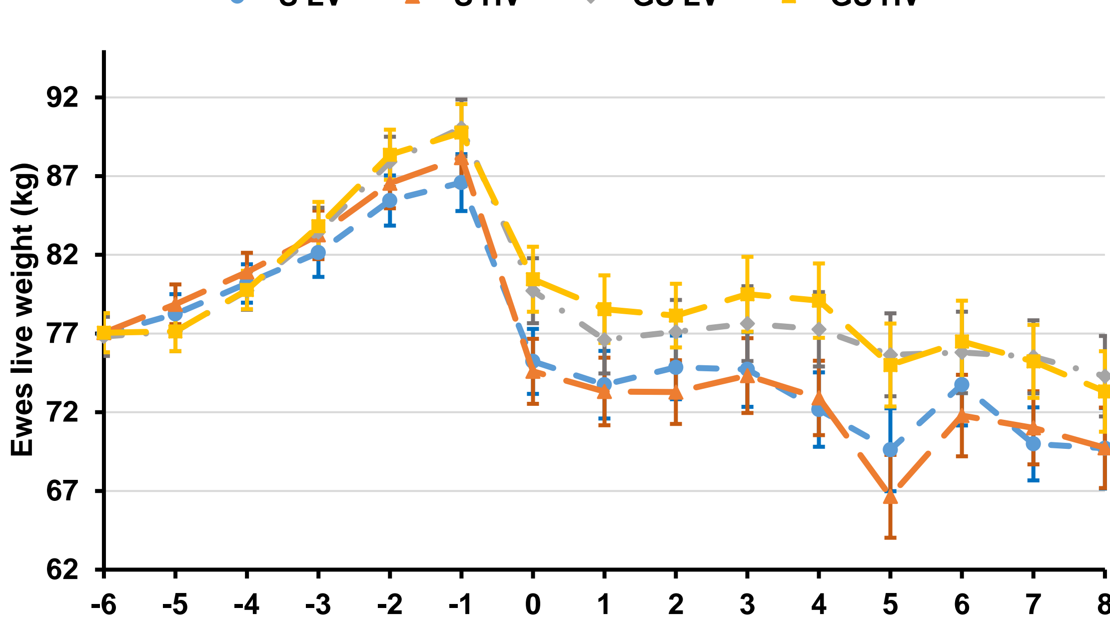


Figure 4. 2. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on ewe live weight. Error bars indicate SED.

Table 4. 9. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on ewe live weight and live weight change.

	S		GS		SED	*P-value*		
	LV	**HV**	**LV**	**HV**		**FT**	**V**	**FT×V**
-6	77.0	77.1	76.8	77.1	1.244	0.939	0.858	0.939
-1	86.6	88.2	90.1	89.8	1.806	0.057	0.614	0.468
0	75.2	74.6	79.7	80.5	2.064	0.001	0.971	0.646
4	72.2	72.9	77.3	79.1	2.363	0.003	0.452	0.749
8	69.7	69.7	74.3	73.3	2.556	0.035	0.799	0.784
Live weight gain(kg)								
-6 to -1	9.64	11.1	13.4	12.7	1.245	0.005	0.654	0.221
0 to 4	-4.01	-2.49	-0.91	-1.36	1.453	0.051	0.613	0.348
0 to 8	-6.48	-5.67	-3.78	-7.14	2.110	0.684	0.403	0.178

S= straw, GS= grass silage, LV=low vitamin E supplementation (2.8 mg/kg LW), HV=high vitamin E supplementation (9.0 mg/kg LW), FT= forage type (straw or silage), V= vitamin E, FT×V = interaction between forages and vitamin E

4.3.4.2 Ewes body condition score (C)

The initial C of the ewes was similar at week -6 of pregnancy without any difference (*P*>0.05) between the treatment groups (Figure 4.3). There was no FT×V interaction (P>0.05) on ewe C. There was also no FT×V interaction (P>0.05) on C change (Table 4.10). However, ewes on diet SHV tended to gain more C than those on the other three treatments (0.02 unit; *P*=0.056). Ewes on diet SLV tended to lose more C than those on the other three treatments (-0.72 unit; *P*=0.062). During the *pre-partum* period (week -6 to -1) there was no effect

(*P*>0.05) of forage type on ewes C. During the *post-partum*, C was higher in ewes on the silage than straw based treatments at week +4 (2.64 vs 2.21 unit; P=0.004) and week +8 (2.98 vs 2.68 unit; P<0.001). Ewes on the silage based treatments tended to gain more C *pre-partum* (week -6 to 0) than those on the straw based treatments (0.12 vs -0.06 unit; P=0.066). There was no effect (*P*>0.05) of vitamin E supplementation on ewe C through the experiment, however, at week+5 ewes supplemented with high vitamin E had a lower C (2.54 vs 2.81 unit; P=0.004).

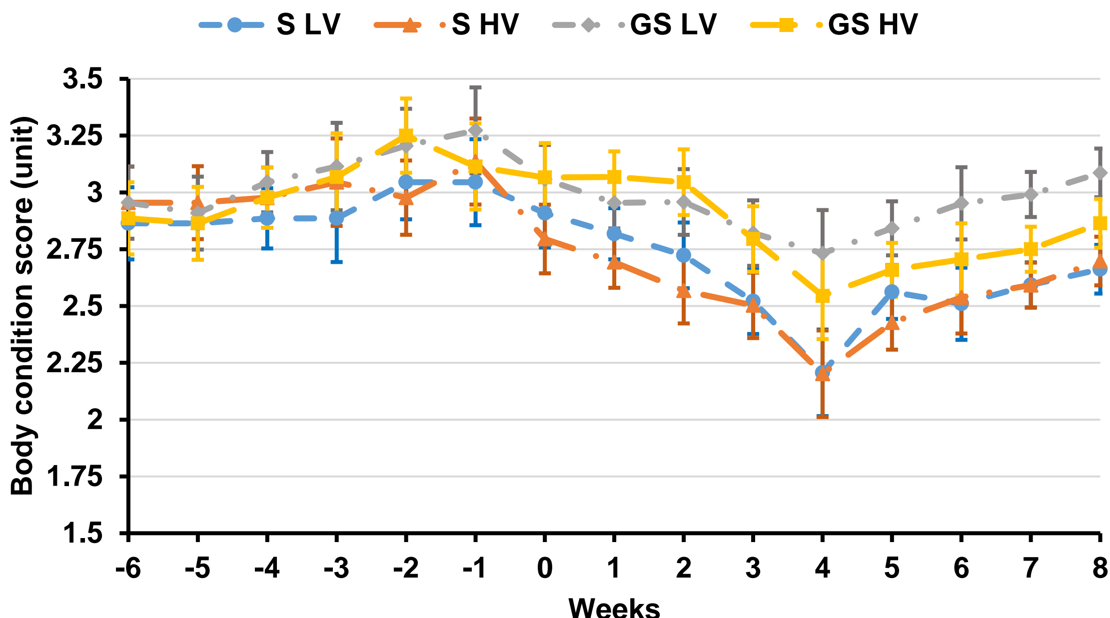


Figure 4. 3. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on the ewe body condition score (C). Error bars indicate SED.

Table 4. 10. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on ewe body condition score (C) and body condition score change.

	S		GS		SED	*P-value*		
	LV	HV	LV	HV		FT	V	FT×V
-6	2.86	2.96	2.96	2.89	0.159	0.920	0.920	0.484
-1	3.05	3.14	3.27	3.11	0.189	0.449	0.799	0.357
0	2.91	2.80	3.06	3.07	0.151	0.060	0.627	0.572
4	2.21	2.20	2.73	2.55	0.191	0.004	0.488	0.505
8	2.66	2.70	3.09	2.86	0.107	<0.001	0.232	0.102
C changes								
-6 to -1	0.18	0.18	0.34	0.22	0.140	0.295	0.549	0.549
0 to 4	-0.72	-0.46	-0.31	-0.56	0.181	0.230	0.999	0.062
0 to 8	-0.26	0.02	-0.01	-0.18	0.157	0.827	0.629	0.054

S= straw, GS= grass silage, LV=low vitamin E supplementation (2.8 mg/kg LW), HV=high vitamin E supplementation (9.0 mg/kg LW), FT= forage type (straw or silage), V= vitamin E, FT×V = interaction between forages and vitamin E

4.3.4.3 Ewe back fat

The initial ewe back fat was similar at week -6 of pregnancy without any difference (*P*>0.05) between the treatment groups (Figure 4.4). There was a FT×V interaction (P<0.05) effect on ewe back fat changes (Table 4.11). The highest back fat was in ewes offered treatment GSLV (3.69 mm; *P*=0.006) at week+4, Ewes offered treatment GSHV had the highest back fat (0.84 mm; *P*=0.046) during the *pre-partum* period (week -6 to -1), while, ewes offered treatment SHV lost the lowest back fat (-0.68 mm; *P*=0.006) during the *post-partum* period. There was no effect (*P*>0.05) of forage type on ewe back fat *pre-partum*, however, at week -3 ewes offered the straw based diets tended to have higher back fat depth than those offered the silage based diets (4.25 vs 3.57 mm; P=0.058). There was an effect (P<0.05) of forage type on ewe back fat depth at weeks +2, and +4, Ewes offered the straw based diets had less back fat at week +2 (3.80 vs 5.11 mm; P=0.003) and week+4 (2.58 vs 3.24 mm; P=0.008) than those offered the grass silage based diets. There was an effect (P<0.05) of forage type on ewe back fat change both *prepartum* and *post-partum.* Ewes offered grass silage gained more back fat *pre-partum* (0.55 vs -0.24 mm; P=0.005), whilst *post-partum* ewes offered the straw based diet tended to loss less back fat during early lactation (-1.24 vs -1.78 mm; P=0.093) and gained more back fat from week 2 to 8 (0.37 vs -0.63 mm; P=0.009). There was no effect (*P*>0.05) of vitamin E supplementation on ewe back fat or back fat change through the experiment.

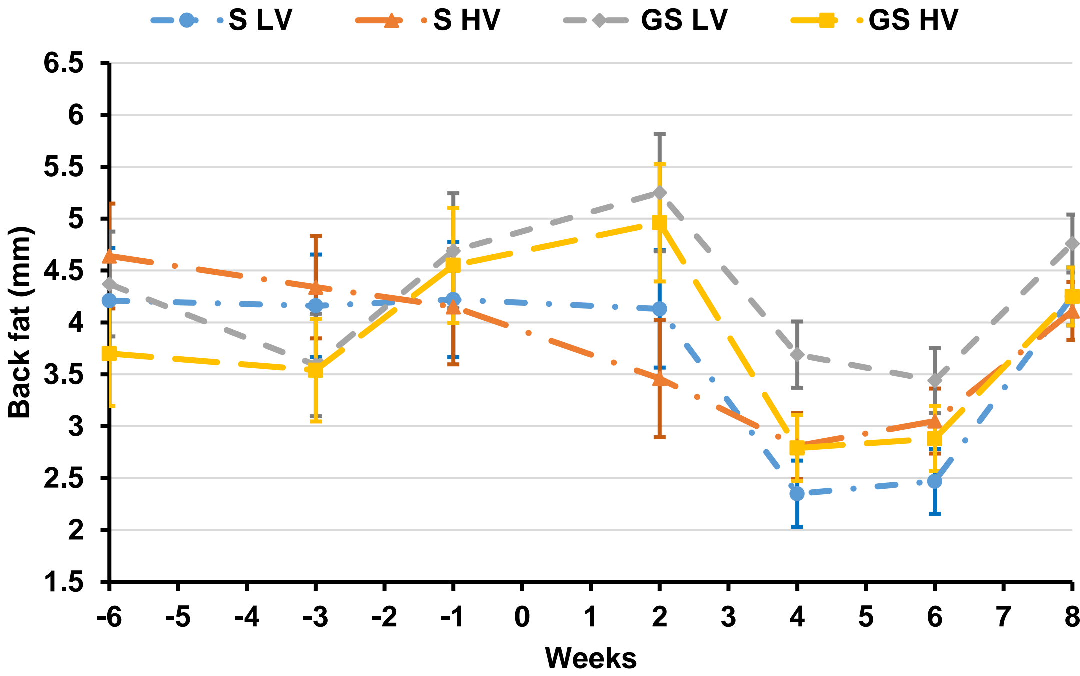


Figure 4. 4. The effect of forage type and vitamin E supplementation during late pregnancy and early lactation on ewe back fat depth. Error bars indicate SED.

Table 4. 11. The effect of forage type and vitamin E supplementation during late pregnancy and early lactation on ewe back fat depth (mm) and back fat change (mm).

	S		GS		SED	*P-value*		
	LV	**HV**	**LV**	**HV**		**FT**	**V**	**FT×V**
-6	4.21	4.64	4.37	3.70	0.505	0.294	0.749	0.139
-1	4.22	4.15	4.69	4.55	0.554	0.278	0.796	0.922
2	4.13	3.46	5.25	4.96	0.565	0.003	0.240	0.638
4	2.35^{a}	2.81^{a}	3.69^{b}	2.79^{a}	0.319	0.008	0.354	0.006
8	4.25	4.11	4.76	4.25	0.279	0.113	0.112	0.346
Back fat change								
-6 to -1	0.01^{ab}	-0.48^{a}	0.25^{ab}	0.84^{b}	0.364	0.005	0.858	0.046
2 to 4	-1.79^{a}	-0.68^{b}	-1.39^{ab}	-2.16^{a}	0.437	0.093	0.593	0.006
2 to 8	0.12	0.62	-0.55	-0.71	0.490	0.009	0.629	0.353

S= straw, GS= grass silage, LV=low vitamin E supplementation (2.8 mg/kg LW), HV=high vitamin E supplementation (9.0 mg/kg LW), FT= forage type (straw or silage), V= vitamin E, FT×V = interaction between forages and vitamin E

[a,b] Means with the same superscript are not significantly different (P<0.05).

4.3.4.4 Ewe eye muscle depth

The initial ewe eye muscle depth was similar at week -6 of pregnancy without any difference (P>0.05) between the treatment groups (Table 4.12). There was no FT×V interaction (P>0.05) on ewe eye muscle depth and eye muscle depth change (Figure 4.5). However, ewes offered treatment GSHV tended to have the highest eye muscle depth (24.3 mm; P=0.061). There was no effect (P>0.05) of forage type or vitamin E supplementation on ewe muscle depth through the experiment.

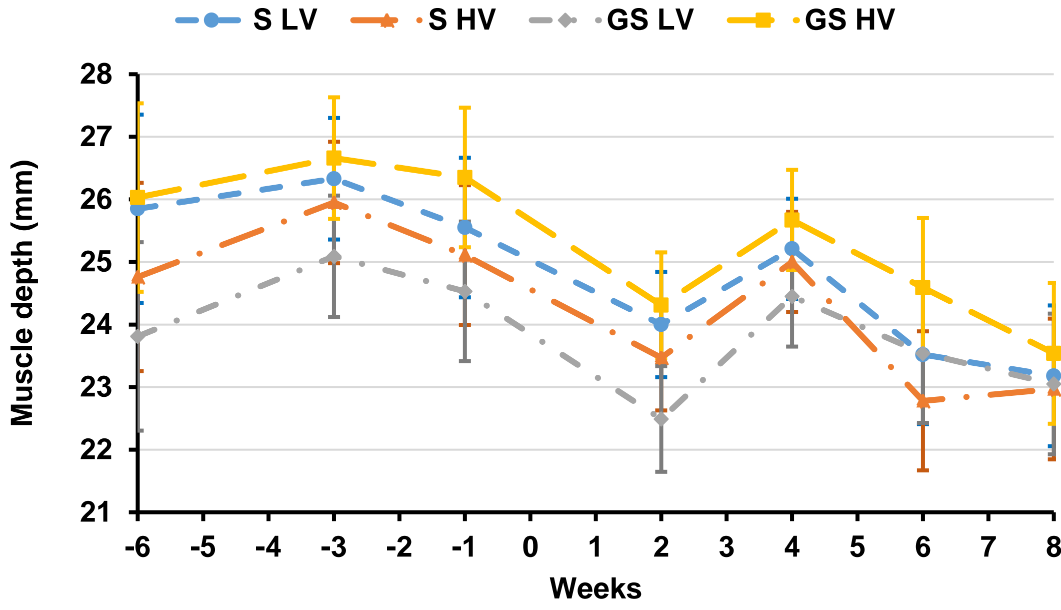


Figure 4. 5. The effect of forage type and vitamin E supplementation during late pregnancy and early lactation on ewe muscle depth. Error bars indicate SED.

Table 4. 12. The effect of forage type and vitamin E supplementation during late pregnancy and early lactation on the ewe eye muscle depth (mm) and eye muscle depth change (mm).

	S		GS		SED	*P-value*		
	LV	HV	LV	HV		FT	V	FT×V
-6	25.9	24.8	23.8	26.0	1.505	0.719	0.597	0.132
-1	25.6	25.1	24.5	26.4	1.116	0.890	0.389	0.161
2	24.0	23.5	22.5	24.3	0.843	0.581	0.294	0.061
4	25.2	25.0	24.5	25.7	0.802	0.945	0.385	0.219
8	23.2	23.0	23.1	23.5	1.125	0.787	0.861	0.670
Eye muscle depth change								
-6 to -1	-0.29	0.35	0.70	0.33	1.772	0.700	0.917	0.690
2 to 4	1.20	1.38	2.38	1.37	0.553	0.148	0.295	0.139
2 to 8	-0.82	-0.65	0.75	-0.77	1.094	0.360	0.396	0.286

S= straw, GS= grass silage, LV=low vitamin E supplementation (2.8 mg/kg LW), HV=high vitamin E supplementation (9.0 mg/kg LW), FT= forage type (straw or silage), V= vitamin E, FT×V = interaction between forages and vitamin E

4.3.4.5. Placenta

There was a FT×V interaction (*P*<0.05) on placenta measurements (Table 4.13). Ewes offered treatment SHV had the highest placenta weight (910 g; *P*=0.022), cotyledon weight (299 g; *P*=0.007), lowest cotyledon efficiency (38.9 g; *P*=0.024) and tended to have highest cotyledon number (100; *P*=0.077). Ewes offered treatment SLV tended to have the highest placenta efficiency (14.2 g; *P*=0.053). There was an effect (*P*<0.05) of forage type on placenta measurements. Ewes offered the silage based treatments had the highest placenta weight (900 vs 804 g; P=0.041) and lowest placenta efficiency (13.3 vs 11.1;

$P<0.001$). There was an effect ($P<0.05$) of vitamin E supplementation on placenta measurements. Ewes supplemented with high vitamin E had a higher placenta weight (904 vs 801 g; $P=0.027$) and cotyledon weight (262 vs 221 g; $P=0.027$).

Table 4. 13. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on the placental weight and placental efficiency.

	S		GS		SED	*P-value*		
	LV	HV	LV	HV		FT	V	FT×V
Placenta weight (g)	699[a]	910[b]	902[b]	898[b]	62.90	0.041	0.027	0.022
Cotyledons number	84.4	100	97.2	94.7	6.950	0.448	0.192	0.077
Cotyledons weight (g)	207[a]	299[b]	234[a]	224[a]	0.025	0.190	0.027	0.007
Placenta efficiency[1]	14.2[c]	12.5[b]	10.8[a]	11.4[ab]	0.795	<0.001	0.294	0.053
cotyledons efficiency[2]	49.1[b]	38.9[a]	43.0[ab]	46.3[ab]	4.006	0.829	0.233	0.024
cotyledons density[3]	0.12	0.11	0.11	0.11	0.011	0.309	0.507	0.414

S= straw, GS= grass silage, LV=low vitamin E supplementation (2.8 mg/kg LW), HV=high vitamin E supplementation (9.0 mg/kg LW), FT= forage type (straw or silage), V= vitamin E, FT×V = interaction between forages and vitamin E

[a,b] Means with the same superscript are not significantly different ($P<0.05$).

[1]Placenta efficiency: gram of lamb produced per gram of placenta

[2]Cotyledon efficiency: gram of lamb produced per gram of cotyledon

[3]Cotyledon density: number of cotyledon/ placenta weight.

4.3.4.6. Colostrum yield and composition

There was no FT×V interaction (*P*>0.05) on colostrum yield and colostrum component yields (Table 4.14). There was a FT×V interaction (*P*<0.05) on colostrum vitamin E concentration, ewes offered treatment SHV had the highest vitamin E concentration (24.4 mg/ kg, *P*=0.045). There was no effect (*P*>0.05) of forage type on colostrum yield, colostrum composition and component yield. There was an effect (*P*<0.05) of vitamin E supplementation on colostrum vitamin E content and yield. High vitamin E supplementation increased colostrum vitamin E concentration (22.2 vs 13.9 mg/ kg; P<0.001) and vitamin E yield (2.30 vs 1.66 mg/ h; P=0.036) compared with low vitamin E supplementation. High vitamin E supplementation tended to increase colostrum fat concentration (128 vs 113 g/ kg; P=0.084), and decrease lactose yield (5.20 vs 6.65 g/ h; P=0.070) compared with low vitamin E supplementation.

4.3.4.7. Milk yield and composition

There was no FT×V interaction (*P*>0.05) on milk yield or composition (Table 4.15). However, ewe fed GSHV treatment tended to have the highest milk secretion rate (131 ml/ h; *P*=0.065). There was a FT×V interaction (*P*<0.05) on milk component yield, ewe fed GSHV treatment had the highest milk lactose yield (6.65 g/ h; *P*=0.041). There was no effect (*P*>0.05) of forage type or vitamin E supplementation on milk yield, milk composition and component yield.

4.3.4.8. Litter weight and lamb growth

There was no FT×V of interaction (*P*>0.05) on litter weight and litter weight change (Table 4.16). However, litters from ewes offered treatment SHV tended to have the highest birthweight (11.0 kg; *P*=0.095). There was an effect (*P*<0.05) of forage type on litter weight. Litter weight was higher in ewes offered the straw based treatments compared with those offered the silage based treatments at weeks +6 (35.77 vs 32.96; P=0.005), +7 (39.5 vs 36.18; P=0.001) and +8 (42.96 vs 39.53; P<0.001). There was an effect (*P*<0.05) of forage type on litter weight gain which was higher in lambs from ewes offered the straw based diets compared with that of those offered the silage based diets from birth to week+8 (32.73 vs 29.91 kg; P<0.001) and from week+4 to week +8 (15.81 vs 13.52 kg; P<0.001). Vitamin E supplementation tended to effect litter birth weight. Litters born to ewes supplemented with high vitamin E tended to have a higher birth weight (10.44 vs 9.78; P=0.056). Litter weight was also higher in high vitamin E supplemented ewes than low at week +8 (42.19 vs 40.30; P=0.023). There was no effect (*P*>0.05) of vitamin E supplementation on litter weight gain. However, litters from ewes offered high vitamin E tended to have a higher weight gain than

those of ewes supplemented with low vitamin E between weeks +4 to +8 (15.28 vs 14.05; P=0.051).

Table 4. 14. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on colostrum yield, colostrum composition and component yields.

	S		GS		SED	*P-value*		
	LV	HV	LV	HV		FT	V	FT×V
Secretion rate (ml/h)	119	99.0	121	110	13.90	0.531	0.134	0.699
Composition (g/kg)								
Total Solid	247	271	267	237	22.84	0.684	0.860	0.115
Fat	109	132	116	123	10.88	0.905	0.084	0.302
Total Protein	81.9	87.9	71.6	78.9	12.02	0.274	0.444	0.940
Lactose	53.2	41.2	57.0	50.8	7.860	0.255	0.134	0.609
Vitamin E (mg/kg)	12.2[a]	24.4[c]	15.6[ab]	19.9[bc]	2.520	0.766	<0.001	0.045
Component yield (g/h)								
Total Solid	29.6	27.2	32.1	26.0	4.583	0.845	0.211	0.573
Fat	13.4	13.1	13.8	13.2	2.264	0.850	0.800	0.919
Total Protein	9.74	9.04	8.45	8.82	1.938	0.591	0.907	0.700
Lactose	6.30	4.30	7.00	6.10	1.020	0.110	0.070	0.497
Vitamin E yield (mg/h)	1.47	2.45	1.84	2.14	0.393	0.932	0.036	0.240

S= straw, GS= grass silage, LV=low vitamin E supplementation (2.8 mg/kg LW), HV=high vitamin E supplementation (9.0 mg/kg LW), FT= forage type (straw or silage), V= vitamin E, FT×V = interaction between forages and vitamin E

Table 4. 15. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on milk yield, milk composition and component yields.

	S		GS		SED	*P-value*		
	LV	HV	LV	HV		FT	V	FT×V
Secretion rate (ml/h)	125	115	116	131	9.090	0.731	0.698	0.065
Composition (g/kg)								
Total Solid	188	200	209	200	11.74	0.212	0.852	0.236
Fat	52.2	59.4	65.7	59.9	7.707	0.211	0.907	0.244
Total Protein	41.7	43.0	44.0	43.2	1.436	0.243	0.816	0.304
Lactose	51.4	51.3	50.1	50.9	0.814	0.164	0.571	0.405
Component yield (g/h)								
Total Solid	23.5	23.1	24.4	26.3	2.379	0.235	0.682	0.504
Fat	6.49	6.85	7.82	7.94	1.145	0.149	0.771	0.886
Total Protein	5.24	5.00	5.11	5.62	0.423	0.419	0.652	0.223
Lactose	6.47	5.93	5.77	6.65	0.460	0.969	0.619	0.041

S= straw, GS= grass silage, LV=low vitamin E supplementation (2.8 mg/kg LW), HV=high vitamin E supplementation (9.0 mg/kg LW), FT= forage type (straw or silage), V= vitamin E, FT×V = interaction between forages and vitamin E

Table 4. 16. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on the ewe litter weights (kg) and litter weight changes (kg).

	S		GS		SED	*P-value*		
	LV	HV	LV	HV		FT	V	FT×V
BW	9.74	11.0	9.80	9.90	0.473	0.135	0.056	0.095
1	13.5	14.4	13.1	13.4	0.902	0.309	0.344	0.653
2	18.5	18.7	17.4	18.3	0.961	0.297	0.411	0.622
3	23.1	23.2	22.2	22.8	0.995	0.344	0.610	0.709
4	27.3	27.0	25.8	26.8	1.079	0.290	0.638	0.427
5	31.1	31.5	29.6	31.2	1.122	0.265	0.225	0.433
6	35.2	36.3	32.7	33.2	1.272	0.005	0.417	0.818
7	39.6	39.4	35.1	37.2	1.234	0.001	0.305	0.194
8	42.3	43.6	38.3	40.8	1.090	<0.001	0.023	0.455
Weight changes								
Birth to W+4	17.6	16.2	16.2	16.9	0.885	0.593	0.585	0.117
Birth to W+8	32.6	32.8	29.0	30.9	0.936	<0.001	0.123	0.205
W+4 to W+8	15.0	16.6	13.1	14.0	0.845	<0.001	0.051	0.570

S= straw, GS= grass silage, LV=low vitamin E supplementation (2.8 mg/kg LW), HV=high vitamin E supplementation (9.0 mg/kg LW), FT= forage type (straw or silage), V= vitamin E, FT×V = interaction between forages and vitamin E

4.3.5. Ewes and lamb plasma vitamin E and IgG

4.3.5.1. Ewe plasma vitamin E

The initial ewe plasma vitamin E concentration was similar at week -6 of pregnancy without any difference (*P*>0.05) between the treatment groups (Table 4.17). There was an effect (P<0.05) of time on ewe plasma vitamin E concentration (Table 4.17). Ewes plasma vitamin E increased from 1.69 (µg/ml) at week -6 to 3.20 (µg/ml) at week -1 then declined to 2.58 (µg/ml) at week 2. There was effect of forage type (*P*<0.05) on plasma vitamin E at week -1. Ewes offered silage had a higher plasma vitamin E concentration than those offered straw (3.61 vs 2.88 µg/ml; P=0.005). There was also an effect of vitamin E supplementation (P<0.05) on ewe plasma vitamin E concentration. High vitamin E supplementation increased ewe plasma vitamin E. There was a FT×V interaction (*P*<0.05) on plasma vitamin E concentration. At week -1 plasma vitamin E was lowest in ewes offered treatment SLV (1.82 µg/ml, *P*=0.028). There was no time × FT interaction (*P*>0.05) on ewe plasma vitamin E. There was a time × V interaction (*P*<0.05) effect on ewe plasma vitamin E. Plasma vitamin E increased in ewes offered the high vitamin E supplemented treatments as the experiment progressed. There was no time × FT × V interaction (*P*>0.05) on ewe plasma vitamin E concentration.

4.3.5.2. Lamb's plasma vitamin E and IgG

There was no FT×V interaction (*P*>0.05) on lamb plasma vitamin E and IgG concentration at week +2 (Table 4.18). There was no effect of forage type (P>0.05) on lamb plasma vitamin E and IgG concentration. There was an effect of vitamin E supplementation (P<0.05) on lamb plasma vitamin E. Lamb from ewes supplemented with high vitamin E had a higher plasma vitamin E concentration. There was no effect (*P*>0.05) of vitamin E supplementation on lamb plasma IgG.

Table 4. 17. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on the ewe blood plasma vitamin E (µg/ml).

Vitamin E	S		GS		SED	*P-value*		
	LV	HV	LV	HV		FT	V	FT×V
Ewes plasma								
Week -6	1.63	1.52	1.73	1.64	0.213	0.479	0.519	0.985
Week -1	1.82[a]	3.94[c]	3.11[b]	4.12[c]	0.307	0.005	<0.001	0.026
Week +2	1.57	3.46	1.94	3.16	0.556	0.940	0.001	0.406

S= straw, GS= grass silage, LV=low vitamin E supplementation (2.8 mg/kg LW), HV=high vitamin E supplementation (9.0 mg/kg LW), FT= forage type (straw or silage), V= vitamin E, FT×V = interaction between forages and vitamin E

[a,b] Means with the same superscript are not significantly different (*P*<0.05).

SED values: FT= 0.193, V=0.193, FT×V=0.273, Time= 0.172, Time×FT=0.276, Time×V=0.276, Time×FT×V=0.392; P-values: FT= 177, V=<0.001., FT×V=0.086, Time= <0.001, Time×FT=<0.324, Time×V=<0.001, Time×FT×V=0.489

Table 4. 18. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on lamb blood plasma vitamin E and IgG at week +2.

Lambs plasma	S		GS		SED	*P-value*		
	LV	HV	LV	HV		FT	V	FT×V
Vitamin E (µg/ml)	3.38	6.46	2.69	5.77	0.930	0.315	<0.001	0.999
IgG (mg/ml)	7.56	6.11	6.39	6.76	1.503	0.809	0.620	0.405

S= straw, GS= grass silage, LV=low vitamin E supplementation (2.8 mg/kg LW), HV=high vitamin E supplementation (9.0 mg/kg LW), FT= forage type (straw or silage), V= vitamin E, FT×V = interaction between forages and vitamin E

4.3.6. Blood plasma metabolites

4.3.6.1. Plasma glucose

There was effect (P<0.05) of time on plasma glucose concentration (Table 4.19). Plasma glucose concentration increased from 2.09 mmol/ l at week -6 to 3.27 mmol/ l at week -1 and 3.47 mmol/ l at week +4 and declined to 3.24 mmol/ l at week +8. There was no effect (P>0.05) of forage type on plasma glucose concentration. There was an effect (P<0.05) of vitamin E supplementation on plasma glucose concentration. High vitamin E supplementation had no effect on plasma glucose concentration in ewes fed straw based diet, while high vitamin E supplementation decreased plasma glucose concentration in ewe offered GS based diet at week +6 (3.60 vs 2.87 mmol/ l; P=0.025) and week +8 (3.66 vs 2.81 mmol/ l; P=0.016). There was a FT×V interaction (P<0.05) on plasma glucose, the highest glucose concentration was in ewes offered treatment GSLV in both weeks +6 (3.60 mmol/ l; P=0.011) and week+8 (3.66 mmol/ l; P=0.003). There was no time × FT, time × V or time × FT × V interaction (P>0.05) effect on plasma glucose.

Table 4. 19. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on the ewe blood plasma glucose (mmol/ l).

Weeks	S		GS		SED	*P-value*		
	LV	HV	LV	HV		FT	V	FT×V
-6	2.12	2.13	2.06	2.04	0.171	0.580	0.984	0.908
-3	2.93	2.98	3.02	2.94	0.129	0.805	0.861	0.536
-1	3.26	3.33	3.20	3.26	0.221	0.665	0.688	0.987
+2	3.36	3.49	3.67	3.42	0.220	0.450	0.675	0.239
+4	3.43	3.50	3.51	3.44	0.173	0.944	0.992	0.581
+6	3.32^{ab}	3.38^{ab}	3.60^{b}	2.87^{a}	0.190	0.411	0.025	0.011
+8	3.19^{ab}	3.30^{ab}	3.66^{b}	2.81^{a}	0.190	0.925	0.016	0.003

S= straw, GS= grass silage, LV=low vitamin E supplementation (2.8 mg/kg LW), HV=high vitamin E supplementation (9.0 mg/kg LW), FT= forage type (straw or silage), V= vitamin E, FT×V = interaction between forages and vitamin E

^{a,b} Means with the same superscript are not significantly different (P<0.05).

SED values: FT= 0.048, V=0.048, FT×V=0.067, Time= 0.091, Time×FT=0.128, Time×V=0.128, Time×FT×V=0.181.

P-values: FT= 0.749, V=0.043, FT×V=0.003, Time= <0.001, Time×FT=<0.831, Time×V=0.136, Time×FT×V=0.087

4.3.6.2. Plasma beta-hydroxybutyrate (BHB)

There was effect (P<0.05) of time on plasma BHB (Table 4.20). Plasma BHB concentration reduced from 1.03 mmol/ l at week -6 to 0.73 mmol/ l at week -1 and 0.68 mmol/ l at week +4 and increased to 0.92 mmol/ l at week +8. There was an effect (P<0.05) of forage type on plasma BHB. Plasma BHB was higher in ewes offered the straw based treatments compared to those offered the silage based treatments in week +4 (0.75 vs 0.61 mmol/ l;

P=0.05) and tended to be lower during week -1 (0.67 vs 0.79 mmol/ l; P=0.099) and week +6 (0.73 vs 0.92 mmol/ l; P=0.090). There was effect (P<0.05) of vitamin E supplementation on plasma BHB. Ewes offered high vitamin E supplementation increased plasma BHB at week +8 (0.93 vs 0.72 mmol/ l; P=0.035) and tended to increase BHB at week +6 (0.93 vs 0.73 mmol/ l; P=0.067). There was no FT×V interaction (P>0.05) effect on plasma BHB. There was no time × FT, time × V or time × FT × V interaction (P>0.05) on plasma BHB.

Table 4. 20. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on the ewe blood plasma beta-hydroxybutyrate (BHB) (mmol/ l).

Weeks	S		GS		SED	*P-value*		
	LV	HV	LV	HV		FT	V	FT×V
-6	1.14	1.18	0.75	1.07	0.311	0.273	0.439	0.529
-3	0.73	0.68	0.59	0.71	0.095	0.345	0.795	0.330
-1	0.68	0.65	0.86	0.72	0.099	0.099	0.218	0.430
+2	0.68	0.78	0.62	0.93	0.179	0.747	0.121	0.442
+4	0.76	0.74	0.59	0.63	0.091	0.050	0.849	0.658
+6	0.65	0.82	0.80	1.04	0.146	0.090	0.067	0.722
+8	0.78	0.85	0.67	1.39	0.126	0.787	0.035	0.158

S= straw, GS= grass silage, LV=low vitamin E supplementation (2.8 mg/kg LW), HV=high vitamin E supplementation (9.0 mg/kg LW), FT= forage type (straw or silage), V= vitamin E, FT×V = interaction between forages and vitamin E
SED values: FT= 0.061, V=0.061, FT×V=0.086, Time= 0.097, Time×FT=0.141, Time×V=0.141, Time×FT×V=0.199.
P-values: FT= 0.762, V=0.041, FT×V=0.137, Time= 0.027, Time×FT=0.216, Time×V=0.265, Time×FT×V=0.494

4.3.6.3. Non-esterified fatty acids (NEFA)

There was effect (P<0.05) of time on plasma NEFA concentration (Table 4.21). Plasma NEFA concentration reduced from 1.37 mmol/ l at week -6 to 0.25 mmol/ l at week -1 then increased to 0.54 mmol/ l at week +4 and declined to 0.20 mmol/ l at week +8.

There was also an effect (P<0.05) of forage type on plasma NEFA concentration. Plasma NEFA concentration was higher in ewes offered the straw based diet compared to those offered the silage based treatments from week -3 to +4. There was no effect (P>0.05) of vitamin E supplementation on plasma NEFA concentration. There was no FT×V interaction (P>0.05) on plasma NEFA concentration throughout the experiment. There was a time × FT interaction (P<0.05) on plasma NEFA concentration. Plasma NEFA concentration in all treatments reduced both *pre-partum* and *post-partum* as the experiment progressed. The reduction was higher in ewes offered the silage based treatments compared with those offered the straw based treatments. There was no time × V or time × FT × V interaction (P>0.05) on plasma NEFA concentration.

Table 4. 21. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on the ewe blood plasma non-esterified fatty acids (NEFA) (mmol/ l).

Weeks	S		GS		SED	*P-value*		
	LV	HV	LV	HV		FT	V	FT×V
-6	1.36	1.46	1.26	1.42	0.330	0.766	0.588	0.902
-3	0.36	0.42	0.13	0.12	0.053	<0.001	0.467	0.391
-1	0.32	0.39	0.13	0.13	0.059	<0.001	0.381	0.419
+2	0.75	0.68	0.28	0.16	0.174	0.001	0.464	0.849
+4	0.68	0.88	0.30	0.30	0.169	0.001	0.398	0.398
+6	0.15	0.12	0.17	0.12	0.040	0.803	0.150	0.636
+8	0.17	0.15	0.10	0.12	0.043	0.116	0.971	0.569

S= straw, GS= grass silage, LV=low vitamin E supplementation (2.8 mg/kg LW), HV=high vitamin E supplementation (9.0 mg/kg LW), FT= forage type (straw or silage), V= vitamin E, FT×V = interaction between forages and vitamin E

SED values: FT= 0.065, V=0.065, FT×V=0.915, Time= 0.087, Time×FT=0.131, Time×V=0.131, Time×FT×V=0.185.

P-values: FT= 0.002, V=0.743, FT×V=0.497, Time= <0.001, Time×FT=0.011, Time×V=0.559, Time×FT×V=0.790

4.3.6.4. Total protein

There was effect (P<0.05) of time on plasma total protein concentration (Table 4.22). Plasma total protein concentration reduced from 58.2 g/ l at week -6 to 53.9 g/ l at week -1, then increased to 56.5 g/ l at week +4 and declined to 55.6 g/ l at week +8. There was an effect (P<0.05) of forage type on plasma protein concentration. Plasma protein concentration was higher in ewes offered the straw based treatment compared with those offered the silage based treatments in week +6 (58.3 vs 54.9 g/ l; P=0.012) and tended to be higher at both week -3 (57.7 vs 55.2 g/ l; P=0.069) and week +8 (56.9 vs 54.2 g/ l; P=0.069). There was an effect (P<0.05) of vitamin E supplementation on plasma protein concentration. High vitamin E supplementation reduced ewe plasma protein concentration at week +6 (54.6 vs 58.6 g/ l; P=0.004) and week +8 (53.6 vs 57.6 g/ l; P=0.013). There was no FT×V interaction (P>0.05) on plasma total protein concentration. However, ewes offered treatment SLV tended to have a highest plasma protein concentration at week +6 (61.4 g/ l; P=0.073). There was no time × FT, time ×V or time × FT × V interaction (P>0.05) on plasma total protein concentration.

Table 4. 22. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on the ewe blood plasma total protein (g/ l)

Weeks	S		GS		SED	*P-value*		
	LV	HV	LV	HV		FT	V	FT×V
-6	60.7	56.5	56.8	58.6	2.552	0.633	0.540	0.117
-3	59.8	55.7	55.0	55.4	1.825	0.069	0.162	0.104
-1	55.3	53.3	53.2	54.3	2.065	0.710	0.740	0.297
+2	57.9	57.9	56.0	57.6	2.758	0.592	0.678	0.686
+4	57.7	53.2	56.3	50.3	6.900	0.676	0.299	0.880
+6	61.4	55.1	55.8	54.1	1.654	0.012	0.004	0.073
+8	59.4	54.5	55.8	52.6	1.996	0.069	0.013	0.555

S= straw, GS= grass silage, LV=low vitamin E supplementation (2.8 mg/kg LW), HV=high vitamin E supplementation (9.0 mg/kg LW), FT= forage type (straw or silage), V= vitamin E, FT×V = interaction between forages and vitamin E

SED values: FT= 0.891, V=0.891, FT×V=1.260, Time= 0.959, Time×FT=1.540, Time×V=1.540, Time×FT×V=2.177.

P-values: FT= 0.064, V=0.074, FT×V=0.056, Time= 0.005, Time×FT=0.723, Time×V=0.138, Time×FT×V=0.763

4.3.6.5. Albumin

There was effect (*P*<0.05) of time on plasma total albumin concentration (Table 4.23). Plasma albumin concentration increased from 28.1 g/ l at week -6 to 30.4 g/ l at week -1 and to 32.2 g/ l at week +4 then declined to 27.3 g/ l at week +8. There was also an effect (*P*<0.05) of forage type on plasma albumin concentration. Plasma albumin concentration was higher in ewes offered the straw based treatments compared with those offered the silage based treatments throughout the experiment. There was no effect (*P*>0.05) of vitamin E supplementation on plasma albumin concentration. There was no FT×V interaction (*P*>0.05) on plasma albumin concentration throughout the experiment. There was a time × FT interaction (*P*<0.05) on plasma albumin concentration. Plasma albumin concentration of ewes offered the straw based treatments was higher than that of ewes offered the silage based treatments silage from week-6 to -1. There was no time × V or time × FT × V interaction (*P*>0.05) on plasma albumin concentration.

Table 4. 23. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on the ewe blood plasma albumin (g/ l).

Weeks	S		GS		SED	*P-value*		
	LV	HV	LV	HV		FT	V	FT×V
-6	28.7	27.9	28.4	29.1	1.146	0.580	0.991	0.375
-3	31.4	31.2	29.5	29.8	0.804	0.010	0.959	0.780
-1	31.2	32.0	29.3	29.1	1.379	0.031	0.810	0.546
+2	33.7	32.7	31.7	31.9	0.878	0.041	0.513	0.349
+4	34.2	32.5	31.3	30.8	0.861	0.002	0.085	0.398
+6	30.2	28.4	27.8	27.6	0.814	0.014	0.110	0.179
+8	28.7	27.7	27.1	25.9	1.019	0.031	0.152	0.887

S= straw, GS= grass silage, LV=low vitamin E supplementation (2.8 mg/kg LW), HV=high vitamin E supplementation (9.0 mg/kg LW), FT= forage type (straw or silage), V= vitamin E, FT×V = interaction between forages and vitamin E

SED values: FT= 0.418, V=0.418, FT×V=0.592, Time= 0.538, Time×FT=0.819, Time×V=0.819, Time×FT×V=1.159.

P-values: FT= 0.005, V=0.174, FT×V=0.295, Time= <0.001, Time×FT=0.050, Time×V=0.627, Time×FT×V=0.472

4.3.6.6. Urea

There was effect (*P*<0.05) of time on plasma total urea (Table 4.24). Plasma urea concentration increased from 3.41 mmol/ l at week -6 to 3.76 mmol/ l at week -1 and to 5.40 mmol/ l at week +4, then declined to 4.86 mmol/ l at week +8. There was effect (*P*<0.05) of forage type on plasma urea. Plasma urea concentration was higher in ewes offered the straw based treatments compared to those offered the silage based treatments in weeks -3, -1 and +2. There was no effect (*P*>0.05) of vitamin E supplementation on plasma urea. However, ewes supplemented with high vitamin E tended to have a lower plasma urea at week -1 (3.34 vs 4.28 mmol/ l; *P*=0.097). There was no FT×V interaction (*P*>0.05) on plasma urea throughout the experiment. There was a trend time × FT interaction (*P*<0.05) effect on plasma urea. During the *post-partum* period, plasma urea increased in ewes offered the straw based treatments as the experiment progressed, while in ewes offered the silage based treatments it reduced. There was no time × V or time × FT × V interaction (*P*>0.05) on plasma urea.

Table 4. 24. The effect of forage type and vitamin E supplementation in late pregnancy and early lactation on the ewe blood plasma urea (mmol/ l)

Week	S		GS		SED	*P-value*		
	LV	HV	LV	HV		FT	V	FT×V
-6	3.57	3.15	4.00	2.93	0.939	0.874	0.281	0.631
-3	4.22	3.53	2.24	1.79	0.713	0.002	0.281	0.818
-1	5.15	4.08	3.41	2.61	0.745	0.009	0.097	0.799
+2	6.45	5.76	4.44	4.11	0.836	0.007	0.408	0.767
+4	6.00	5.91	5.36	4.32	1.015	0.141	0.445	0.513
+6	4.61	5.27	4.12	4.88	1.037	0.558	0.347	0.947
+8	4.31	4.76	5.15	5.24	1.217	0.454	0.755	0.841

S= straw, GS= grass silage, LV=low vitamin E supplementation (2.8 mg/kg LW), HV=high vitamin E supplementation (9.0 mg/kg LW), FT= forage type (straw or silage), V= vitamin E, FT×V = interaction between forages and vitamin E
SED values: FT= 0.375, V=0.375, FT×V=0.531, Time= 0.416, Time×FT=0.662, Time×V=0.662, Time×FT×V=0.936.
P-values: FT= 0.034, V=0.420, FT×V=0.895, Time= <0.001, Time×FT=0.056, Time×V=0.402, Time×FT×V=0.831

4.3.7. Diet digestibility

There was no FT×V interaction (P>0.05) on any of the digestibility coefficients (Table 4.25). There was an effect (P<0.05) of forage type on DM, OM, NDF, ADF, GE and vitamin E digestibility. The grass silage based treatments had higher DM digestibility (0.79 vs 0.73; P=0.027), organic matter (0.80 vs 0.74; P=0.023), NDF digestibility (0.77vs 0.69; P=0.009), ADF digestibility (0.77 vs 0.57; P<.001), gross energy digestibility (0.79 vs 0.65; P<.001) and vitamin E digestibility (0.62 vs 0.42; P=0.003) than the straw based treatments. Vitamin E supplementation had an effect (P<0.05) on vitamin E digestibility. High vitamin E supplementation increased vitamin E digestibility (0.62 vs 0.42; P=0.003).

Table 4. 25. The effect of forage type and vitamin E supplementation in late pregnancy on diet digestibility

Digestibility	S		GS		SED	*P-value*		
	LV	HV	LV	HV		FT	V	FT×V
Diet digestibility (kg/ kg)								
DM	0.72	0.73	0.79	0.78	0.036	0.027	0.943	0.594
OM	0.73	0.75	0.81	0.80	0.034	0.023	0.981	0.587
CP	0.70	0.71	0.74	0.73	0.041	0.294	0.986	0.639
NDF	0.68	0.69	0.77	0.76	0.039	0.009	0.973	0.774
ADF	0.55	0.58	0.76	0.77	0.055	<0.001	0.691	0.753
GE (MJ/kg DM)	0.63	0.66	0.79	0.78	0.048	<0.001	0.842	0.608
Vitamin E (mg/ mg)	0.30	0.54	0.54	0.70	0.073	0.003	0.003	0.487
Energy values (MJ/kg DM)								
DE	11.7	11.9	11.9	11.3	1.109	0.833	0.769	0.619
ME	9.47	9.60	9.66	9.14	0.899	0.833	0.769	0.619

S= straw, GS= grass silage, LV=low vitamin E supplementation (2.8 mg/kg LW), HV=high vitamin E supplementation (9.0 mg/kg LW), FT= forage type (straw or silage), V= vitamin E, FT×V = interaction between forages and vitamin E

4.4. Discussion

In the current experiment, straw intake was lower than expected both *pre-partum* and *post-partum*. However, it was in agreement with the results of Capper *et al.* (2007). In addition, straw intake was lower than grass silage intake. The rations were formulated on the basis that the grass silage DM intake would be approximately 1.0 and 1.5 kg/day during late pregnancy and lactation according to AHDB (2016). However, they were considerably higher than anticipated. Helander *et al.* (2014) documented a higher grass silage intake both *pre-partum* and *post-partum* than recorded in the current study. Decreasing particle size of grass silage increases feed intake in dairy cows (Tayyab *et al.*, 2018) and pregnant ewes (Deswysen *et al.*, 1978; Elizalde and Henríquez., 2009; Helander *et al.*, 2014 and AHDB, 2016). In the current experiment, the short chop length of the grass silage may have contributed to the high grass silage intake during late pregnancy. This could be due to a reduction in the time required for chewing, prior to swallowing (Tayyab *et al.*, 2018). The current findings are similar to previously published works on the effect of chop length (grass silage or maize silage) on DMI in dairy cattle (Tayyab *et al.*, 2018).

During the *pre-partum* period, ewes offered the grass silage based diets had a higher total dry matter intake and gained more LW and back fat than those on the straw based diets. Similarly, *post-partum* ewes offered the grass silage based diet had a higher LW, C, and back fat depth. In addition, they lost less LW during early lactation. In the current experiment, the diets were formulated to provide similar levels of ME and MP. The higher than expected DMI intake of ewes on grass silage based diets meant that ME and MP supply were considerably higher in this group. Blood plasma glucose, glucagon, insulin, NEFA, BHB, triglycerides and total lipids are predictors of energy status in the body (Caldeira *et al.*, 2007). Substantial information on an animals energy status can be provided by blood plasma insulin, glucose and NEFA diagnosis (Caldeira *et al.*, 2007). The concentration of blood plasma NEFA reflects energy balance change (Pedernera *et al.*, 2008; Giuliodori *et al.*, 2011) and mobilisation of lipid (Butler, 2014). High plasma NEFA concentration is a clear indicator of negative energy balance (Caldeira *et al.*, 2007). In the current experiment, the relatively low ewes plasma NEFA concentration during late pregnancy indicate that ewes experienced positive energy balance and gained more weight and back fat on the silage based diet. In addition, they tended to loss less LW during early lactation.

In the current experiment, forage type had no effect on litter birth weight. However, ewes offered the grass silage based diets had a higher placenta weight and lower placenta efficiency. In addition, ewes offered the straw based diets mobilised more body tissue to maintain similar colostrum and milk component yields. However, the lambs grew faster.

Lambs in all treatments were offered creep *ad-libitum* from weeks 4 to 8. Lambs daily creep intakes may have been higher in the straw than silage based diets. This could be the reason for higher weight gain during this period, and the higher final weaning weight of lambs on the straw based treatments.

In the UK, concentrate feed for pregnant and lactating ewes typically contain 100-150 mg/kg DM of vitamin E (EBLEX, 2014; AHDB, 2016). For pregnant ewes, the NRC (2007) recommend that the minimum vitamin E requirements for ewes during late pregnancy and lactation should be 5.6 IU/kg body weight. The level of vitamin E supplied by the low vitamin E treatments was slightly higher at 240 mg/kg DM (2.8 mg/kg LW) than the typical supplementation level in the UK, but lower than the NRC (2007) recommendations. Vitamin E supply from the high vitamin E treatments was higher at 750 mg/day (9.0 mg/kg LW) than both the NRC (2007) requirements and current UK vitamin E supplementation levels. Diets were formulated to ensure that the low and high levels of vitamin E supply were similar on both the straw and grass silage based treatments, by blending together different proportions of the stock concentrates (Table 4.6). However, as the amount of vitamin E in the straw (30.4 mg/kg DM) was considerably lower than that in the silage (147 mg/kg DM), and the amount of concentrate fed was considerably higher, the nature of vitamin E supplied by the two forage treatments was different. For ewes offered the straw based diets the majority of the vitamin E was supplied as α-tocopherol acetate from the concentrate fraction. However, for ewes offered the grass silage based diets, a far higher proportion of vitamin E was supplied as *RRR* tocopherol from the forage fraction (Table 4.7).

Forages are considered to be an important natural source of vitamin E (Elgersma *et al*., 2012). The naturally occurring form of vitamin E, *RRR*-α-tocopherol is the most bioactive available among the tocopherols that can be found in grains and forages (Yang, 2003; Azzi, 2018), and easier for absorption (Burton and Traber, 1990). In the current experiment, grass silage and straw were supplemented as a natural source of vitamin E (*RRR*-α-tocopherol) containing a high or low concentration of naturally occurring vitamin E, while concentrates were fed as a synthetic source of vitamin E (all-*rac*-α-tocopherol acetate). The absorption rate of *RRR*-α-tocopherol is higher than the other stereoisomers (EFSA, 2010). Whereas, α-tocopherol acetate is less biologically active and is required to be hydrolysed in the gut to the phenol (free tocopherol) form prior to absorption in the small intestine (Burton and Traber, 1990). Another reason for higher plasma concentration of vitamin E in the grass silage treatment is α-tocopherol stereoisomers, vitamin E from natural sources has *RRR* isomers while the synthetic form of α-tocopherol (all-*rac*-α-tocopherol) consists of a racemic mixture of the eight stereoisomers (*RRR, RRS, RSR, RSS, SSS, SRS, SSR and SRR*) (Meglia *et al*., 2006; Wilburn *et al*., 2008 and Yang, 2003). Hepatic protein (α-TTP) which is responsible for the uptake of α-tocopherol from the hepatic cells into the very low-density

lipoprotein (VLDL) prefer to bind with *RRR* isomer than the other isomers (Burton and Traber, 1990). Therefore, vitamin E supplementation from grass silage may have been more efficient.

Vitamin E absorption rate is between 30 to 70%, which means that a high proportion of ingested vitamin E is excreted in the faeces before absorption, as the main route of vitamin E excretion (Bramley *et al.*, 2000; Debier and Larondelle, 2005). At high levels of vitamin E inclusion vitamin E is also excreted by both biliary secretions and mucosal cells into faeces (Bramley *et al.*, 2000; Debier and Larondelle, 2005). The efficiency of vitamin E absorption was higher in the high vitamin E supplemented treatments, and, in the silage compared with the straw base diet treatment.

The results of studies on the effect of oral vitamin E supplementation of ewes in late pregnancy indicate that plasma α-tocopherol concentration can be increased by vitamin E supplementation. Ewes plasma vitamin E in the current study was in accordance with those of Merrell, (1998), Daniels *et al.* (2000), Capper *et al.* (2005), Rooke *et al.* (2009) and Dønnem *et al.* (2015) who documented that ewes plasma vitamin E increased with supplementation of vitamin E during late pregnancy. In the current experiment, the plasma vitamin E concentration in ewes offered the HV diets was approximately 4.0 µg/ml at week -1, which is considered higher than the NRC (2007) critical value (2.0 µg/ml) and higher than ewes offered the LV treatments (2.5 µg/ml). However, vitamin E concentration in ewes offered LV treatment was higher than NRC (2007) critical value (2.0 µg/ml). In the current experiment, source of vitamin E also had an effect on plasma vitamin E concentration. For example, the plasma vitamin E concentration of ewes offered the SLV treatment was lower than the NRC (2007) critical value (2.0 µg/ml) and lower than the plasma vitamin E concentration of ewes offered the GSLV treatment, even though the level of vitamin E supplementation was slightly higher in the straw compared with the grass silage based diets (3.80 *vs* 2.70 mg/kg LW in SLV vs GSLV) and (11.2 *vs* 9.70 mg/kg LW in SH vs GSHV) respectively. The current results are in accordance with Kasapidou *et al.* (2009) who documented that high vitamin E supplementation increased plasma vitamin in growing lambs, in addition, plasma vitamin E was higher in grass silage based diet that concentrates. The main vitamin E supplement added to concentrate diets (Alpha-tocopherol acetate) is biologically inactive and needs to be hydrolysed to the α-tocopherol form before absorption in the small intestine (Burton and Traber, 1990), The longer time required for hydrolysis may reduce the efficiency of absorption in the small intestine. Therefore, vitamin E supplementation from natural sources may be more efficient.

During the *post-partum* period the plasma vitamin E concentration of ewes on all treatments was lower than *pre-partum*, and in the LV diets, lower than the NRC (2007) critical value (2.0 µg/ml) (Table 4.17); in addition, increasing vitamin E supplementation up to 700 mg/day

from both grass silage and concentrate was required to keep ewe plasma α-tocopherol above NRC (2007) critical value (2.0 µg/ml). Vitamin E is easily and rapidly transferred from plasma into colostrum and milk, this might be the reason for the lower plasma vitamin E concentration *post-partum* (Liu *et al.*, 2014). The NRC (2007) recommendations currently make no distinction between pregnant and lactating ewes and assume that the vitamin E requirement of both pregnant and lactating ewes is similar. In light of the above results, the vitamin E requirements of lactating ewe is higher than current recommendation of NRC (2007) based on plasma vitamin E levels .

In the current experiment, the high level of vitamin E supplementation had no effect on ewe performance parameters (LW, C, back fat and eye muscle depth) during both the *pre-* and *post-partum* periods. In previous studies, inconsistent results have been documented on the effect of vitamin E on ewe performance. The current results are consistent with those of Merrell *et al.* (1998) who found that high vitamin E supplementation had no effect on ewes weight and C. However, they disagree with the results of Capper *et al.* (2007) who reported that supranutritional supplementation of vitamin E increased C loss, plasma NEFA and BHB concentrations, and milk protein content during early lactation as a result of body fat mobilisation. Similarly, the results of Nieto *et al.* (2016) disagree with the current results, as they reported that ewes supplemented with high vitamin E tended to loss less weight during pregnancy and early lactation.

The previously published works are inconsistent with regard to whether vitamin E crosses through the placenta in any appreciable amounts. Lamb plasma vitamin E concentration at birth was less than 15% of ewe plasma concentration (Njeru *et al.*, 1994a; Kumagai and White, 1995; Schenker *et al.,* 1998 and Sterndale *et al.,* 2018). Therefore, supplementation of pregnant ewes with vitamin E *pre-partum* is an inefficient method to supplement neonates with vitamin E before suckling (Kumagai and White, 1995; McDowell *et al.*, 1996; Rooke *et al.*, 2004). However, Capper *et al.* (2005) reported that appreciate amount of vitamin E do cross the placental barrier and it is avidly assimilated into neonatal body tissues. Capper *et al.* (2005) documented that maternal supra nutritional supplementation of vitamin E increased vitamin E concentration in neonatal brain ($P<0{\cdot}05$) and *semimembranosis* muscle ($P<0{\cdot}05$). This means that plasma vitamin E concentrations is not a reliable indicator of vitamin E status in the body. The results of previously published studies on the effect of vitamin E supplementation on lamb birth weight are inconsistent. In the current experiment, the high level of vitamin E supplementation increased placental weight, cotyledon weight and tended to increase lamb birth weight, particularly in ewes offered the straw based diets. Vitamin E supplementation during late pregnancy has been shown to increase the placental vascular network and angiogenesis which leads to an increase in the growth rate of the foetus by providing more nutrients (Kasimanickam *et al.*, 2010). Alpha-tocopherol

supplementation has also been shown to increase the expression of mRNA for placental growth factor (PIGF), endothelial nitric oxide synthase (eNOS) and hypoxia inducible factors (HIF-1α) in cotyledons (Kasimanickam *et al*., 2010). The above mechanism may explain why high vitamin E supplementation had a positive effect on both placental parameters and litter birth weight. The trend towards higher lamb birth weight (P=0.056) in the current experiment could be due to an increase in placental weight and other aspects of placental efficiency. In the current study, the effects of vitamin E supplementation on litter birth weight are consistent with those of Capper *et al.* (2005) and Nieto *et al.* (2016) who reported that supplementation of ewes with vitamin E during late pregnancy increased lamb birth weight. However, they disagree with the results of Merrell *et al.* (1998); Daniels *et al.* (2000)*;* Dafoe *et al.* (2008) and Rooke *et al.* (2009), who documented that there is no effect of high vitamin E supplementation during late pregnancy on lamb birth weight.

The main sources of nutrients for the growing lamb during the first few weeks of life are colostrum and milk. In previously published experiments, supra-nutritional levels of vitamin E supplementation had no effect on colostrum and milk yield and composition in ewes (Capper *et al*., 2005; Capper *et al*., 2007), or milk production in Holstein dairy cows (Al-Mabruk *et al*., 2004; Politis *et al*., 2004). Similarly, in the current experiment, high vitamin E supplementation did not appear to have an effect on colostrum and milk vitamin E yield. However, as expected the colostrum vitamin E yield was higher in the high vitamin E supplemented treatments. Liu *et al.* (2014) documented that vitamin E is easily and rapidly transferred from plasma into colostrum and milk, this might be the reason for the higher colostrum vitamin E yield in ewes offered HV treatments. Plasma vitamin E was approximately twice as high in lambs from ewes that were supplemented with high compared with low vitamin E 2 weeks *post-partum*. High vitamin E supplementation increased lambs weight gain from week 4 to 8 and weaning weight at week 8. As an antioxidant, vitamin E reduces oxidative stress and improves the immunity of neonatal and growing lambs (Liu *et al*., 2014). A higher vitamin E intake during weeks 0 to +4 may have reduced oxidative stress in lambs resulting in higher creep intakes and enhanced performance later on.

Ewes offered treatment SHV appeared to respond to vitamin E supplementation. They lost the highest back fat *pre-partum*. In addition, they lost less C and back fat *post-partum*. In the current experiment, ewes in SHV treatment had the highest placenta weight, cotyledons weight, tended to have the highest cotyledons number and 11% higher litter birth weight. Vitamin E supplementation during late pregnancy can improve reproductive performance, prevent pathology of the reproductive system or foetus, and reduced myocardial necrosis (Buchanan-Smith *et al.,* 1969), In addition, vitamin E supplementation has a role to prevent reproductive problems like retained placenta and endometritis (Hemingway, 2003).

Protection of ewes from the reproduction problems resulting from oxidative stress and tissues damage may help to improve performance.

4.5. Conclusion

The LW and C of ewes on the GS based diets was higher than that of ewes on the S based diets, owing to a higher DMI and nutrient supply than predicted. As expected, high vitamin E supplementation increased α-tocopherol concentration in ewe plasma during late pregnancy; particularly in those offered, the GS based diets. During lactation ewe plasma α-tocopherol was lower than the NRC (2007) critical value for deficiency (2.0 μg/ml) in LV supplemented treatments. This suggests that the vitamin E requirements of lactating ewes is higher than the current recommendation of NRC (2007). In addition, high vitamin E supplementation increased α-tocopherol concentration in colostrum and lamb plasma. The higher litter weight of ewes offered the high level of vitamin E supplementation may have resulted from an increase in placental size and efficiency. High vitamin E supplementation had an effect on ewe performance characteristics particularly those on the S based diet treatments. Ewes fed the SHV treatment tended to lose the lowest C during lactation, in addition, they lost less back fat during early lactation. Further studies are required to investigate the effect of source and level of vitamin E supplementation on absorption and availability of vitamin E from the forages.

Chapter 5

Effect of source and level of vitamin E supply on vitamin E absorption and availability in growing lambs.

5.1. Introduction

Vitamin E (tocochromanols) consists of various biological isomers (four tocopherols and four tocotrienols); which occur naturally as free alcohols in the lipid fraction of green leaves and seeds (DellaPenna, 2005; Fritsche *et al.*, 2017). Among all the vitamin E isomers, *RRR*-α-tocopherol is considered the most important biologically active form that can be found in grains and forages (NRC, 2007; Azzi, 2018), and appear in significant levels in blood and animal tissues (Hidiroglou *et al.*, 1992; Scherf *et al.*, 1996).

Animal diets contain various concentrations of vitamin E and concentrates are often fortified with the synthetic form of α-tocopherol, which consists of chemical esterification of α-tocopherol with acetate to produce α-tocopherol acetate (McDowell *et al.*, 1996; NRC, 2007), which is then supplied in animals feeds (Meglia *et al.*, 2006; Wilburn *et al.*, 2008 and Yang, 2003). Work with growing lambs has concluded that the availability of vitamin E from forages may be considerably higher than that from concentrates (Kasapidou *et al.*, 2009). However, the mechanism is poorly understood. Before absorption, pancreatic lipase or intestinal mucosal esterase may be required to hydrolyse α-tocopherol acetate to α-tocopherol (Schmölz, 2016), which might reduce its availability. The form in which vitamin E is supplied may be an important factor influencing vitamin E availability and therefore responses to additional vitamin E supply.

In the UK, EBLEX (2014) suggested that vitamin E should be supplemented at higher levels than the ARC (1980) recommendations (15-20 mg/kg DM) when there is a higher demand, for example, during late pregnancy and early lactation, or when concentrates and conserved forages are fed, the concentrates should be supplemented with 100 mg/kg DM. Clinical legions associated with white muscle disease are normally associated with blood serum levels of <1.0 to 1.5 µg/ml, although concentrations of <2.0 µg/ml are generally considered deficient (Rice and McMurray, 1982). Recent estimates suggest that a dietary supply of 5.3 mg/kg live weight may be required to maintain plasma levels of >2.0 µg/ml (NRC, 2007). For a 30 kg lamb consuming approximately 1.0 kg/day DM, this equates to a dietary concentration of 150 mg/kg DM. To enhance immune competence, lamb birth weight or extend the shelf life of meat, the requirement should be increased further to 10 mg/kg live weight (NRC, 2007). Again, for a 30 kg growing lamb, this equates to a dietary concentration of approximately 300 mg/kg DM (NRC, 2007). In experiment one, where ewes were offered a straw based diet with concentrates containing either 50 or 500 mg/kg vitamin E, the higher

dietary level was required to maintain plasma vitamin E levels >2.0 µg/ml. During week -1 *pre-lambing*, plasma vitamin E levels were 0.85 and 2.06 µg/ml for the low and high vitamin treatments respectively (Chapter three). Although ewe plasma vitamin E levels were higher on the high vitamin E treatments (2.06 µg/ml), they were only twice those of ewes on the low vitamin E treatments, suggesting that level of vitamin supply may also influence vitamin E absorption and availability. In addition, Ewe plasma α-tocopherol concentration was higher on the grass silage based diet which contained a high proportion of natural vitamin E than the straw based diet in experiment two (chapter four). Therefore, the objective of the current experiment was to investigate the effect of source and level of vitamin E supply on vitamin E apparent absorption and availability in growing lambs.

5.2. Materials and methods

5.2.1. Experimental design

The experiment was conducted at Harper Adams University between October and January 2018 in the Animal Production, Welfare and Veterinary Sciences (APWVS) ruminant metabolism building. Local ethical approval was obtained from the Animal Welfare and Ethical Review Board (AWERB). Twelve castrate lambs with a live weight of approximately 25 kg ±SD=1.12 were housed in raised floor pens (1.5 x 2.0 m) in the sheep metabolism room and allocated to one of four treatments in 2 x 2 factorial design. The experiment was run over two periods with three lambs being allocated to each treatment in each period, giving six replicates per treatment. In order to minimise carry over effects between each period, lambs were maintained either on the low or high vitamin E treatments throughout the experiment.

Silage (0.60) + concentrates (0.40)	Low vitamin E (5.3 mg/kg LW) (GSLV)
Silage (0.60) + concentrates (0.40)	High vitamin E (10.0 mg/kg LW) (GSHV)
Straw (0.20) + concentrates (0.80)	Low vitamin E (5.3 mg/kg LW) (SLV)
Straw (0.20) + concentrates (0.80)	High vitamin E (10.0 mg/kg LW) (SHV)

In May 2017, grass silage was made from a first cut perennial ryegrass (*Lolium perenne*) sward. The ryegrass was mown at the leafy stage, then wilted for 24 h. A precision chop self-propelled forage harvester (John Deere 7840i, Nottinghamshire UK) was used to chop the harvested ryegrass. Then, two litres per tonne of the additive (Axphast Gold, Biotal, Worcestershire, UK) was added to grass silage which were ensiled in separate roofed concrete clamps.

Prior to diet formulation, samples of forages were collected and analysed in duplicate according to the methods of A.O.A.C (2016) as described in chapter 2 for dry matter

(Section 2.1.1), ash (Section 2.1.2), crude protein (Section 2.1.3), ether extract (Section 2.1.4), NDF (Sections 2.1.5), ADF (Section 2.1.6) and GE (Section 2.1.7). Forage samples were also analysed for vitamin E using high performance liquid chromatography as described in Chapter two (Section 2.9.2). Two stock concentrates with the same raw material composition were formulated to provide either a low (201 mg/kg DM) or high (1217 mg/kg DM) level of vitamin E (Table 5.1). The grass silage and straw were measured to contain approximately 30 and 140 mg/kg DM vitamin respectively (Table 5.2). When fed with either the straw or grass silage the two stock concentrates were blended together to provide a dietary vitamin E content of either 272 (LV) of 540 (HV) mg/kg DM) (Table 5.3). Each diet was then fed at 1.5 x maintenance to supply either 160 (LV) or 318 (HV) mg/day vitamin E (5.4 and 10.6 mg/kg LW respectively) (Table 5.4). Water was provided *ad-libitum*. Concentrates and forages were fed twice daily as two equal feeds. Feed samples were taken weekly for further analysis.

5.2.2. Digestibility Trial Diets

The diets were formulated for castrate lambs with a mean live weight of 30.0 kg and a feeding level of 1.5 times maintenance estimating DMI = 0.590 kg/day (Table 5.4). Calculated ME requirement = 6.72 MJ/day according to (AFRC, 1993) (Table 5.4).

Table 5. 1. Raw material and predicted chemical composition of concentrate diets.

	LV	HV
Raw materials (g/kg)		
Barley	600	600
Sugar beet pulp	203	179
Soya bean meal	114	120
Molasses	50.0	50.0
Urea	6.00	6.00
Minerals & vitamins[1]	25.0	25.0
Vitamin E Top [2]	2.30	20.0
Total	**1000**	**1000**
Predicted[3] chemical composition (g/kg DM)		
Dry matter (g/kg)	868	870
Crude protein	180	181
ERDP (0.05)	128	128
DUP (0.05)	34.0	34.0
Ether extract	13.0	13.0
Ash	74.0	72.0
NDF	213	206
Starch	341	341
Vitamin E (mg/kg DM)	201	1217
ME (MJ/kg DM)	12.6	12.3
FME (MJ/kg DM)	11.7	11.4
ERDP/FME (g/MJ)	11.0	11.2

[1] Mineral/ vitamins supplement (25 kg/ton) (BESTMIX[TM]) containing Vit. A 400,000.00 IU/kg, Vit. D3 80,000.00 IU/kg Vit. B12 2,800.00 mcg/kg, Vit. E 2,400.00 IU/kg, Vit. B1 40.00 mg/kg, calcium 315.00%, chloride 17%, ferrous sulphate 26,670.00 mg/kg, manganous oxide 4,840.00 mg/kg, molybdenum 200.00 mg/kg, sodium 11.00 %, selenium 12.00 mg/kg, iodine 200.00 mg/kg, iron 8000.00 mg/kg, manganese 3000 mg/kg, salt 28%, and zinc oxide 4,800.00mg/kg.
[2] Vitamin E Top supplement (BESTMIX[TM]) containing Vit. E 50,000.00 IU/kg, calcium 35.57 %.
[3] Diet chemical composition predicted according to AFRC (1993).

Table 5. 2. Analysed chemical composition (g/kg DM) of grass silage (GS) and straw (S)

	GS	S
Dry matter (g/kg)[1]	275	850
Crude protein[1]	120	42.0
NDF[1]	532	717
Vitamin E (mg/kg DM)[1]	140	30.0
ME (MJ/kg DM)[2]	10.6	6.40

[1] The chemical composition was analysed prior to diet formulation
[2] Typical value taken from AFRC (1993).

Table 5. 3. Raw material and chemical composition of lamb diets.

	GSLV	GSHV	SLV	SHV
Raw materials (g/kg)				
Straw	**-----**	**-----**	200	200
Grass silage	600	600	**-----**	**-----**
Concentrate (Low vitamin E)	290	25.0	695	425
Concentrate (High vitamin E)	110	375	105	375
Total	**1000**	**1000**	**1000**	**1000**
Chemical composition (g/kg DM)				
Dry matter (g/kg)	513	515	865	867
Crude protein	144	144	153	153
NDF	405	406	314	316
Vitamin E (mg/kg DM)[1]	274	539	271	541
ME (MJ/kg DM)	11.4	11.3	11.3	11.3

[1] Values are calculated using predicted vitamin E concentrations.

Table 5. 4. Daily fresh feeding offered levels (g/day) and offered nutrient supply for 30 kg growing lamb

	GSLV	GSHV	SLV	SHV
Feeding levels				
Dry matter intake	590	590	590	590
Dry matter intake (g/kg $wt^{0.75}$)	46.0	46.0	46.0	46.0
Grass silage	**1287**	**1287**	**----**	**----**
Straw	**----**	**----**	**139**	**139**
Concentrate (LV) (g/day)	**197**	**16**	**472**	**289**
Concentrate (HV) (g/day)	**75.0**	**254**	**71.0**	**254**
Fresh intake (g/day)	1559	1557	682	682
Fresh intake (g/kg $wt^{0.75}$)	122	121	53.0	53.0
Nutrient supply (g/day)				
Dry matter	590	590	590	590
Crude protein	85.0	85.0	90.0	90.0
NDF	238	239	185	185
Vitamin E (mg/day)	162	318	159	319
ME (MJ/day)	**6.71**	**6.66**	**6.68**	**6.64**
Vitamin E (mg/kg LW)	**5.40**	**10.6**	**5.30**	**10.6**
Vitamin E from forage (%) [1]	**31.0**	**16.0**	**2.00**	**2.00**

[1] Values are calculated using analysed vitamin E concentrations.

5.2.3. Experimental routine:

All lambs were weighed weekly as described in chapter 2 (Section 2.4). The experimental diets were offered according to $LW^{0.75}$ to provide approximately 1.5 x maintenance energy requirements (AFRC, 1993) in two equal feeds at approximately 09:00 and 16:00 h respectively. Each diet was offered to three lambs per treatment over two periods. Each period consisted of a 14 days adaptation period, where lambs were adapted to the

experimental treatment, followed by a 7 days faecal collection period. The apparent whole tract nutrient digestibility of each treatment was determined using the method of Sinclair *et al.* (2003). Briefly, faeces were collected using the bag and harness technique (Sinclair *et al.*, 2003). During each faecal collection period, bags were emptied once daily, before the morning feed, with the total weight of faecal output being recorded. A subsample of each lamb's daily output (0.1) was stored at -20 °C before further analysis. Throughout the experiment, feed intake was also recorded and samples collected and stored at -20 °C before bulking, subsampling and chemical analysis. Blood samples were collected 2 hours after the morning feed by jugular venepuncture as described in chapter 2 (Section 2.2) at the beginning of the experiment and on days 10 and 17 of each period. Blood samples were collected into two types of tubes potassium oxalate for determination of glucose and sodium heparin for the determination vitamin E, total protein, albumin, BHB, NEFA and urea. The plasma was removed and stored in 1.0 ml eppendorf tubes at -20 C before further analysis.

5.2.4. Chemical analysis:

All feed and faecal samples were defrosted and analysed in duplicate according to the methods of A.O.A.C (2016) as described in chapter 2 for dry matter (Section 2.1.1), ash (Section 2.1.2), crude protein (Section 2.1.3), ether extract EE (Section 2.1.4), NDF (Sections 2.1.5), ADF (Sections 2.1.6) and GE (Section 2.1.7). They were also analysed for vitamin E using high performance liquid chromatography as described in chapter two Section (2.9.2). Following processing, lamb blood plasma was analysed for glucose (Randox Laboratories kit, GL1611), beta-hydroxybutyrate (BHB) (Randox Laboratories kit, RB1008), non-esterified fatty acid (NEFA) (Randox Laboratories kit, FA115), urea (Randox Laboratories kit, UR 221), albumin (Randox Laboratories kit, AB 362), and total protein (Randox Laboratories kit, Tp 245), by using Cobas-Mira (Mira plus ABX Diagnostics) blood analyser. Plasma vitamin E was determined as described in chapter two (Section 2.9.1).

5.2.5. Calculations and statistical analysis:

The apparent whole tract digestibility of each chemical component was calculated from the component intake and faecal output over each 7 days collection period as follows:

$$\text{Digestibility (g/g)} = \frac{\text{Intake (g)} - \text{faecal output (g)}}{\text{Intake (g)}} \qquad \text{Eq. 5.2.5}$$

Digestible energy was calculated from the GE content of each diet multiplied by the GE digestibility coefficient. Metabolisable energy (ME) was estimated as DE x 0.81 (AFRC, 1993). The results were analysed by ANOVA as a 2 x 2 factorial experiment using a cross

over design by Genstat 18th, edition (VSN Int. Ltd., Hempstead. UK), main effects being forage type and vitamin E level. Differences between means were determined using the protected least significant difference (LSD) (Snedecor and Cochran, 1989).

5.3. Results

5.3.1. Animal health

All lambs were in good condition throughout the experiment.

5.3.2. Diet composition

The analysed chemical composition of the experimental diets is shown in Table 5.5. The straw had a higher dry matter, NDF and protein content than expected in Table 5.2. The grass silage had a higher crude protein, lower vitamin E, dry matter and NDF than expected in Table 5.2. The chemical composition of the concentrates was slightly different to that expected from the formulated diet in Table 5.1. In all concentrates, dry matter was higher than expected. ether extract, crude protein and NDF was slightly lower, organic matter in GSHV was lower than expected. Vitamin E content in GSHV and GSLV concentrates was lower than predict in Table 5.3.

Table 5. 5. Chemical composition (g/kg DM) of the forages and concentrates used in the experiment.

Chemical composition	Straw	Silage	SLV	SHV	GSLV	GSHV
Dry matter (g/kg)	872	257	881	881	885	881
Organic matter	960	909	926	922	926	916
Crude protein	55.2	138	168	167	168	168
NDF	813	462	170	170	171	171
ADF	511	292	81.9	86.9	81.9	80.9
Fat g/kg DM	5.10	29.4	9.60	10.5	10.8	11.1
GE (MJ/kg DM)	18.1	17.2	16.9	16.9	17.0	16.7
Vitamin E (mg/kg DM)	34.3	104	235	542	257	479
Selenium (mg/kg DM)	0.02	0.01	0.30	0.32	0.24	0.31

S= straw, GS= grass silage, SL= straw low vitamin E (mg/kg DM), SV= straw high vitamin E (mg/kg DM), GSL= grass silage low vitamin E, GSH= grass silage high vitamin E

5.3.3. Lamb live weight, dry matter intake and faecal output.

The concentrate, forage and total dry matter intake (TDMI) of lambs on the straw based treatments were similar to expected (Table 5.4). The concentrate dry matter intake (DMI) of lambs on the grass silage based treatments was similar to expected, but forage and TDMI

were lower than those shown in Table 5.4. There was no FT×V interaction (*P*>0.05) effect on lamb weight, metabolic weight, dry matter intake DMI, metabolic dry matter intake DMI $^{0.75}$ and faecal output (Table 5.6). There was an effect (*P*<0.05) of forage type on dry matter intake (g) and faecal output. Lambs offered the straw based treatments had a higher concentrate DMI (389 vs 192 g/day DM; P<.001), metabolic concentrates DMI$^{0.75}$ (35.8 vs 17.5 g/day; P<.001), total DMI (489 vs 458 g/day; P<.001), metabolic total DMI$^{0.75}$ (44.9 vs 41.7 g/day; P<.001) and faecal output (136 vs 124 g/day; P<0.029) than those offered the grass silage based treatments, while forage DMI (99.7 vs 266 g/day; P<.001) and metabolic forage DMI$^{0.75}$ (9.16 vs 24.2 g/day; P<.001) were lower in lambs offered the straw based treatments than those offered the grass silage based treatments. There was an effect (*P*<0.05) of vitamin E supplementation on lamb weight, metabolic lamb weight $^{0.75}$, forage DMI, total DMI, and a trend towards an effect on concentrate DMI and faecal output. Lambs offered treatment HV had a lower LW (23.4 vs 25.2 kg; P<.001), metabolic LW$^{0.75}$ (10.6 vs 11.2 kg; P<.001), forage DMI (176 vs 189 kg; P<0.023), total DMI (458 vs 490 kg; P<0.030), and tended to have a lower concentrate DMI (281 vs 301 kg; P<0.063) than those offered treatments LV while, lambs on the HV treatment tended to have lower faecal output than (134 vs 126 kg; P<0.073) LV treatment.

Table 5. 6. The effect of forage type and vitamin E level on live weight (kg), dry matter intake (g/ day) (g) and faecal output (g) of lambs used in the experiment.

	S		**GS**		**SED**	***P-value***		
	LV	**HV**	**LV**	**HV**		**FT**	**VE**	**FT×VE**
Mean LW	25.0	23.3	25.4	23.4	0.543	0.382	<0.001	0.752
Mean MW (LW$^{0.75}$)	11.2	10.6	11.3	10.7	0.184	0.379	<0.001	0.750
Conc. DMI	398	381	204	181	11.86	<0.001	0.063	0.675
Conc. DMI$^{0.75}$	35.7	35.8	17.8	17.3	0.841	<0.001	0.779	0.585
Forage DMI	105	94.5	273	258	6.254	<0.001	0.023	0.525
Forage DMI$^{0.75}$	9.26	9.05	24.4	24.0	0.377	<0.001	0.363	0.731
Total DMI	502	476	477	440	16.51	0.004	0.030	0.588
Total DMI$^{0.75}$	45.0	44.9	42.1	41.3	1.153	<0.001	0.614	0.610
DM faecal	131	140	120	128	4.974	0.029	0.073	0.888

S= straw, GS= grass silage, LV=low vitamin E supplementation (5.3 mg/kg LW), HV=high vitamin E supplementation (10.0 mg/kg LW), FT= forage type (straw or silage), V= vitamin E, FT×V = interaction between forages and vitamin E
Mean MW (LW $^{0.75}$) = Mean Metabolic weight (live weight $^{0.75}$)
Conc. DMI $^{0.75}$ = Concentrate dry matter intake g/kg$^{0.75}$.
Forage DMI $^{0.75}$ = Forage dry matter intake g/kg$^{0.75}$.
Total DMI $^{0.75}$ = Total dry matter intake g/kg$^{0.75}$.

5.3.4. Vitamin E intake

There was a FT×V interaction (P<0.05) on vitamin E intake (Table 5.7). The highest concentrate vitamin E intake was on treatment SHV (210.90 mg/kg DM; P=0.002) and lowest on treatment GSLV (56.2 mg/kg DM; P=0.002). Similarly, vitamin E intake (mg/kg BW) was highest for lambs on treatment SHV (8.78; P<0.001) and the lowest for lambs on treatment GSLV (3.45; P<0.001). The proportion of vitamin E intake from the forages was highest for lambs on treatment GSLV (33.1 %; P=0.015) and lowest for lambs on treatment SHV (1.60 %; P=0.015). There was an effect (P<0.05) of forage type on vitamin E intake. Lambs offered the straw based diets had a higher concentrate vitamin E (151 vs 78.1 mg/kg DM; P<0.001) and vitamin E intake per kg LW (6.34 vs 4.38 mg/kg LW; P<0.001) intake than grass silage based diets. Vitamin E intake from forage was lower in lambs offered the straw based diets than the silage based diets (3.42 vs 27.5 mg/kg DM; P<.001) Lambs offered the silage based diets had a higher proportion of vitamin E intake from forage (27.3 % vs 2.60 %; P<.001) than those offered the straw based diets. There was an effect (P<0.05) of vitamin E supplementation on vitamin E intake. Lambs offered the high vitamin E diets had higher concentrate vitamin intake (155 vs 73.9 mg/kg DM; P<0.001) and vitamin E intake per kg live weight (7.05 vs 6.67 mg/kg LW; P<.001) than those offered low vitamin E. whereas, the proportion of vitamin E intake from the forages was lower in the high vitamin E supplemented treatment (11.5 vs 18.4 mg/kg DM; P=0.004).

5.3.5. Nutrients intake and faecal nutrient output

There was FT×V interaction (P<0.05) on nutrient intake (Table 5.8). Lambs on treatment SHV had the highest total vitamin E intake (214 mg/kg DM; P=0.002), and the lambs on treatment GSLV the lowest (84.0 mg/kg DM; P=0.002). There was an FT×V interaction (P<0.05) on both faecal NDF and ADF output (Table 5.8). The highest faecal NDF and ADF output were on treatment SHV (71.3, *P*=0.038; 45.0 *P*=0.048 g/ day; P<.001). There was an effect of forage type (P<0.05) on nutrient intake and output. Lambs offered the straw based diets had a higher intake of organic matter (456 vs 419 g/day; P=0.010), crude protein (71.6 vs 67.1 g/day; P=0.039) and gross energy (8.37 vs 7.81 MJ/day; P=0.012) and total vitamin E intake (155 vs 106 mg/kg DM; P<0.001) than those offered the grass silage based diets. Lambs offered the straw based diets tended to have a lower intake of both NDF (147 vs 156 g/day; P=0.058) and ADF (86.6 vs 94.6 g/day; P=0.056) than those offered the grass silage based diets. Lambs offered the straw based diets had a higher faecal NDF (66.8 vs 51.0 g/day; P<.001), ADF (43.0 vs 32.8 g/day; P<.001) and GE (2.39 vs 2.24 MJ/day; P=0.045) output than those offered the grass silage based diets. There was an effect of vitamin E supplementation (P<0.05) on nutrient intake. Lambs offered the high vitamin E diets had a higher total vitamin intake (171 vs 89.5 mg/kg DM; P<0.001) than those offered

low vitamin E diets. There was an effect of vitamin E supplementation ($P<0.05$) on faecal nutrient output. Lambs supplemented with high vitamin E had a higher faecal NDF (61.8 vs 56.0 g/day; $P=0.005$) and ADF (39.1 vs 36.7 g/day; $P=0.019$) output; and tended to have a higher faecal vitamin E output (48.4 vs 38.1 mg/kg LW; $P=0.073$), than those on the low vitamin E supplemented treatments.

Table 5. 7. The effect of forage type and vitamin E level on vitamin E intakes of lambs used in the experiment.

Vitamin E intake (mg/day)	S		GS		SED	*P-value*		
	LV	HV	LV	HV		FT	VE	FT×VE
Concentrate	91.7^{b}	211^{c}	56.2^{a}	99.9^{b}	6.920	<0.001	<0.001	0.002
Forage	3.42	3.42	27.7	27.2	0.430	<0.001	0.48	0.472
Vitamin E (mg/kg BW)	3.89^{a}	8.78^{c}	3.45^{a}	5.31^{b}	0.180	<0.001	<0.001	<0.001
Forage VE proportion %	3.60^{a}	1.60^{a}	33.1^{c}	21.5^{b}	1.664	<0.001	0.004	0.015

S= straw, GS= grass silage, LV=low vitamin E supplementation (5.3 mg/kg LW), HV=high vitamin E supplementation (10.0 mg/kg LW), FT= forage type (straw or silage), V= vitamin E, FT×V = interaction between forages and vitamin E

[a,b] Means with the same superscript are not significantly different (*P*<0.05).

Table 5. 8. The effect of forage type and vitamin level on nutrient intake and faecal nutrient output (g/day) of lambs used in the experiment.

	S		**GS**		**SED**	***P-value***		
	LV	**HV**	**LV**	**HV**		**FT**	**VE**	**FT×VE**
Nutrient intake (g/day)								
Organic matter	456	455	424	413	11.25	0.010	0.471	0.569
Crude protein	71.7	71.5	67.9	66.3	2.091	0.039	0.590	0.653
NDF	147	147	157	154	4.526	0.058	0.697	0.638
ADF	85.9	87.3	96.0	93.3	4.261	0.056	0.836	0.533
GE (MJ/day)	8.37	8.36	7.92	7.69	0.182	0.012	0.397	0.434
Vitamin E (mg/ day)	95.1^{a}	214^{c}	84.0^{a}	127^{b}	7.046	<0.001	<0.001	0.002
Nutrient output (g/day)								
Organic matter	81.6	93.8	102	106	21.75	0.357	0.625	0.805
Crude protein	23.1	24.0	23.9	24.4	1.597	0.625	0.574	0.862
NDF	62.2^{b}	71.3^{c}	49.7^{a}	52.4^{a}	1.484	<0.001	0.005	0.038
ADF	40.9^{b}	45.0^{c}	32.5^{a}	33.1^{a}	0.870	<0.001	0.019	0.048
GE (MJ/day)	2.34	2.43	2.20	2.28	0.071	0.045	0.177	0.917
Vitamin E (mg/ day)	38.5	49.7	37.7	47.1	6.029	0.705	0.073	0.837

S= straw, GS= grass silage, LV=low vitamin E supplementation (5.3 mg/kg LW), HV=high vitamin E supplementation (10.0 mg/kg LW), FT= forage type (straw or silage), V= vitamin E, FT×V = interaction between forages and vitamin E

^{a,b} Means with the same superscript are not significantly different (*P*<0.05).

5.3.6. Diet apparent digestibility

There was no FT×V interaction ($P>0.05$) on any of the digestibility coefficients or energy values (DE and ME) (Table 5.9). There was an effect ($P<0.05$) of forage type on NDF, ADF, and vitamin E digestibility. The grass silage based diets had a higher NDF digestibility (0.67 vs 0.55 g/g; $P<0.001$) and ADF (0.65 vs 0.50 g/g; $P=0.001$) digestibility than the straw based diets. However, vitamin E digestibility was higher on the straw based diets (0.68 vs 0.59 mg/ mg; $P=0.022$) than the grass silage based diet. Vitamin E supplementation had an effect ($P<0.05$) on DM, NDF and vitamin E digestibility, and energy values. High vitamin E supplementation reduced DM digestibility (0.72 vs 0.74 g/ g; $P=0.037$), NDF digestibility (0.59 vs 0.63 g/ g; $P=0.024$), digestible energy (11.7 vs 12.6 MJ/ kg DM; $P=0.024$) and metabolisable energy (9.50 vs 10.2 MJ/ kg DM; $P=0.024$) compared to the low vitamin E supplementation. High vitamin E supplementation increased vitamin E digestibility (0.70 vs 0.58 mg/ mg; $P=0.008$) compared to the low vitamin E supplementation.

Table 5. 9. The effect of forage type and vitamin E level on apparent diet digestibility and energy values

	S		GS		SED	*P-value*		
	LV	**HV**	**LV**	**HV**		**FT**	**VE**	**FT×VE**
Diet digestibility (g/ g)								
DM	0.73	0.71	0.74	0.72	0.010	0.423	0.037	0.746
OM	0.82	0.79	0.76	0.74	0.047	0.163	0.539	0.880
CP	0.68	0.67	0.65	0.63	0.029	0.200	0.519	0.945
NDF	0.58	0.52	0.68	0.66	0.017	<0.001	0.024	0.195
ADF	0.52	0.48	0.66	0.64	0.026	0.001	0.187	0.565
GE	0.72	0.71	0.72	0.70	0.012	0.772	0.138	0.690
Vitamin E (mg/ mg)	0.60	0.77	0.55	0.63	0.036	0.022	0.008	0.140
Energy values (MJ/kg DM)								
DE	12.7	11.8	12.4	11.7	0.367	0.379	0.034	0.675
ME	10.3	9.55	10.0	9.44	0.297	0.379	0.034	0.675

S= straw, GS= grass silage, LV=low vitamin E supplementation (5.3 mg/kg LW), HV=high vitamin E supplementation (10.0 mg/kg LW), FT= forage type (straw or silage), V= vitamin E, FT×V = interaction between forages and vitamin E

5.3.7. Lambs blood plasma metabolites and vitamin E concentration

There was no FT×V interaction (P>0.05) on lamb blood plasma metabolites and vitamin E concentrations (Table 5.10). However, lambs on treatment SLV tended to have the highest plasma NEFA (0.32 mmol/ l, *P*=0.097). There was an effect (P<0.05) of forage type on lamb blood plasma metabolites. Lambs offered the straw diets had a lower blood plasma urea (4.40 vs 5.63 mmol/l; P=0.013), and tended to have a lower plasma vitamin E concentration (1.95 vs 2.33 µg/ml; P=0.075) than those offered the grass silage based diets. There was an effect (P<0.05) of vitamin E supplementation on lamb plasma vitamin E concentration and a trend effect on plasma metabolites. Lambs supplemented with high vitamin E had a higher plasma vitamin E concentration (2.38 vs 1.90 µg/ml; P=0.030), but tended to have a lower albumin concentration (23.8 vs 27.9 g/ l; P=0.093) than those offered the low vitamin E diets.

Table 5. 10. The effect of forage type and vitamin E level on lamb blood metabolites

	S		GS		SED	*P-value*		
	LV	HV	LV	HV		FT	VE	FT×VE
Total protein (g/ l)	60.0	50.6	57.7	53.1	6.056	0.970	0.205	0.549
Albumin (g/ l)	28.6	23.2	27.2	24.5	2.512	0.974	0.093	0.428
Urea (mmol/ l)	4.87	3.93	5.93	5.33	0.611	0.013	0.176	0.682
Glucose (mmol/ l)	3.75	4.53	3.80	3.90	0.441	0.297	0.267	0.269
BHB (mmol/ l)	0.43	0.52	0.48	0.46	0.063	0.895	0.601	0.231
NEFA (mmol/ l)	0.32	0.27	0.24	0.33	0.061	0.786	0.702	0.097
Vitamin E (µg/ml)	1.76	2.14	2.04	2.62	0.275	0.075	0.030	0.620

S= straw, GS= grass silage, LV=low vitamin E supplementation (5.3 mg/kg LW), HV=high vitamin E supplementation (10.0 mg/kg LW), FT= forage type (straw or silage), V= vitamin E, FT×V = interaction between forages and vitamin E

5.3.8. Lamb plasma vitamin E concentration throughout the experiment

There was an effect (P<0.05) of time on lamb plasma vitamin E concentration (Figure 5.1). Plasma vitamin E concentration reduced from 2.47 µg/ml at the beginning of the first period to 2.21 µg/ml by the end of period 1 and to 1.89 µg/ml by the end of the second period. There was an effect of forage type (P<0.05) on lamb plasma vitamin E concentration. Lamb offered the grass silage based diets had a higher plasma vitamin E, than those offered the straw based diets at the end of second period (2.21 vs 1.57 µg/ml; P=0.007) and tended to have a higher plasma vitamin E concentration at the end of the first period (2.59 vs 1.87 µg/ml; P=0.089). There was an effect of vitamin E supplementation (P<0.05) on plasma vitamin E concentration. High vitamin E supplementation increased plasma vitamin E concentration at the end of the first period (2.92 vs 1.51 µg/ml; P=0.014), at the beginning

of collection of the second period (1.82 vs 1.08; µg/ml *P*=0.002) and at the end of second period (2.24 vs 1.53 µg/ml; P=0.009). There was no FT×V interaction (*P*>0.05) on plasma vitamin E concentration. There was no time × FT, time × V or time × FT × V interaction (*P*>0.05) on plasma vitamin E concentration. However, lambs offered HV treatment tended to increase plasma vitamin E from 2.46 µg/ml at the beginning of the first period to 2.92 µg/ml at the end of the first period then reduced to 2.21 µg/ml. Plasma vitamin E of lamb offered LV treatment reduced from 2.48 µg/ml at the beginning of the first period to 1.50 µg/ml and 1.52 µg/ml at the end of the first and end of the second period respectively.

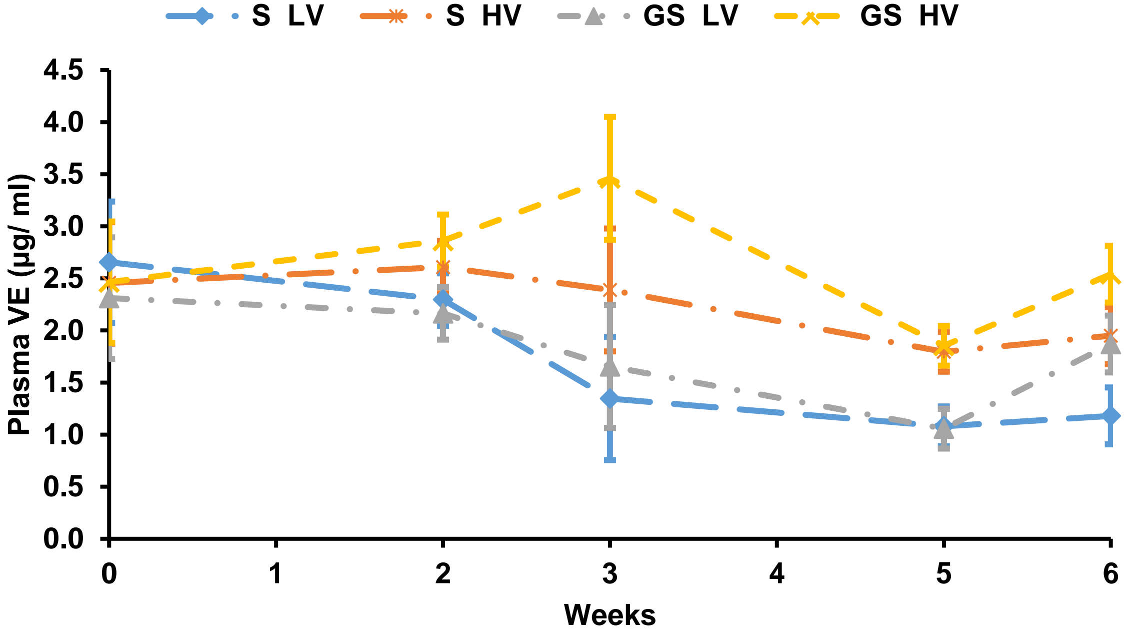


Figure 5. 1. The effect of forage type and vitamin E level on plasma vitamin E concentration throughout the experiment

SED values: FT= 0.092, V=0.092, FT×V=0.130, Time= 0.188, Time×FT=0.260, Time×V=0.280, Time×FT×V=0.382.
P-values: FT= 0.027, V=<0.001, FT×V=0.147, Time= 0.006, Time×FT=0.173, Time×V=0.073, Time×FT×V=0.598

5.4. Discussion

In the current experiment, lambs were fed according to metabolic weight ($LW^{0.75}$) to provide approximately 1.5 x with their maintenance allowance (AFRC, 1993). The proximate analyse and vitamin E content of the two forages were determined prior to diet formulation which were 140 and 30 mg/kg DM in both the grass silage and straw respectively. Two stock concentrates were formulated with the same raw material composition but different levels of vitamin E content to provide either a low (e.g. 201 mg/kg DM) or high (e.g. 1217 mg/kg DM) level of vitamin E. For each treatment, the stock concentrates were blended in appropriate proportions to ensure similar levels of vitamin E supply (5.3 or 10.0 mg/kg LW) but from different sources. The analysed chemical composition of the concentrates was slightly different to predicted throughout the experiment. Concentrate NDF and ADF were lower than anticipated. In addition, vitamin E in concentrates and grass silage were lower than anticipated throughout the trial. However, the natural source of vitamin E content in grass silage was higher than straw.

The proportion of concentrate to forage intake was approximately twice in the straw than GS based diets, while, on the GS based diets the proportion of grass silage to concentrate intake was higher. This was the reason that the nutrients intake (organic matter, crude protein, gross energy) were higher in the straw based diets. While, both NDF and ADF intake was lower in straw than grass silage based diets.

In the current experiment, there was no effect of forage type on lambs LW, $LW^{0.75}$. However, the NDF and ADF intake tended to be lower in the straw than the grass silage based diets, and the NDF and ADF digestibility coefficient were lower in the straw than the grass silage based diets. The NDF in the straw probably consists of a higher proportion of more resistant cell wall components such as lignin and cellulose (McDonald *et al.*, 2011),. In addition, approximately 72% of the raw materials that were used in the concentrate formulation were barley and soya bean meal, which contain a high proportion of starch (McDonald *et al.*, 2011). Forage digestibility can be reduced by supplementation with high starch diets as a readily available carbohydrate source (McDonald *et al.*, 2011). Rumen fermentation of starch increases the production of volatile fatty acids (VFA) and lactate, which reduces the ruminal pH to 6 or lower (Plaizier *et al.*, 2008; McDonald *et al.*, 2011; Gómez Luis *et al.*, 2016). This reduction of ruminal pH inhibits the activity cellulolytic microorganisms that are responsible for the digestion of fibre (McDonald *et al.*, 2011; Gómez Luis *et al.*, 2016). This could be another reason for the lower NDF and ADF digestibility coefficients in straw than grass silage based diet treatment.

Lambs on the straw based diets had a higher concentrate and total vitamin E intake than those on the GS based diet. However, the proportion of vitamin E intake from the forages was higher in lambs on the grass silage based diets than those on the straw based diets. Similarly, the proportion of vitamin E supply from concentrates was higher for lambs on the high vitamin E diets. Vitamin E intake was 3.89, 8.78, 3.45 and 5.31 mg/kg LW for lambs on treatments SL, SH, GSL and GSH respectively. The vitamin E content of the grass silage was measured to be 140 mg/kg DM prior to the experiment commencing. However, the vitamin E content of the grass silage samples taken throughout the experiment was only 103 mg/kg DM. This resulted in a lower than anticipated vitamin E supply to lambs offered the grass silage based diets.

During period 1, lambs on the high vitamin E diets lost more weight than those on the low vitamin E diets. The lower weight was then used to calculate the feeding level during period two. The current results are consistent with those of Wulf *et al.* (1995) who reported that lamb weight gain reduced when they were supplemented with 1000 mg/ /day rather than 500 or 0 mg/day vitamin E. The reason by which high vitamin E supplementation reduced lamb weight is unclear and requires more investigation. All diets were fed according to lamb metabolic weight. The high vitamin E diets reduced NDF digestibility, with consequent effects on both the digestible and metabolisable energy content of the diets. The reason why high vitamin E supplementation reduced NDF digestibility and metabolisable energy values is unclear and requires more investigation. The current results disagree with the results of Belanche *et al.* (2016) who found that vitamin E supplementation of 50 IU/L of α-tocopherol acetate in an *in vitro* study improved rumen fermentation, increased rumen gas production, total VFA, protozoal activity and resulted in a small increase in feed digestibility (+8%). This level of supplementation was the best in between different levels (0.5, 5, 50 or 500 IU/L). Similarly, the current results also disagree with Belanche *et al.* (2017) who documented that supplementation of vitamin E (50 IU/L) caused a small increase in feed degradability in an *in vitro* study experiment.

In the current experiment, lamb plasma vitamin E concentration and vitamin E digestibility were higher in lambs on the high compared to the low vitamin E diets. Plasma vitamin E concentration in lambs on the high vitamin E diets was approximately 1.3 times higher than the low vitamin E diets. In addition, Lamb plasma vitamin E concentration was 1.2 times higher on the grass silage compared with the straw based diets. However, as a result of the lower than expected vitamin E content of the grass silage, the vitamin E intake of lambs on the grass silage diets was lower than that of lambs on the straw diets which provides evidence that the availability of α-tocopherol from the forages is higher than concentrates. Forages are considered to be an important natural source of vitamin E (Elgersma *et al.*, 2012). The naturally occurring *RRR*-α-tocopherol is the most bioactive available among

tocopherols found in grains and forages (Yang, 2003; Azzi, 2018), and easier for absorption (Burton and Traber, 1990). The bioavailability of *RRR*-α-tocopherol is higher than α-tocopherol acetate (1.49 vs 1.00 IU/mg) (Yang, 2003). In the current experiment, grass silage and straw were supplied as a natural source of vitamin E (*RRR*-α-tocopherol) containing a high or low concentration of naturally occurring vitamin E, respectively, while concentrates were fed as a synthetic source of vitamin E (all-*rac*-α-tocopherol acetate). Alpha-tocopherol acetate is biologically inactive and is required to be hydrolysed in the gut to the phenol (free tocopherol) by the enzyme pancreatic carboxyl ester hydrolase, prior to absorption in the small intestine (Burton and Traber, 1990; Schneider, 2005). High supplementation of all-*rac*-α-tocopherol acetate reduces absorption of α-tocopherol and results in lower concentration of α-tocopherol in blood plasma (Machlin and Gabriel, 1982; Bjørneboe *et al*., 1990). However, in the current experiment the digestibility of vitamin E in GS based diet was lower compared to S based diet. Total vitamin E intake was lower in GS than S based diet, this could be the reason of lower vitamin E absorption in GS than S based diet. The higher plasma concentration of vitamin E in lambs on the grass silage based diets is that the α-tocopherol stereoisomers of Vitamin E from natural sources consists of *RRR* isomers, while the synthetic form of α-tocopherol (all-*rac*-α-tocopherol) consists of a racemic mixture of the eight stereoisomers (*RRR, RRS, RSR, RSS, SSS, SRS, SSR and SRR*) (Meglia *et al*., 2006; Wilburn *et al*., 2008 and Yang, 2003). Hepatic protein (α-TTP) which is responsible for the uptake of α-tocopherol from hepatic cells into the very low-density lipoprotein (VLDL) has a higher affinity for the *RRR* isomer than the other isomers (Burton and Traber, 1990). Therefore, vitamin E uptake from grass silage was more efficient.

A considerable but variable proportion of ingested vitamin E (30 to 70%) is excreted in faeces prior to absorption as the main route of excretion (Bramley *et al*., 2000; Debier and Larondelle, 2005). In the current experiment, the availability of Vitamin E was between 55 to 77% which is consistent with the results of Jensen *et al.* (1999) who reported that the absorption coefficient of α-tocopherol acetate was approximately 70% in broiler chickens regardless of the supplemented levels of α-tocopherol acetate (50, 10, 150 or 200 mg/kg feed). In addition, the current results agree with those of (Zeitz *et al*., 2016) who documented that tocopherol digestibility ranged between 68.7 to 77.9 % in rats fed diets containing approximately 60 mg/kg vitamin E with or without conjugated linoleic acid. Lambs' plasma vitamin E levels were similar to those reported by Kasapidou *et al.* (2009) who documented a higher plasma α-tocopherol concentration in lambs' supplemented a mixed diet of concentrates and silage compared to those offered α-tocopherol acetate from concentrate diet. Similarly, the current results are similar to (Weiss *et al*., 2009) who found that calf

plasma concentration of natural vitamin E (*RRR* α-tocopherol) was 1.24 to 1.43 times higher than synthetic vitamin E (all-*rac*-α-tocopherol).

High levels of dietary α-tocopherol lead to high levels of α-tocopherol excretion via mucosal cells and biliary secretions into faeces (Bramley *et al*., 2000; Debier and Larondelle, 2005).

High levels of α-tocopherol are excreted by the liver such that plasma vitamin E does not rise more than 2-4 fold (Fuller *et al*., 1996; Bruno *et al*., 2005; Imai *et al*., 2011). The high levels of α-tocopherol plasma are converted to α-carboxyethylhydroxychromanol (CEHC) which is the end product of hepatic metabolism (Schubert *et al*., 2018) which is then excreted via both faeces and urine (Chiku *et al*., 1984; Swanson *et al*.,1999; Traber, 2013; Schubert *et al*., 2018). Therefore, high levels of vitamin E supplementation could increase absorption efficiency, but it does not necessarily increase plasma α-tocopherol in similar rate.

5.5. Conclusion

The uptake of naturally occurring vitamin E from grass silage into blood plasma was more efficient than uptake of alpha tocopherol acetate from concentrates. High vitamin E supplementation reduced diet DM and fibre digestibility resulting in a reduction in DE and ME content. However, the mechanism is unclear. The availability of vitamin E was higher in GS than S based diet, and higher in high than low supplemented treatments.

Chapter 6

General Discussion

6.1. General Discussion

The objective of this series of experiments was to investigate the effect of body tissue reserves and source of vitamin E on the response of pregnant and lactating ewes to supra-nutritional levels of vitamin E supplementation.

The National Research Council NRC (1985) estimated that the vitamin E requirement of ruminants was between 10 to 60 mg/kg DM, and recommended supplementing 20 IU/ kg DM. The National Research Council NRC (2007) recommend that the vitamin E requirements of ewes should be increased to 5.6 mg/kg LW in order to keep plasma α-tocopherol level above a critical values of ≥2 µg/ml, which is considered necessary to protect animals from white muscle disease (WMD). For an 85 kg ewe, this equates to approximately 450 mg/day. In the UK, concentrate feeds for pregnant and lactating ewes typically contain 100-150 mg/kg DM vitamin E (EBLEX, 2014; AHDB, 2018), which equates to 1.3 to 2.0 mg/ kg LW.

One of the notable results in the current study was the effect of source and level of vitamin E supplementation on ewe and lamb blood plasma α-tocopherol concentrations. In experiment 1, vitamin E supplementation of 500 mg/kg DM increased plasma α-tocopherol concentration to 2.10 and 1.92 µg/ml during late pregnancy and lactation respectively. This high level of vitamin E was required to maintain plasma α-tocopherol levels at >2.0 µg/ml (NRC, 2007) during late pregnancy (Figure 6.1). This equates to approximately 476 mg/kg DM (5.6 mg/kg LW) for an 85 kg sheep, which is similar to the NRC (2007) recommendation of 5.6 mg/kg LW, and significantly higher than fed in most UK ewe rations. In experiment 1, vitamin supplementation of 540 mg/day was sufficient to maintain plasma levels above the critical value of 2.0 µg/ml recommended by NRC (2007). In experiment 2, vitamin E supplementations of 250 mg/day in pregnant ewes in combination with a natural source of vitamin E from grass silage increased ewe plasma vitamin E to 3.11 µg/ml, which was sufficient to maintain plasma α-tocopherol levels above 2.0 µg/ml. Whereas, ewe plasma vitamin E was 1.84 µg/ml when they were supplemented with 240 mg/day of vitamin E from concentrate on the straw based diet. In addition, in experiment 3, vitamin E supplementation of 84 mg/day to growing lambs in combination with a natural source of vitamin E from grass silage increased lambs' plasma vitamin E to 2.04 µg/ml, which was sufficient to maintain plasma α-tocopherol levels above 2.0 µg/ml. Whereas, lamb plasma vitamin E was 1.76 µg/ml when they were supplemented with 95 mg/day of vitamin E from concentrate on the straw based diet. In experiment 2, vitamin E supplementation of 725mg/ day increased the

vitamin E concentration of ewes plasma to approximately 4.0 µg/ml, which is considerably higher than recommended by NRC (2007). During both the *pre-partum* and *post-partum* periods, ewes supplemented with high vitamin E had a higher plasma vitamin E, particularly when offered grass silage. The NRC (2007) requirements are similar for both pregnant and lactating ewes (5.3 mg/ kg LW). In the current study, vitamin E content of both concentrates was similar during late pregnancy and lactation. The results of the current study suggest that the vitamin E requirements of lactating ewes might be higher than NRC (2007) recommendations. In experiment 1, during lactation the high level of vitamin E supplementation (up to 950 mg/day) failed to maintain ewe plasma α-tocopherol above the critical value (2.0 µg/ml) (Figure 6.2). Whereas, in experiment 2 during the *post-partum* period increasing vitamin E supplementation up to 700 mg/day from both grass silage and concentrate was required to keep ewe plasma α-tocopherol above (2.0 µg/ml) (Figure 6.2).

Vitamin E is easily and rapidly transferred from plasma into colostrum and milk. This might be the reason for the lower plasma vitamin E concentration *post-partum* (Liu *et al.*, 2014). In light of the above results, the vitamin E requirements of lactating ewes are higher than the current recommendation of NRC (2007). In addition, a lower level of vitamin E is required when it is supplemented in combination with forages that contains a high proportion of natural vitamin E.

The current results are consistent with those of Merrell, (1998), Daniels *et al.* (2000), Capper *et al.* (2005), Rooke *et al.* (2009) and Dønnem *et al.* (2015) who reported that high vitamin E supplementation of ewe during late pregnancy and lactation increased plasma α-tocopherol concentration as described in Section 1.2.2.2. In addition, these results are similar to those of Kasapidou *et al.* (2009), who reported that the plasma α-tocopherol concentration of lambs was higher on grass silage than concentrate based diets. In experiment 2, diets that provided a higher proportion of natural vitamin E from grass silage increased plasma α-tocopherol concentration compared to those that provided a higher proportion of vitamin E from concentrates. Vitamin E from natural sources is biologically more active and absorbed more efficiently from the small intestine than the synthetic form of vitamin E as described in Section 1.3.2.2. Differences in the relative proportions of α-tocopherol stereoisomers is another reason for the higher plasma α-tocopherol concentrations in ewe that fed grass silage based diets. The naturally occurring form of vitamin E consists of the *RRR* isomers, while the synthetic form of vitamin E consists of a racemic mixture of eight stereoisomers (Yang, 2003; Meglia *et al.*, 2006 and Wilburn *et al.*, 2008) as described in Section 1.3.2.2. Hepatic protein (α-TTP) which is responsible for the uptake of α-tocopherol from the hepatic cells into the very low-density lipoprotein (VLDL) prefer to bind with *RRR* isomer than the other isomers (Burton and Traber, 1990). Therefore, vitamin E supplementation from grass silage was more efficient.

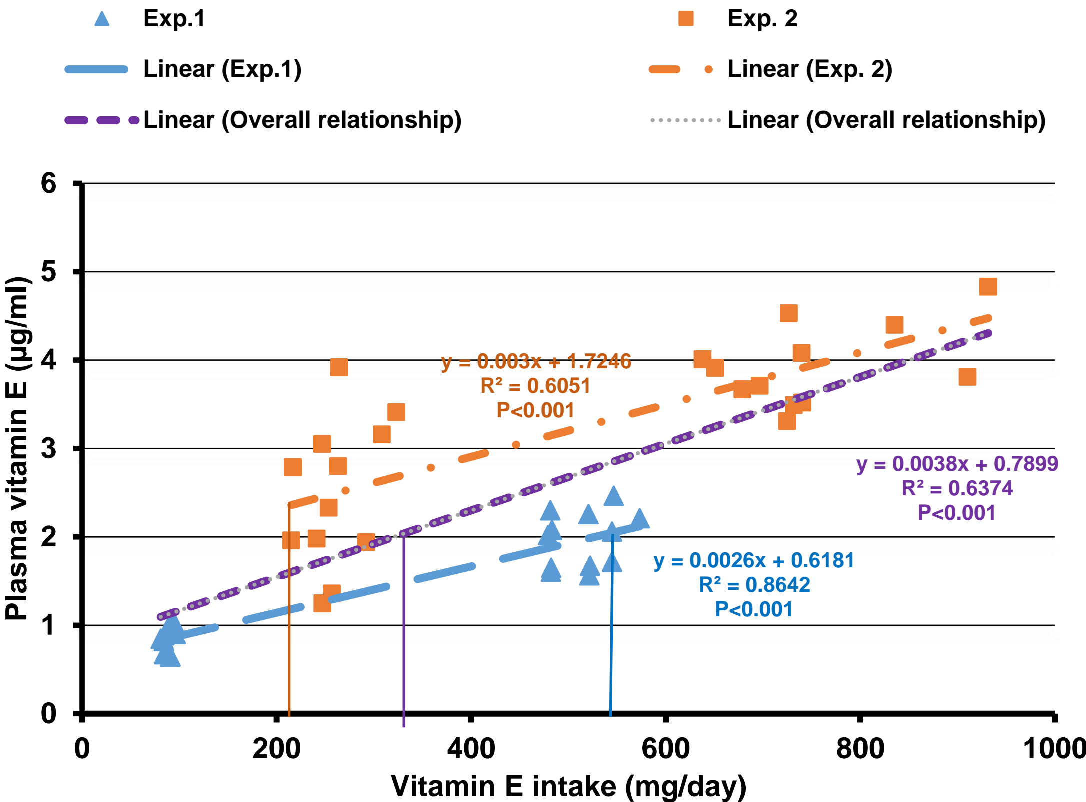


Figure 6. 1. Relationship between dietary vitamin E supply (mg/day) and plasma vitamin E concentration (µg/ml) for ewes in late pregnancy offered the diets fed in experiments 1 and 2.

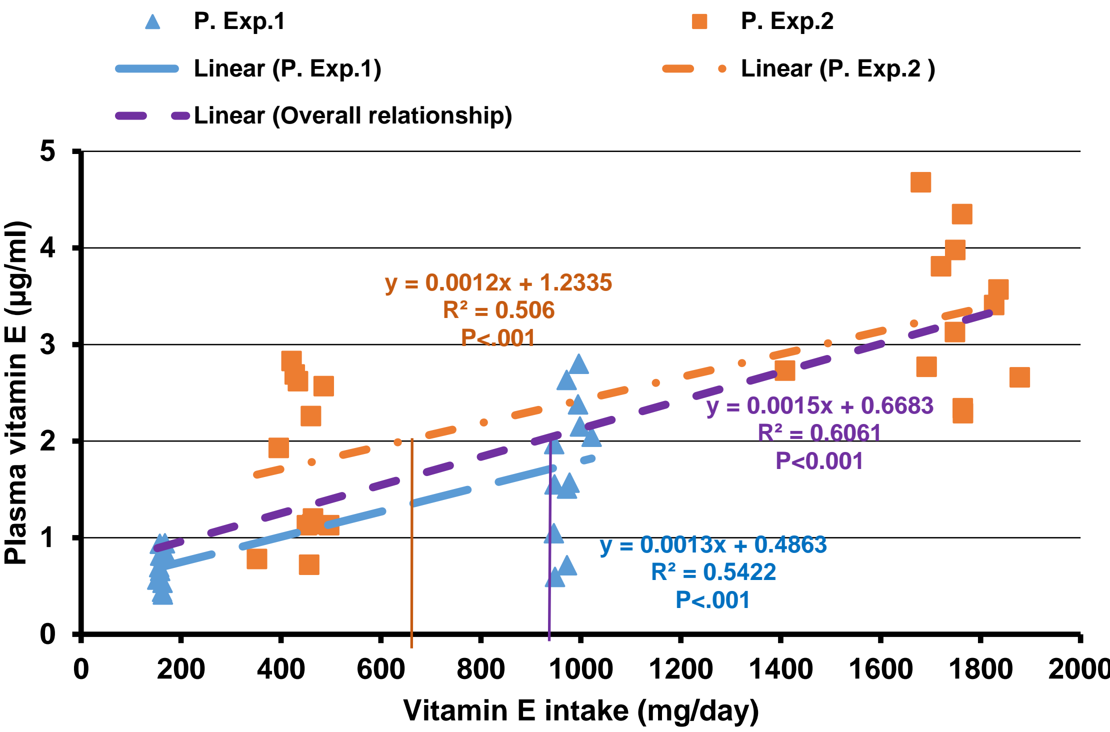


Figure 6. 2. Relationship between dietary vitamin E supply (mg/day) and plasma vitamin E concentration (µg/ml) for ewes in early lactation offered the diets fed in experiments 1 and 2.

As previously discussed (Section 1.2.4.1), α-tocopherol supplementation during late pregnancy has been reported to increase the placental vascular network and angiogenesis leading to an increase in nutrient supply to the growing foetus (Kasimanickam *et al.*, 2010). Alpha-tocopherol supplementation increases the expression of mRNA for placental growth factor (PIGF), endothelial nitric oxide synthase (eNOS) and hypoxia inducible factors (HIF-1α) in cotyledons (Kasimanickam *et al.*, 2010). This mechanism potentially explains the results observed in experiment 2, where the high level of vitamin E supplementation increased placental weight, cotyledon weight and tended to increase lamb birth weight (Figure 6.3). In experiment 2, the plasma vitamin E concentration of ewes offered the high vitamin E diets was approximately 4.0 µg/ml at week -1, which is considered higher than the NRC (2007) critical value (2.0 µg/ml). This may be the reason for the response to high vitamin E supplementation. Whereas, in experiment 1, the high level of vitamin E supplementation was only sufficient to maintain plasma vitamin E at the NRC (2007) recommended levels (2.0 µg/ml) in late pregnancy, but not to elicit a supra-nutritional response, this could be the reason that high vitamin E supplementation in experiment 1 had no effect on litter birth weight. The results of previously published studies on the effect of vitamin E supplementation on lamb birth weight are inconsistent. The effects of vitamin E supplementation on litter birth weight in experiment 2 are consistent with those of Capper *et al.* (2005) and Nieto *et al.* (2016) who reported that vitamin E supplementation of ewes

during late pregnancy increased lamb birth weight. However, the effects of vitamin E supplementation on litter birth weight in experiment 1 are similar to those of Merrell *et al.* (1998); Daniels *et al.* (2000); Dafoe *et al.* (2008); Rooke *et al.* (2009), who documented no effect of high vitamin E supplementation during late pregnancy on lamb birth weight.

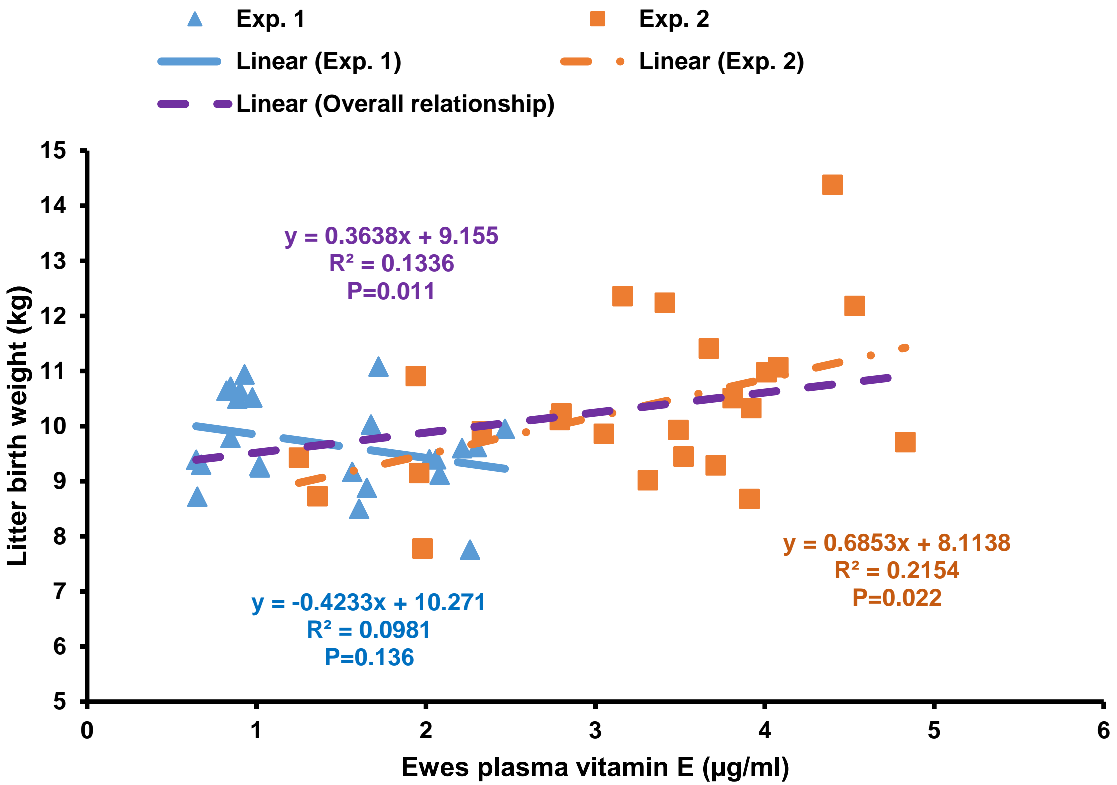


Figure 6. 3. Relationship between ewes plasma vitamin E (μg/ml) during late pregnancy and litter weight (kg) for ewes offered the diets fed in experiments 1 and 2.

In experiment 1, increasing vitamin E supplementation from 89 to 540 mg/day increased colostrum vitamin E concentration (~6 to 17 mg/ kg) during lactation (Figure 6.4), and ultimately increased lamb plasma vitamin E concentration from 1.02 to 1.84 μg/ml (R^2 = 0.4494) (Figure 6.5), which means that high vitamin E supplementation failed to maintain lamb plasma vitamin E concentration above the critical value (2.0 μg/ml) recommended by NRC (2007). In experiment 2, increasing vitamin E supplementation from 260 to 950 mg/day increased colostrum vitamin E from ~14 to 22 mg/kg colostrum during lactation (Figure 6.4), and ultimately increased lamb plasma vitamin E from 3.01 to 6.11 μg/ml, however, there was no relationship between colostrum vitamin E and lamb plasma vitamin E (R^2 = 0.0696) (Figure 6.5). In addition, the plasma vitamin E concentration of lambs from ewes offered the low vitamin E diets was higher (3.01 μg/ml) than that of lambs from ewes offered the high vitamin E diets in experiment 1 (1.84 μg/ml). However, the colostrum vitamin E concentration of ewes offered the high vitamin E diets in experiment 1 was only slightly higher than that of ewes offered the low vitamin E diets in experiment 2 (16.77 vs

14.25 mg/kg respectively). The higher lamb plasma vitamin E concentrations in experiment 2 could be due to higher levels of ewe vitamin E supplementation increasing the vitamin E concentration of colostrum.

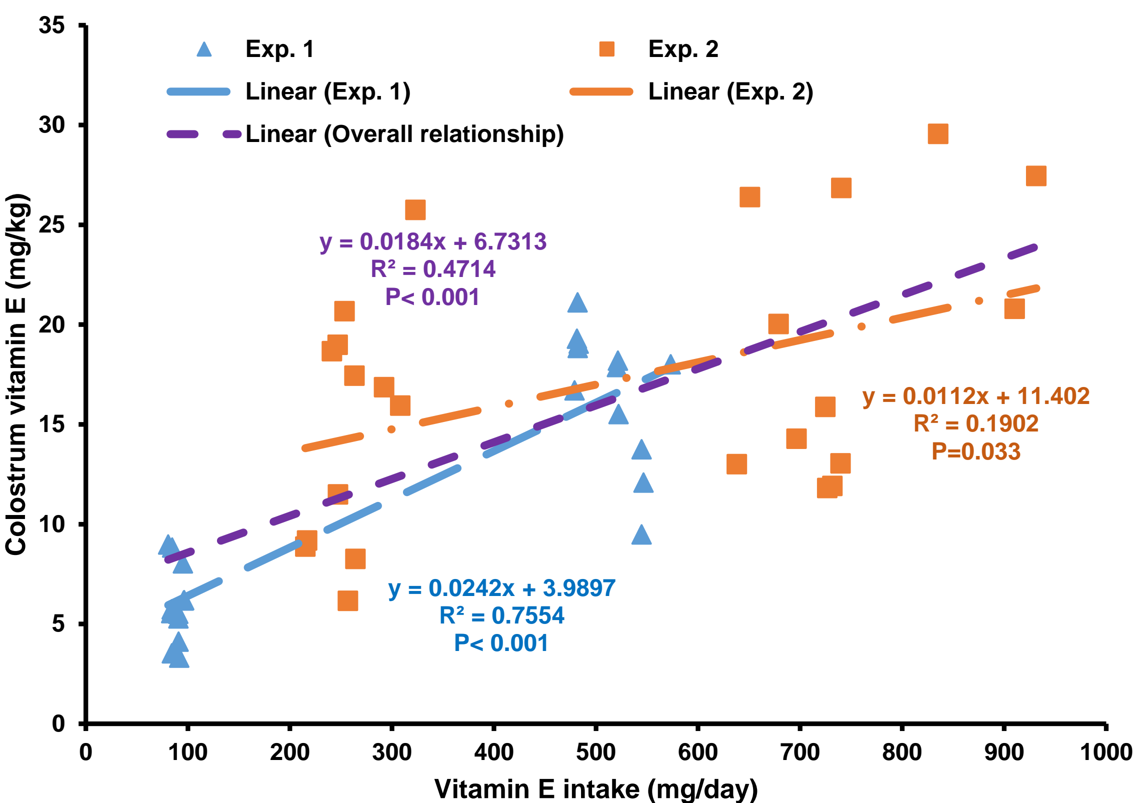


Figure 6. 4. Relationship between ewe vitamin E intake (mg/day) and colostrum vitamin E concentration (mg/kg) for ewes offered the diets fed in experiments 1 and 2.

In experiment 1, vitamin E supplementation did not affect lamb weaning weight. Whereas, in experiment 2, lamb weaning weight increased with high vitamin E supplementation. As an antioxidant, vitamin E reduces oxidative stress and improves the immunity of neonatal and growing lambs (Liu *et al*., 2014). As mentioned in the above paragraph, lamb plasma α-tocopherol in experiment 1, was slightly lower than the critical value (2.0 µg/ml) recommended by (NRC, 2007). Whereas, in experiment 2, the higher vitamin E intake during weeks 0 to +4 increased lamb plasma α-tocopherol concentration to 6.11 µg/ml. This may have reduced oxidative stress and enhanced lamb performance. In experiment 1, the effects of vitamin E supplementation on lamb weaning weight were similar to those of Daniels *et al.* (2000), Capper *et al.* (2007), Dafoe *et al.* (2008) and Rooke *et al.* (2009) who found that there was no effect of vitamin E supplementation in late pregnancy and lactation on lamb weaning weight. Whereas, the results in experiment 2 are in agreement with those of Merrell *et al.* (1998) and Kott *et al.* (1998) who reported that vitamin E supplementation of ewes during late pregnancy and lactation increased lambs weaning weight.

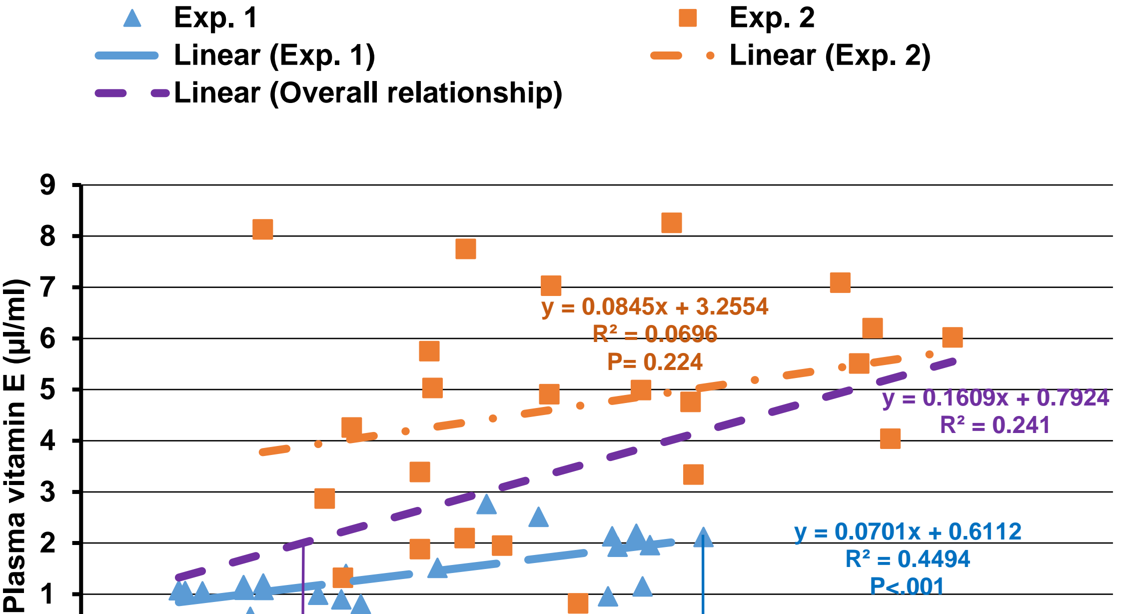


Figure 6. 5. Relationship between colostrum vitamin E concentration (mg/kg) and lambs' plasma vitamin E concentration (µl/ml) for ewes offered the diets fed in experiments 1 and 2.

As discussed previously in Section 3.4., vitamin E supplementation during late pregnancy improves reproductive performance by preventing pathology of the reproductive system or foetus and reducing myocardial necrosis (Buchanan-Smith *et al.,* 1969). In addition, vitamin E supplementation may help to prevent reproductive problems like retained placenta and endometritis (Hemingway, 2003). Protection of ewes from reproductive problems where oxidative stress may be a contributory factor may help to improve performance, especially in ewes that have experienced nutritional restriction. A further significant finding of the current study was the effect of high vitamin E supplementation on ewe performance. In experiment 1, ewes that experienced nutritional restriction and received the high level of vitamin supplementation tended to gain more live weight *pre-partum*, and maintain live weight during early lactation. In addition, they lost less C. In experiment 2, ewes offered the straw based diets with the high level of vitamin E supplementation lost less C and tended to lose less back fat *post-partum.*

A number of vitamin E functions has been documented. As an antioxidant, vitamin E prevents the oxidation of polyunsaturated fatty acids (PUFA) and lipoproteins in cell membranes to hydroperoxides and protects animal cell membrane from oxidative damage (Bramley *et al.,* 2000; Mcdowell, 2000). High vitamin E supplementation improved feed conversion efficiency in Morkaraman (Macit *et al.,* 2003a) and Awassi (Macit *et al.,* 2003b)

sheep. The above functions might be the reasons that high vitamin E supplementation had an effect on ewes performance in the current study.

In previous studies, inconsistent results have been documented on the effect of vitamin E on ewe performance. The results of the current study are similar to the results of Nieto *et al.* (2016) who reported that ewes supplemented with high vitamin E tended to loss less weight during pregnancy and early lactation. Whereas, they are inconsistent with those of Merrell *et al. (*1998) who reported that there was no effect of vitamin E on ewes C change, and Capper *et al.* (2007) who found that a high level vitamin E supplementation increased C loss.

6.2. General Conclusion

1- High vitamin E supplementation increased ewe plasma vitamin E, colostrum vitamin E and lamb plasma vitamin E concentration particularly in ewes offered the grass silage based diets which provided a higher proportion of *RRR*-α-tocopherol as a natural source of vitamin E rather than all-*rac*-α-tocopherol, a synthetic vitamin E from concentrates.

2- The vitamin E requirements of lactating ewes are considerably higher than the current NRC (2007) recommendation. The current study results show that supplementation of 540 mg/day of all-*rac*-α-tocopherol acetate was sufficient to meet NRC (2007) requirements during late pregnancy. Whereas, supplementation above 950 mg/ day might be required during lactation. However, supplementation of 700 mg/ day in combination with a high proportion of *RRR*-α-tocopherol from forages is enough to meet NRC (2007) requirements during lactation.

3- High vitamin E supplementation tended to increase ewe LW gain during late pregnancy and reduce C loss during early lactation, particularly in ewes that had experienced nutritional restriction during mid-pregnancy.

4- Supplementation of pregnant ewes with 9.0 mg/ kg LW may enhance litter birth and weaning weight via increasing placental size and efficiency. This is equivalent to the 9.0-10.0 mg/kg LW recommended by NRC (2007) to enhance immune competence and shelf life in meat.

6.3. Areas for further research

1- In experiment 2, supra-nutritional vitamin E supplementation tended to increase lamb birth. This could be a result of an increase in placental size and efficiency. During pregnancy 80% of placental growth occurs in mid pregnancy. Vitamin E supplementation during mid-pregnancy may also influence placental size and lamb birthweight.

2- During lactation, in experiment 1 and 2, ewes supplemented with supra-nutritional vitamin E lost less C. In addition, supra-nutritional vitamin E supplementation increased lamb weaning weight in experiment 2. However, the vitamin E requirements to elicit a supra-nutritional response during lactation may be higher than that was in the current study. Vitamin E supplementation at higher levels than in current study may increase the response of lactating ewes and their lambs.

3- The response of ewes and their lambs to supra-nutritional vitamin E supplementation in current study could be due to the antioxidant function of vitamin E. Supplementation of alternative antioxidants may also improve ewe and lamb performance.

4- In experiment 3, high vitamin E supplementation reduced dry matter and NDF digestibility coefficient in growing lambs fed at maintenance. The mechanisms by which this reduction took place require further investigation.

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