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Effect of Different Levels of Undegradable Protein on Performance, Reproductive Activity and Rumen Fermentation in Ewes during Flushing Period

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Abstract

The objective of this study was to investigate the effect of different protein sources with different degradability ratios during flushing period of ewes on body weight change, reproductive performance, and rumen fermentation parameters. 35 multiparous native crossbred ewes (BW = 54±2.5kg) were randomly allocated to five dietary treatments (7 ewes/treatment) for 3 months. Experimental diets were isonitrogenous (12.27% CP) and isocaloric (2.22Mcal ME)/kg DM. In diet I (control), solvent extracted soybeans (SESM 33% RUP of CP), II feed grade urea (FGU 31% RUP), III slow release urea (SRU 31% RUP). As sources of undegradable protein, extruded expeller SBM-EESM 40 (37% RUP) and extruded expeller SBM-EESM 60 (41% RUP) were used in groups IV and V, respectively. Ewes fed a 37% RUP diet gained more ($p<0.05$) weight compared with ewes fed a 31% RUP diet (6.71 vs. 2.92kg). Ewes in EESM 40 had the highest twinning percentage, while the urea groups had the highest fertility rate. Protein source and RUP levels in ewes' diets had no significant effect ($P< 0.05$) on feed intake, CP, ME, BCS, progesterone level, blood biochemical parameters, and rumen kinetics. In conclusion, the dietary level of RUP (37% of CP) in EESM 40 was the best for producing twins, gaining weight, and having the highest level of progesterone hormone and a lower degree of degradation of branched chain amino acids, while SRU (31% of RUP) had the best fertility rate, which was confirmed by the highest levels of blood glucose and progesterone.

Keywords

Ewes, Flushing, Reproductive Indices, Rumen Kinetics, Undegradable Protein

1. Introduction

Reproduction efficiency plays a critical role in determining profit potential for livestock production systems. The high reproductive rate of sheep is among its most significant benefits. Three environmental factors that influence ewe fertility have been extensively studied: photoperiod, weather, and nutrition. Due to its immediate and indirect effects on physiology and reproductive function, nutrition is crucial. For a very long time, ruminant nutrition was only focused on quantity, not quality, which resulted in a glaring discrepancy between the needs and the feeds that were readily accessible. Therefore, it is widely accepted that any effort to increase their output should focus on improving the feeding system **(Muniz et al., 2008)**.

One of the key factors that directly affects agricultural animals' productivity and reproductivity is the quality of their nutrition. Breeds with greater productivity typically require more nutrients, including protein, which is necessary for tissue deposition. Because they provide a supply of amino acids in the form of rumen undegradable protein (RUP) and a nitrogen source from rumen degradable protein (RDP) for the synthesis of microbial protein, dietary proteins are essential in ruminant nutrition **(Kaur and Arora, 1995)**.

A realistic management technique to increase ovulation rate and subsequently, prolificacy of sheep production systems is to manipulate fertility through diet. In order to boost nutrient consumption (especially in terms of energy and or protein) before, during or following mating, nutritional flushing is described as the temporary supply of additional feed. Flushing diets act via the hypothalamus pituitary-gonadal axis affecting the secretion of hormone including insulin, progesterone and estrogen **(Scaramuzzi et al., 2006)**.

The beneficial impact of high protein supplements on the rate of ovulation in both cows and sheep has been the subject of numerous studies. According to **Lazarin et al. (2012)**, supplementing Santa Ines ewes' diets with SBM and CGM as a RUP source elevated their ovulation rate. This finding is in line with that of **Rodrigues et al. (2015)**, who came to the same conclusion about the significant effects on the metabolic and reproductive responses of ewes consumed various levels of dietary protein during the mating season. Additionally, **Daghigh Kia et al. (2016)** found that supplementing corn gluten meal as a source of rumen undegradable protein prior to mating enhanced ovarian function, follicular growth, and ovulation rate due to elevated insulin and maybe certain amino acids, which would lead to higher rates of fertility, fecundity, and twin births. Adequate levels of protein can improve the energy balance of the animal, and also promote the synthesis of lipoproteins **(Auboiron et al., 1995)** which are essential agents for the transport of cholesterol, a molecule necessary for steroidogenesis, besides participating in the direct stimulation of insulin growth factor I (IGF-I) secretion by luteal cells **(Castañeda-Gutiérrez et al., 2007)**.

Ruminants have a unique ability to ferment fodder in the rumen before moving to the rest of the gut. Volatile fatty acids (VFA), microbial proteins (MP), and ammonia nitrogen (NH3) are the end results of fermentation **(Gleghorn, 2003; Diego et al., 2018)**. Volatile fatty acids and pH are impacted by the degree of protein degradation **(Jasim, 2019)**, but other research found no evidence of a significant relationship between the protein source and these variables **(Shain et al., 1998)**. Despite the fact that microbial protein is the main source of protein for ruminants, boosting dietary RUP can raise the flow of AA over microbial AA supply. There are no documented requirements for RUP in sheep diets or its effect on rumen fermentation or its relationship with reproduction, but the **NRC (2007)** reports that raising dietary RUP from 3.4 to 9.3% of DM reduces dietary CP requirements, indicating improved efficiency of N use from RUP. So, this study was conducted to investigate the impact of various protein ingredients with different ratios of protein degradability on ewes' reproductivity and rumen fermentation characteristics during the flushing period. Moreover, the effect of undegradable protein on body weight changes and blood parameters of ewes were investigated.

2. Materials and Methods

All animal procedures were conducted in accordance with the standards set forth in the guidelines for the care and use of experimental animals. **The study protocol was approved by the Animal Ethics Committee at Faculty of Veterinary Medicine, Beni-Suef University, Egypt (number 022-435).**

2.1. Animals and Housing

The experiment was carried out in the sheep experimental farm of the Animal Reproduction Research Institute, El-Ahram, Giza, Egypt. Thirty-five non pregnant native mixed-breed ewes (Barki x Rahmani), aging between 2 and 3 years, with a body condition score (BCS) about 2.5, and body weight of 54±2.5 kg, were used. The animals had a clinical examination and were found to be both internally and externally parasite-free and in apparent healthy condition. Five equal groups of experimental ewes were divided randomly; each group was housed in a separate pen with natural lighting and temperature. One ewe was excluded from group number (V) due to reproductive disorder. The ewes were synchronized at the start of the experiment using an intravaginal sponge containing 60 mg of medroxyprogesterone acetate inserted for 14 days. Preventing vaginitis Oxytetracycline 0.25 mL was administrated into each sponge. Teaser rams were used to observe the estrous manifestations at 8:00 and 17:00 for at least of 15 minutes each time beginning twelve hours after the sponge was withdrawn. 17 days after the sponge was removed, adult rams were introduced and used to mate ewes naturally. One month after mating pregnancy was detected then one month later confirmed using ultrasonography.

2.2. Diets

Before the trial commenced, diets were gradually offered to the ewes over a preparatory two-week period before the flushing period to accustom the animals and measure the level of feed intake. The flushing period covered three months, four weeks before mating and continued for eight weeks after and until the pregnancy was detected and confirmed. The five experimental groups were fed on diets differing in the source of protein and its degradability. Physically the diets were composed of the roughages hay and wheat straw and the concentrates yellow corn, molasses and supplements the source of protein in diet I (the control) was solvent extract soybeans and designated by SESM (24.49% RUP of CP), feed grade urea (FGU; 0.23% RUP of CP) in II and slow release urea (SRU; 0.27% of RUP of CP) in III. As a source of undegradable protein extruded expeller SBM – EESM 40 and extruded expeller SBM – EESM 60 were used in group IV and V respectively. As related to the bypass protein EESM 40 contains 43.69 % RUP of crude protein and EESM 60 contains 61.51%, commercially 40 and 60 %. RUP of the five diets reached 33, 31, 31, 37, and 41% of CP, in respective order as shown in the following.

Table 1. Animal groups

Group		N	Dietary protein supplement	RUP : RDP (% of CP)
I	(SESM – Control)	7	Solvent extracted soybean meal	33.05 : 66.95
II	(FGU)	7	Feed grade urea	30.83 : 69.17
III	(SRU)	7	Slow release urea	30.68 : 69.32
IV	(EESM 40)	7	Extruded-expeller soybean meal (40)	37.23 : 62.77
V	(EESM 60)	6	Extruded-expeller soybean meal (60)	41.01 : 58.99

SESM: Solvent extracted soybean meal **FGU**: Feed grade urea **SRU**: Slow release urea **EESM 40**: Extruded-expeller soybean meal 40% bypass protein **EESM 60**: Extruded-expeller soybean meal 60% bypass protein **RUP**: Rumen undegradable protein **RDP**: Rumen degradable protein

Table (2) shows the analysis of the feed ingredients while Table (3) the physical and chemical composition of the five experimental diets. The experimental groups received the feed ingredients in total mixed rations (TMR) twice a day at 8:00 and 16:00, ad libitum. The diets were balanced to be isoenergetic isonitrogenous containing on the average 2.22 Mcal ME/kg DM and 12.27 % CP. Ewes have unlimited access to fresh water.

2.3. Measurements

2.3.1. Diet analyses, Determination of Ewe Performance and Lambing Rate

The feed ingredients used in the formulation and TMR were sampled and analyzed for Dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to **AOAC (1995)**. The in vitro nitrogen degradability properties of the TMR components were measured before the experiment started. These findings allowed for the calculation of the amount of undegradable protein (UDP) supplied by each treatment **(Roe et al., 1990)**, and ME (Mcal/kg) estimated using **NRC of sheep (1985)** and of the diets calculated. The higher levels of dietary protein was nominated to cover the needs of ewes, and the recommended percentage in the NRC plus the used to be added safety margin. To calculate the DM intake, the weekly amount of TMR provided and rejected by ewes was gathered and weighed before the morning meal. Before morning feeding on the first day the diets were provided, and subsequently once a month until the finish of the flushing period, ewes were weighed and their body condition scores (BCS) were also measured. Handling over and around the backbone of ewes was used to evaluate the BCS on a scale of 0 to 5 according to **Gordon (1997)**. Lambing and fertility rates and twinning percentage were traced and recorded according to **Ababakri et al. (2021)**.

2.4. Chemical Analysis of Blood and Rumen Fluid

Blood samples were obtained by jugular venipuncture 3hrs post morning feeding on days 3, 6, 9, 12, and 15 of the next reproductive cycle after CIDR removal to measure progesterone hormone (also on day 17). Serum was separated and stored at -20°C until being used. Thereafter, glucose, blood urea nitrogen, total protein, albumin were measured spectrophotometrically using chemical test kits according to the manufacture instructions of each test kit **(Reactivos GPL Barcelona, España)**, and (globulin was calculated). Serum progesterone analysis was done by using a commercial kit based on Enzyme Linked Immune Sorbent Assay (ELISA) **(AB Diagnostic Systems GmbH, Germany)**.The glucose was measured immediately after sampling. Approximately 100 ml of ruminal fluid were collected as described by **Saeed and Latif (2008)**. Samples were withdrawn at the last week of the flushing period before morning feeding (zero time), 1, 2, and 4 hrs post feeding. Immediately after collection of rumen fluid, ruminal pH value was determined using a pH meter. Ruminal ammonia nitrogen (NH_3-N) **Con-way (1948),** total volatile fatty acids (TVFA) **Abou-Akkada and El-shazly (1964)** were determined. Gas chromatography **(model GC-2014, Shimadzu, Tokyo, Japan)** was used to determine the molar ratios of each particular VFA.

2.5. Statistical Analysis

All data were subjected to statistical analysis including the calculation of the mean and standard error and to determine significant differences among treatment groups. Using the **SPSS (2007)**, data were analyzed using one-way ANOVA, which suggests a randomized full block design.

Table 2. Chemical composition (% on DM basis) and metabolizable energy value (Mcal/kg) of feed ingredients used in the experimental rations.

Ingredient	DM	CP	NDF	ADF	Ash	NFC	EE	ME	RUP	RDP	RUP (% CP)
Roughages											
Alfalfa hay (early bloom)	91.55	13.57	48.82	24.36	8.70	27.16	1.75	2.03	3.99	9.58	29.40
Wheat straw	91.64	5.20	78.40	54.58	8.70	7.35	0.35	1.48	1.04	4.16	20.00
Concentrates											
Yellow corn (coarse ground)	91.08	9.60	10.10	3.68	1.64	75.36	3.30	3.15	5.94	3.66	61.88
Soybean meal (solvent extraction)	91.25	47.69	15.84	7.58	6.00	28.76	1.71	3.18	11.68	36.01	24.49
Extruded expeller soybean meal 40	92.72	47.26	19.72	8.82	7.90	21.62	3.50	3.07	20.65	26.61	43.69
Extruded expeller soybean meal 60	94.49	46.38	29.94	9.00	5.00	14.72	3.96	3.18	28.53	17.85	61.51
Feed grade urea	99.19	256	0.02	0.01	0.00	0.00	0.00	0.00	0.59	255.41	0.23
Slow release urea (Optigen II)*	99.72	266	0.04	0.02	0.00	0.00	11.80	0.00	0.71	256.29	0.27
Molasses	71.43	3.00	0.66	0.33	9.77	86.37	0.20	2.86	0.09	2.91	3.00

DM = dry matter, **CP** = crude protein, **NDF** = neutral detergent fiber, **ADF** = acid detergent fiber, **NFC** = nonfibrous carbohydrate calculated according to the equation **= 100 – (% NDF+% CP+ % EE+ % ash)**, **EE** = ether extract, **ME =** metabolizable energy tabulated according **NRC of Sheep (1985), RUP=** rumen undegradable protein, **RDP**= rumen degradable protein. *Optigen II: each kg contains urea 855 gm, vegetable oil (soybean) 125 gm, beta-carotene 10 gm, and BHT 10 gm. The product is manufactured by ALLTECH, Inc. USA. Mineral & vitamin premix: Each 3 kg of mineral and vitamin premix contain Mn: 20000 mg; Fe: 30000 mg; Zn: 20000 mg; Cu: 7000 mg; I: 100 mg; Se: 100 mg; Mg: 20000 mg; Co: 100 mg; vitamin A: 5000000 IU; vitamin D_3: 1000000 IU; vitamin E: 20000 mg; and $CaCO_3$ as a carrier added up to 3 kg. The premix manufactured by Egypt Pharma for Prymix & Feed Additives Industrial.

Table 3. Physical composition (% on as fed basis) and Chemical composition (% on DM basis) of rations fed to ewes during flushing period

Ingredient	Experimental diet				
	I (C)	II (FGU)	III (SRU)	IV (EESM 40)	V (EESM 60)
Physical composition (% on as fed basis)					
Alfalfa hay	45.20	45.20	45.20	45.25	45.29
Wheat straw	20.16	20.16	20.16	20.17	20.20
Yellow corn	22.57	25.59	25.59	22.59	22.61
Solvent extracted soybean meal 44%	5.56	1.99	1.99	-	-
Extruded expeller soybean meal 40	-	-	-	5.48	-
Extruded expeller soybean meal 60	-	-	-	-	5.38
Feed grade urea	-	0.54	-	-	-
Slow release urea (Optigen II)	-	-	0.54	-	-
Molasses, sugarcane	4.94	4.95	4.95	4.94	4.95
Common salt	0.63	0.63	0.63	0.63	0.63
Di-calcium phosphate	0.40	0.40	0.40	0.40	0.40
Mineral & vitamin premix	0.54	0.54	0.54	0.54	0.54
Chemical composition (% on DM basis)					
Dry matter	89.10	89.15	89.15	89.19	89.29
Crude protein	12.23	12.34	12.40	12.21	12.16
Neutral detergent fiber	41.51	41.24	41.24	41.73	42.30
Acid detergent fiber	23.54	23.38	23.38	23.61	23.62
Ash	6.84	6.67	6.67	6.95	6.78
Nonfibrous carbohydrate	36.00	37.22	37.22	35.60	35.21
Ether extract	1.72	1.76	1.83	1.82	1.85
Metabolizable energy (Mcal/kg)	2.23	2.21	2.21	2.23	2.23
Rumen undegradable protein	4.04	3.81	3.81	4.55	4.99
Rumen degradable protein	8.19	8.53	8.59	7.66	7.17
Rumen undegradable protein (% CP)	33.05	30.83	30.68	37.23	41.01
Rumen degradable protein (% CP)	66.95	69.17	69.32	62.77	58.99

C = control**, FGU** = feed grade urea, **SRU**= slow release urea, **EESM 40** = extruded expeller soybean meal 40% of CP bypass protein, **EESM 60** = extruded expeller soybean meal 60% of CP bypass protein.

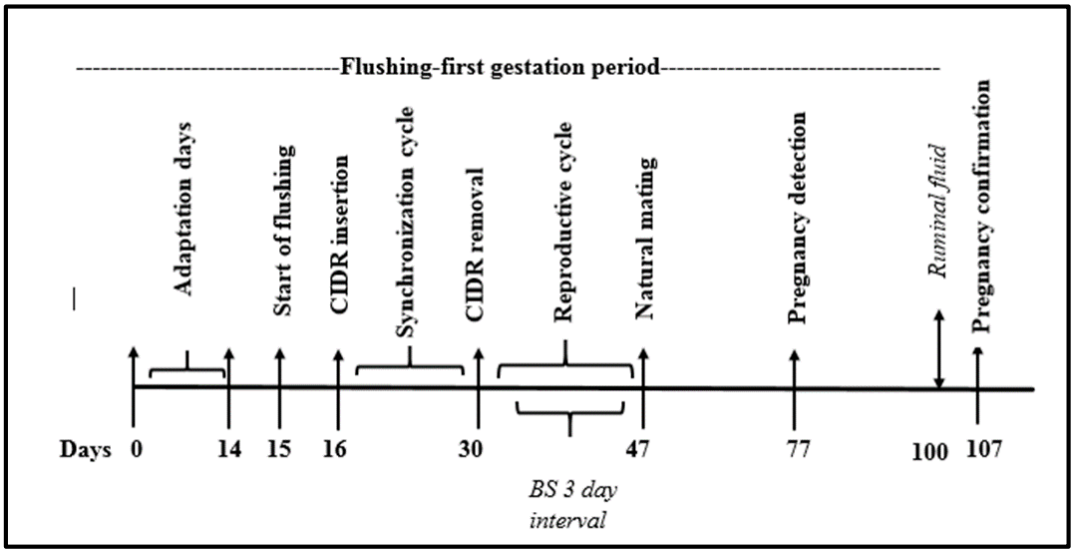

Fig. 1. Schematic figure showing the experimental plan in flushing period.

3. Results

The daily dry matter ranges in the five groups from 1035 to 1250g with a difference of 215g over three months feeding, a difference considered not so high especially for animals of a weight varied from 54 to 57kg. In spite of this when the DM intake is calculated in relation to mean body weight of the groups **(Table, 4)** the control group and EESM 40 one are found to be the higher in intake with a rate of 2.3 and 2.2% followed by the groups EESM 60 and SRU which are nearly equal 2%. FGU group remains to be the lowest in consumption 1.89%. Crude protein and metabolizable energy levels of intake was the groups and the lowest in the urea groups. As to the RUP intake the urea groups were the lowest, about 40 g/ animal/ day which seems to be sufficient and covers the needs of the animal. As long as the dietary CP is around 12%, the RUP around 30% is optimal.

As to the body weight change the excel group is EESM 40 as it is the highest in mean body weight (57kg) and in body weight change where it increased 6.7kg while the control scored 54.5kg mean and 5.6kg change and EESM 60, 56.5kg mean and 4.3kg change. The two urea groups are the lowest, FGU 55 and 3.1kg and SRU 54 and 2.9kg. As the control group surpasses EESM 60 in body weight change so it is not a matter of RUP supply; what is in the control diet covers the needs and more. The decrease in body weight change in FGU and SRU groups is a matter of urea itself, feed grade or slow released and this might be related to their lowest feed intake.

Reproductive indices were represented by two rates, lambing and fertility and another percentage the twinning one. The total ewes exposed for mating were 7 in each group except 6 in EESM 60. The lambing ewes in the groups differed and were 4 in the control, EESM 40 and EESM 60 and 6 in urea groups FGU and SRU, giving a percentage rate of 57 in control and EESM 40, 67 in EESM 60 and 86 in the urea groups. So it seems that the fertility rate is not affected by the supply of RUP, on the reverse it was the highest in urea supplements containing nearly no RUP. Lambing rate is 100 % in all groups while the twinning percentage was the highest in EESM40 (50 % and one ewe born triplet) compared with the other groups ranging from 0 to 25%.

As shown in **Table (5)** progesterone hormone was determined every 3 days starting 3 days after synchronization, for 5 times and at day 17 during the estrous cycle. The level at 3 days started at equal levels from 0.30 to 0.61 ng/ml in control, FGU and ESSM 60 and significantly high ones in SRU (1.27) and EESM 40 (1.32). The levels at the start (3 d) increased gradually to peaks, at the day 12, equal in control, FGU, and ESSM 60 and ranging from 4.94 to 6.54 ng/ml, and significantly high in SRU (10.21) and EESM 40 (10.63). After reaching the peak the figures decreased at first slightly at the day 15, reaching 3.72 to 4.33 ng/ml in control, FGU, and EESM 60; and 8.43 ng/ml in SRU and 8.33 ng/ml in EESM 40, and then decreased a lot to reach at the day 17 less than 1.0 in the three groups and 1.74 and 1.84 ng/ml in SRU and EESM 40 respectively. It is noted that all the three groups (control, FGU, and EESM 60) were statistically at the same levels all over the days, but the groups SRU and EESM 40 showed the highest ones.

Reviewing **Table (6)** for blood biochemical parameters, serum glucose concentration of the groups FGU, EESM 40 and EESM 60 were nearly equal 36, 36 & 39 mg/dl in order, while the group SRU was exceptionally high (57) followed by the control (43). In overall means of BUN, all groups were statistically equal (18 – 21 md/dl) except EESM 60 group showed a lower urea N value (14 md/dl) which can be attributed to its very high RUP concentration. Regarding total protein findings, in the overall means FGU, SRU, and EESM 40 were equal and the control and EESM 60 were of the highest equal levels. The five groups' results of albumin were equal ranging from 2.6 to 3.5 g/dl with the two diets FGU and SRU had statistically high values. Regarding globulin concentrations the control and EESM 60 as they were the highest (6.89 & 6.60 g/dl) while the rest of three groups FGU, SRU, EESM 40 reached in succession to 4.41, 4.66 & 5.22 g/dl.

Regarding ruminal fluid parameters **Table (7)**. In the overall post feeding pH values, the lowest pH was in the EESM groups, and the other three groups are equal.

Ruminal ammonia in the five groups post feeding were equal in SRU, EESM 60, and control and ranged from 8.82 to 7.94 mg/100 ml, while, it increased to 10.04 in EESM 40 and even more to 11.83 in FGU. The TVFAs in mequiv/100 ml in the five groups post feeding ware equal in control, EESM 40 and EESM 60 and ranged from 14.22 to 15.19, while, it decreased to 12.69 in FGU and even to 9.52 in SRU. The VFAs were found to be mostly composed of acetic acid reaching from 59.62 to 64.71 % and followed by the propionic at from 21 to 23 % with no differences between groups. *The butyric acid* was only from 9.97 to 12.64 % with the highest ratios in EESM 60 and EESM 40. *Isobutyric* varied from 0.66 to 2.64 % with the lowest figures in EESM 40 and SRU. *The valeric* varied from 0.93 to 2.95 % with the lowest figures also in EESM 40, 60 and SRU. *Isovaleric* reached less than 1% except in FGU it reached 1.38.

In conclusion the acids are mostly acetic about 64% and propionic about 23% and the rest is mostly butyric and isobutyric reaching about 11% highest in control and EESM 40

Table 4. Feed intake, body weight and body condition score change, and reproductive performance in ewes offered different levels of undegradable protein during flushing period.

	Control	FGU	SRU	EESM 40	EESM 60
Feed intake g/animal/day	1250	1035	1080	1250	1140
Intake CP g /animal/day	152.90	127.70	134.00	152.60	138.60
Intake RUP g/animal/day	50.53	39.37	41.11	56.81	56.84
Intake ME Mcal/animal/day	2.79	2.29	2.39	2.79	2.54
Mean of body weight	54.46	54.90	53.96	56.79	56.48
Body weight change	5.64±1.17^{ab}	3.07±0.49^{c}	2.92±0.22^{c}	6.71±0.46^{a}	4.25±1.05^{bc}
BCS change	0.50±0.15^{a}	0.36±0.14^{a}	0.36±0.09^{a}	0.64±0.09^{a}	0.58±0.15^{a}
Reproductive indices					
Total ewes	7	7	7	7	6
Lambing ewes	4	6	6	4	4
Total offspring	4	7	7	7	5
Lambing rate (%)	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Fertility rate (%)[1]	57.14^{b}	85.71^{a}	85.71^{a}	57.14^{b}	66.67^{ab}
Twining (%)[2]	0^{b}	16.67^{b}	16.67^{b}	50^{a}	25^{ab}

[1] **Fertility rate:** number of ewes giving birth out of total number of ewes x 100

[2] **Twinning rate:** number of ewes delivering twin / number of ewes delivering in the group x 100

$^{a,b,c,...}$ Means ± S.E within each row bearing different superscripts differ significantly (P< 0.05).

Table 5. Serum progesterone level hormone (ng/ml) changes during estrous cycle after Synchronization.

Collection days	Control	FGU	SRU	EESM 40	EESM 60
3	0.61±0.10^{b}	0.47±0.06^{b}	1.27±0.09^{a}	1.32±0.22^{a}	0.30±0.05^{b}
6	0.58±0.16^{b}	0.61±0.07^{b}	2.37±0.67^{a}	2.71±0.69^{a}	0.60±0.20^{b}
9	1.59±0.37^{b}	2.50±0.45^{b}	4.51±0.75^{a}	6.37±1.01^{a}	1.70±0.35^{b}
12	4.94±0.62^{b}	6.54±1.29^{b}	10.21±1.46^{a}	10.63±1.06^{a}	5.15±0.61^{b}
15	4.03±0.80^{b}	4.33±0.21^{b}	8.43±1.37^{a}	8.33±0.69^{a}	3.72±0.62^{b}
17	0.91±0.20^{b}	0.59±0.10^{b}	1.74±0.19^{a}	1.84±0.21^{a}	0.73±0.30^{b}

$^{a,b,...}$Means ± S.E within each row bearing different superscripts differ significantly (P< 0.05).

Table 6. Overall means of blood biochemical parameters of experimental ewes in the flushing period.

Parameters	Control	FGU	SRU	EESM 40	EESM 60
Glucose (mg/dl)	43.11±3.78^{b}	35.94±1.78^{c}	56.66±6.53^{a}	35.68±1.78^{c}	38.60±2.12^{c}
Blood urea nitrogen (mg/dl)	18.18±0.47^{b}	20.67±0.65^{a}	19.63±0.51^{ab}	20.44±0.63^{a}	14.12±0.52^{c}
Total protein (g/dl)	9.71±0.36^{a}	7.87±0.31^{b}	8.17±0.21^{b}	8.07±0.23^{b}	9.24±0.40^{a}
Albumin (g/dl)	2.81±0.05^{b}	3.46±0.11^{a}	3.49±0.10^{a}	2.85±0.04^{b}	2.64±0.08^{b}
Globulin (g/dl)	6.89±0.35^{a}	4.41±0.23^{b}	4.66±0.18^{b}	5.22±0.24^{b}	6.60±0.39^{a}

$^{a,b,c,...}$ Means ± S.E within each row bearing different superscripts differ significantly (P< 0.05).

Table 7. Post feeding overall mean of ruminal parameters and individual volatile fatty acids of ewes at the last week of the flushing period.

Parameters	Control	FGU	SRU	EESM 40	EESM 60
pH	6.63±0.02^{ab}	6.76±0.05^{a}	6.74±0.04^{a}	6.47±0.04^{c}	6.59±0.04^{b}
Ammonia (N) (mg/100 ml)	7.94±0.25^{c}	11.83±1.04^{a}	8.82±0.89^{c}	10.04±0.64^{b}	8.55±0.33^{c}
Total volatile fatty acids (mequiv/100ml)	14.22±0.80^{a}	12.69±0.41^{b}	9.52±0.55^{c}	15.19±0.0.28^{a}	14.57±0.48^{a}
Fatty acids (%)					
Acetic	64.04±1.31^{ab}	59.62±2.27^{c}	63.98±2.58^{ab}	64.71±1.71^{a}	62.86±2.62^{b}
Propionic	21.65±0.77^{ab}	23.02±1.05^{a}	22.76±1.19^{a}	21.05±0.44^{b}	21.47±1.31^{ab}
Isobutyric	1.48±0.25^{b}	2.46±0.29^{a}	0.91±0.07^{d}	0.66±0.13^{d}	1.19±0.10^{c}
Butyric	9.97±0.87^{b}	10.58±1.26^{b}	10.35±1.45^{b}	12.21±1.49^{a}	12.64±1.47^{a}
Isovaleric	0.91±0.10^{b}	1.38±0.15^{a}	0.73±0.06^{c}	0.44±0.04^{d}	0.71±0.07^{c}
Valeric	1.95±0.09^{b}	2.95±0.15^{a}	1.27±0.09^{c}	0.93±0.07^{d}	1.14±0.05^{cd}

$^{a,b,c,...}$ Means ± S.E within each row bearing different superscripts differ significantly (P< 0.05).

4. Discussion

Regarding the dry matter intake, our findings in agreement with numerous publications that concluded that diverse protein sources with varying amounts of undegradable protein had no impact on dry matter intake **Ward et al. (2008), Chegeni et al. (2013),** and **Ababakri et al. (2021)**. While earlier researchers discovered that supplementing with various protein sources with varying degrees of degradability enhanced the intake of dry matter, this finding was not observed in our trial, **Haddad et al. (2005), Ponnampalam et al. (2005), Khalid et al. (2011),** and **Lazarin et al. (2012)** working on sheep. As with the FGU group, which had the lowest feed consumption of all the other groups, **Lee et al. (2011a)** reported a decrease in intake of cow due to urea supplementation and the substitution of soybean meal with urea as a source of degradable protein. The present study indicates that the inclusion of a certain feedstuff with defined protein degradability between 31 and 41%, of a dietary protein around 12%, does not disturb the needs of the microbial population as long as it does not affect feed intake,

except for FGU diet as the urea by itself has a negative effect. The five tested diets contain satisfactory combinations of fermentable carbohydrates, nitrogen compounds, vitamins, and minerals.

Better performance may be attained by boosting feed efficiency, which is the measure of animal product output per unit of feed intake **(Kabir et al. 2004)**. As to the body weight change, it was clear that the EESM 40 group had the highest body weight change as it had the highest feed intake too, and this is in line with **Haddad et al. (2005)**, finding that ewes' levels of feed intake of rations containing the same amount of rumen undegradable protein led to an increase in their body weight. This is in agreement with the findings of previous studies, which observed significant weight gains in beef cattle and ewes supplemented with UP from different sources **(Kridli et al., 2001; Wiley et al., 1991)** respectively. According to **Hoaglund et al. (1992)**, the increase in ewes' body weight and nutritional status was presumably due to either an increase in microbial activity, which might have been brought on by feeding amino acids to the rumen's microbial population, or an increase in microbial production. According to other investigations, improving animal performance was achieved by increasing the amount of dietary undegradable nitrogen (UDN) **(Flis and Wattiaux, 2005; Gulati, et. al., 2005)**. While **Saunders et al. (2010), Lazarin et al. (2012),** and **Ababakri et al. (2021)** reported that using various protein sources with varying degrees of degradability had no impact on ewes' body weight change.

Regarding the body condition score, similar findings were attained by **Saunders et al. (2010), Lazarin et al. (2012),** and **Jabbar et al. (2013)** when studying Santa Ines ewes and Nili-Ravi buffaloes, respectively. On the other hand, it was argued that increased RUP feeding causes the mobilization of body fats in animals **(Schroeder and Gagliostro; 2000)**, which ultimately results in a low body condition score (BCS).

Regarding fertility rates, groups of urea supplementation (FGU and SRU 31% RUP) recorded the highest percent about 86% among the supplemented groups, this was the direct effect of improved conception and ovulation rates. These findings are in line with those of **Mandibela et al. (1995)**, who noticed that ewes fed large amounts of urea during the pre-ovulatory phase had raised plasma concentrations of urea and glucose, which were linked to more advanced development of fertilized sheep ova. On the contrary, **Mohammed et al. (2012)** observed that dietary urea negatively affected the quantity and size of ovarian follicles in sheep, as well as the quality of their oocytes and the levels of serum P4, and, **Smith and Chase (2010)** noticed that female dairy and beef cattle supplemented with DIP, either as protein or as urea, had lower conception rates. The reduction in fertility in dry cows or heifers can be overcome by providing enough energy for the excretion of excess urea or ammonia **(Funston 2007)**, and this conclusion explains our results as the experimental ration contains a good quality carbohydrate source, which might overcome the deleterious effect of urea and result in the improvement of pregnancy and fertility rates in urea groups. The time and how long the urea supplementation will be provided might also be contributing factors to these variations in findings. According to research by **Dawuda et al. (2002)** and **Laven et al. (2002)**, when urea supplements extends more than ten days, dairy cows might adjust to its detrimental effects. But this will rely on other things as well, such the kind and quantity of dietary urea that is included. In our study, it was 0.5%, which is lower than the inclusion level in cow studies, which reached 1%. This might explain the increase in fertility rate in the urea groups.

Among the SBM supplemented groups, fertility rates did not differ significantly from the control group (33% RUP). Similarly, utilizing several sources of nitrogen with various degradability ratios as flushing supplements in grazing Dohne merino ewes did not affect ovulation, conception, or lambing rate, according to **Marais (2011)** and **Lazarin et al. (2012)**. **Haddad et al. (2005), Daghigh Kia, et al. (2016), Rodrigues et al. (2015)**, and **Ababakri et al. (2021)** found an enhancement of reproductive performance in ewes supplemented with RUP. The present findings are in contradiction to these studies.

Among the experimental groups, EESM 40 & control groups had the highest and lowest twinning rate (50 and 0) **(Table, 3),** respectively. This result is in agreement with **Daghigh Kia et al. (2016)** and **Ababakri et al. (2021)**. Considering the nitrogen and calorie content of each experimental treatment was very similar **(Table, 1),** it is possible that the protein quality was the main factor contributing to of the animals' varied responses. The amino acid composition of dietary protein and its digestibility both affect its quality. However, a ruminant's requirement for protein is influenced by its physiology and rate of production. The amount of crude protein consumed by each flushing diet in the current investigation was almost the same; however, the protein's quality varied greatly.

The majority of extruded expeller soybean meal protein is able to bypass the rumen's degradation process, but urea is only considered a non-protein nitrogenous source and is only utilized in the production of microbial protein (MP). **Merchen and Titgemeyer (1992)** claim that MP, which is extremely low in branched chain amino acids (BCAA), is the primary supply of absorbable amino acids in ewes given substantial levels of rumen-degradable protein. Extruded expeller soybean meal has a low ruminally degradable protein content and is high in BCAA. As a result, it can improve the flow of BCAA through the duodenum and help these AA get absorbed through the intestinal wall **(Tagari et al. 1995).** There is strong evidence that BCAAs are beneficial for reproduction. According to **Downing et al. (1995),** BCAA can increase the ovarian production, both directly by impacting ovarian activity and indirectly by raising insulin levels. Due to BCAAs' ability to boost insulin production, particularly leucine, they may help increase ovulation rates **(Kuhara et al. 1991).** It has been proven that lambs given BCAA infusions **(Kuhara et al. 1991)** or excessive amounts

of SBM-based diets **(Molle et al. 1995)** had elevated insulin plasma concentrations**. Hinch and Roelofs (1986)** concluded that insulin infusion enhanced the ovulation rate in ewes.

Regarding progesterone level during the reproductive cycle, we found no remarkable significant variation in progesterone levels between the experimental groups; although, groups EESM 40 and SRU had the highest progesterone concentrations which is matching with the greatest reproductive findings in these groups (fertility rate for SRU group and twinning rate for EESM40 group). Increased levels of progesterone promote fertility, sustain fetuses, and improved lambing rate. So, serum progesterone concentrations did not vary among ewes fed diets low or high in rumen undegradable protein.

The greater biological value of the protein from SBM, particularly when taking into account the characteristics of indispensable amino acids such as lysine and methionine, might be associated with the mechanism relevant to elevation of progesterone concentration in ewes fed with bypass protein. Lipoproteins, specifically low and high density lipoprotein, which are abundant in methionine and lysine **(Auboiron et al., 1995),** are crucial for carrying of cholesterol, the precursor to the synthesis of steroids, including estrogen and progesterone **(Hall, 1994).** Similar findings of high levels of progesterone were observed by **Lazarin et al. (2012)** and **Daghigh Kia et al. (2016)** in regards to ewes fed diets with RUP percentages of 36 and 41, respectively, the same percent as in EESM 40 and 60 groups.

Regarding SRU group with high progesterone level and fertility rate. Non-protein nitrogenous compounds intake especially urea has not been associated with any adverse impacts on gonadotrophin hormone (GH) or luteinizing hormone (LH) release patterns, plasma oestradiol, IGF-I, or insulin levels, according to **Dawuda et al. (2014).** In contrast, **Mohammed et al. (2012)** found that dietary urea had a detrimental impact on serum P4 concentrations. These contradictory findings imply the possibility of other mechanisms, including the reduction or amplification of GnRH, FSH, LH, oestrogen, progesterone, and prostaglandin F2-alpha receptors in selected organs. The time and how long of the urea supplementation will be fed might also be a contributing factor in these variations. According to investigations conducted by **Dawuda et al. (2002) and Laven et al. (2002),** dairy cows can adapt to the harmful effects of urea when treatment lasts longer than ten days. Additionally, it will rely on whatever region of the hypothalamo-pituitary-ovario-uterine axis the urea treatment targets.

Because its significance as generate energy during the proestrus, estrus, and implantation phases, blood glucose can play a significant role in boosting gonadotropin concentrations, follicle size, and development, ultimately raising the ovulation rate **(Hess et al., 2005).** This line with the SRU group had the highest glucose level and fertility rate among the experimental groups. During ruminal fermentation, slow-release urea diets prolong the microbial utilisation of nitrogen sources. As a result, there may be a better synchronization between ruminal NH3-N release and carbohydrate availability, which would increase microbial protein synthesis **(Cherdthong and Wanapat 2010).** The increased microbial protein may raise glucose levels through gluconeogenesis when it reaches the lower portion of the gastrointestinal tract. According to **Milis et al. (2005)** and in accordance with the SRU findings, high ruminal degradable protein concentration increased gluconeogenesis and, as a result, blood glucose levels.

On the other hand, **Downing et al. (1995), Molle et al. (1995),** and **Jahani-Moghadam et al. (2009)** observed that the glucose level increased as the bypass protein content increased. However, other authors reported the opposite findings. Research by **Rusche et al. (1993)** and **Milis et al. (2005)** in beef cows and sheep respectively, reported that various ingredients of RUP lowered blood glucose without having an impact on hormones or reproductive status. **Amanlou et al. (2011) and Daghigh Kia et al. (2016)** demonstrated that ewes' blood glucose levels were not significantly affected by changes in the RUP/RDP ratio**,** which is consistent with the findings of high RUP groups.

The higher content of BUN in the urea consuming animals, which used urea as a protein source in their rations, was not much surprising; however, the higher content of BUN in the SBM groups (control and EESM 40) was unexpected. Possibly, a high BUN can indicate that the rumen's RDP and carbohydrates are not properly synchronized. Large quantities of ammonia are consequently exiting the rumen and entering the blood circulation **(Hongerholt and Muller, 1998).** But according to **Cunningham et al. (1994)** and **Bruckental et al. (2000),** BUN wasn't influenced by the amount of RUP in the diet, and this agrees with our result except for the group of EESM 60 (the highest RUP% among the experimental groups) with the lowest BUN level. Similarly, **Ababakri et al. (2021)** found a significant decrease in BUN Level in response to using 40% RUP in the diets of the ewes. **Milis et al. (2005), Lazarin et al. (2012),** and **Daghigh Kia et al. (2016)** similarly found that ewes given diets with a high RDP: RUP ratio had higher concentrations of plasma urea nitrogen (PUN).

According to the findings, the group of ewes fed a low RDN diets (EESM 60) and control one had a greater TP content without any significant difference. Regarding the group of the highest bypass protein, according to studies by **Jahani-Moghadam et al. (2009), Saeed (2011),** and **Daghigh Kia et al. (2016),** raising the RUP/RDP ratio in the diet results in a linear rise in serum protein. Lower CP degradability in these diets may be responsible for the elevation in TP observed in diets with low RDN: UDN ratios **(Ali et al., 2005)**. Compering urea groups and EESM 40, no response was reported due to the feeding of a low RDP: UDP ratio on TP, and this was consistence with the studies of **Salih (2007) and Shamoon et al. (2009).**

The increase in ruminal pH in urea groups agrees with **Al-Malah (2007),** who had higher levels in serum samples collected from 25 lambs given rations with a higher RDN % compared to those with a low RDN. As a result of feeding various amounts of dietary undegradable protein, researchers **Cherdthong et al. (2011a), Nejad et al. (2017),** and **Paula et al. (2017)** found that ruminal pH was unaffected. According to **Bach et al. (2005),** protein degradation is decreased in rumens with pH levels below 5.5. In all five groups of the experiment, ruminal pH never fell below 6.0 in all supplements; this is beyond the range that is considered sufficient to support cellulolytic bacteria and maintain fiber digestion, as mentioned by **Yokoyama and Johnson (1988).**

Regarding control and urea groups, ruminal ammonia appears to be closely connected to how quickly dietary CP degrades. **Taylor-Edwards et al. (2009), Cherdthong et al. (2011a),** and **Gardinal et al. (2016)** found lower ruminal ammonia concentrations for steers fed Optigen than for steers fed unprotected urea. According to these scientists, Optigen is a ruminally protected source of NPN with controlled-release properties in the rumen that doesn't interfere with other ruminal fermentation products and may even help rumen microbes function more effectively. Likewise, **Puga et al. (2001)** came to the conclusion that controlled-release ammonium supplement (CRUS) actually improved fermentation in sheep.

Regarding bypass protein groups, **Hassan and AL-Sultan (1996)** reported that increasing the undegradable nitrogen ratio caused a dramatic drop in ruminal ammonia, which is similar to our results (EESM 60) and in agreement with **Gidlund et al. (2015) and Silva et al. (2018).** Additionally**,** **Chen et al. (2002)** observed that increasing undegradable protein through extruded soybean meal decreased the NH3-N in rumen consequentially. As a result, the addition of RUP had no adverse impacts on rumen dynamics because the ruminal ammonia nitrogen concentration was kept at levels that supported fibrolytic activity, and the availability of true rumen degradable protein, which amylolytic bacteria prefer to utilize, was not detrimentally affected by dietary RUP amounts **(Lascano and Heinrichs, 2009).**

The results of the present study showed that, total VFA concentration increased quadratically with time. This may be regarded as the stimulation of microbial activity following feed administration. The time of decreasing NH3-N concentration was largely correlated with the period of increasing total VFA concentration and vice versa. These findings could be the result of concurrent NH3-N microbial consumption during periods of elevated microbial activity and VFA synthesis **(Ceconi et al., 2015).** According to **Brossard et al. (2003)** TVFA declined as degradability increased, which is consistent with the findings of the SRU and FGU groups. Other authors have demonstrated no change in the total ruminal VFA concentrations, indicating no deleterious fermentation by dietary addition of FGU or SRU **(Xin et al., 2010; Cherdthong et al., 2011a; Gardinal et al., 2016).**

Regarding the effect of RUP on total volatile fatty acids, **Paula et al. (2017)** and **Silva et al. (2018)** concluded that there were no impacts of dietary RUP on the total volatile fatty acid content, and ruminal fermentation was maintained under normal circumstances. Similar to our findings, **Nejad et al. (2017)** observed that increasing the amount of extruded soybean meal in dairy calves' diets didn't seem to affect their rumen fermentation and VFA levels. This was in line with the results of others who showed that heat processed SB had no effect on rumen VFA in dairy cows **(Giallongo et al., 2015).**

Results of FGU group (31 RUP) revealed that, the molar proportion of propionic acid was improved with mathematical decrease of acetic: propionic ratio and this in agreement with **(Hassan and saeed, 2012).** They observed a decrease of acetate and propionate proportions of ruminal fluid due to increasing degradability, and this might be explained by improved ruminal nutrition and or function causing more effective microbial activity **(Chumpawadee, et. al., 2006).** Other studies **(Heldt et al., 1999; Mathis et al., 2000)** support the reduction in the molar percentage of acetate (along with a rise in propionate) with DIP supplementation.

Regarding the branch chain volatile fatty acids (BCVFA) (isobutyrate, isovalerate, and valerate), similar to the group of feed grade urea, **Mathis et al. (2000),** and **Bandyk et al. (2001),** observed a rise in the molar percentage of these volatile fatty acids. Based on the oxidative deamination and decarboxylation of branched-chain AA (valine, isoleucine, and leucine), ruminal BCVFA are produced **(Allison and Bryant, 1963)**. Therefore, these BCVFA are associated with degradation of the branched-chain AA leucine, isoleucine, and valine, suggesting more degradation of these branched-chain AA for FGU diets. There is strong evidence that BCAAs are beneficial for reproduction, especially the induction of twinning, which was the lowest in this group. While **Bodine et al. (2000)** did not report an effect of DIP on any branch-chain VFA.

The effects of RUP (bypass protein) supplementation on rumen kinetics have been reported for growing beef cattle and lactating dairy cows **(Batista et al., 2016; Rufino et al., 2016).** In the relation between acetic, propionic and butyric, **Paula et al. (2017)** and **Silva et al. (2018)** reported that dietary RUP quantities had no effect on individual VFA concentrations, and ruminal fermentation was maintained under normal circumstances, our findings are consistent with. However, regarding to the proportion of BCVFA, EESM 40 reported the lowest concentration followed by EESM 60 then control group, This is in line with the findings of **Broderick et al. (2015), Gidlund et al. (2015),** and **Paula et al. (2017)**, who came to the conclusion that using extracted canola meal as a source of bypass protein decreased the molar percentage of isovalerate and total branched-chain volatile fatty content when compared to SBM diet. Contrarily, **Lascano and Heinrichs (2009)** reported that the levels of isoacids and valerate remained constant throughout the treatments. The results of BCVFA confirm the results of twinning rate as the

highest was for EESM 40 (50%) and EESM 60 (25%) groups and this confirm the relation between the levels of BCVFA and induction of twinning and bypass protein and its amino acids profile. Our findings suggest that RUP sources did not affect total production or ruminal concentrations of VFA.

5. Conclusion

The obtained results indicate that the dietary level of RUP (37% of CP) in EESM 40 was the best for producing twins, gaining weight, and having the highest level of progesterone hormone and a lower degree of degradation of branched chain amino acids, while SRU (31% of RUP) had the best fertility rate, which is confirmed by the highest levels of blood glucose and progesterone. Also, the findings of the present study suggest that the RUP sources and ratio (31-41% RUP of CP) did not affect the rumen fermentation dynamics.

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7. Conflict of Interest

The authors declare no conflict of interest.

8. References

Ababakri R, Dayani O, Khezri A, Naserian AA (2021). Effects of extruded flaxseed and dietary rumen undegradable protein on reproductive traits and the blood metabolites in Baluchi ewes. Journal of Animal and Feed Sciences, 30: 214–222. https://doi.org/10.22358/jafs/139153/2021.

Abou-akkada AR, El-shazly K (1964). Effect of absence of cilite protozoa from the rumen microbial activity and growth of lamds. Appl. Micobiol., 12: 384.

Ali MF, El-Saidy B,Mohsen MK, Khalafalla MME (2005). Performance of lambs fed on ration containing soybean meal treated with formaldehyde and probiotics. II. Productive and reproductive performance. Egyp. J. Nutr. Feed, 8(1): 511-527.

Allison MJ, Bryant MP (1963). Biosynthesis of branched chain amino acids from branched-chain fatty acids by rumen bacteria. Arch. Biochm. Biophys. 101:269–277. https://doi.org/10.1016/S0003-9861(63)80012-0

Al-Mallah ODM (2007). Effect of protein levels in formaldehyde treated rations on coefficient of digestion and performance in Awassi lambs. Ph.D.Thesis, University of Mosul.

Amanlou H, Karimi A, Mahjoubi1 E, Milis C (2011). Effects of supplementation with digestible undegradable protein in late pregnancy on ewe colostrums production and lamb output to weaning. Journal of Animal Physiology and Animal Nutrition 95: 616–622. https://doi.org/10.1111/j.1439-0396.2010.01092.x

AOAC (Association of Official Analytical Chemists) (1995). Official Methods of Analysis of Official Analytical Chemists International16th ed., Arlington, VA, USA.

Auboiron S, Durand D, Robert JC, Chapman MJ, Bauchart D (1995). Effects of dietary fat and L-methionine on the hepatic metabolism of very low density lipoproteins in the pre ruminant calf. Bos spp. Reproduction Nutrition Development.35 (2):167-178.

Bach A, Calsamiglia S, Stern MD (2005). Nitrogen metabolism in the rumen. J. Dairy Sci. 88(E Suppl. 1):E9–E21. https://doi.org/10.3168/jds.S0022-0302(05)73133-7

Bandyk CA, Cochran RC, Wickersham TA, Titgemeyer EC, Farmer CG, Higgins JJ (2001). Effect of ruminal vs post ruminal administration of degradable protein on utilization of low-quality forage by beef steers. J. Anim. Sci. 79:225–231. https://doi.org/10.2527/2001.791225x

Batista ED, Detmann E, Titgemeyer EC, Filho SCV, Valadares RFD, Prates LL, Rennó LN, Paulino MF (2016). Effects of varying ruminally undegradable protein supplementation on forage digestion, nitrogen metabolism, and urea kinetics in nellore cattle fed low-quality tropical forage. J. Anim. Sci. 94: 201–216. https://doi.org/10.2527/jas2015-9493.

Bodine TN, Purvis HT, II, Ackerman CJ, Goad CL (2000). Effects of supplementing prairie hay with corn and soybean meal on intake, digestion, and ruminal measurements by beef steers. J. Anim. Sci. 78:3144–3154. https://doi.org/10.2527/2000.78123144x

Broderick GA, Faciola AP, Armentano LE (2015). Replacing dietary soybean meal with canola meal improves production and efficiency of lactating dairy cows. J. Dairy Sci. 98:5672–5687. https://doi.org/10.3168/jds.2015-9563

Bruckental I, Holtzman M, Kaim M, Aharoni Y, Zamwell S, Voet H, Arieli A (2000). Effect of amount of undegradable crude protein in the diets of high-yielding dairy cows on energy balance and reproduction. Livest. Prod. Sci. 63: 131–140. https://doi.org/10.1016/S0301-6226(99)00124-4

Castañeda-Gutiérrez E, Benefield BC, de Veth MJ, Santos NR, Gilbert RO, Butler WR, Bauman DE (2007). Evaluation of the mechanism of action of conjugated linoleic acid isomers on reproduction in dairy cows. J. Dairy Sci. 90:4253–4264. https://doi.org/10.3168/jds.2007-0117

Ceconi I, Ruiz-Moreno MJ, DiLorenzo N, DiCostanzo A, Crawford GI (2015). Effect of slow-release urea inclusion in diets containing modified corn distillers grains on total tract digestibility and ruminal fermentation in feedlot cattle. J. Anim. Sci. 2015.93:4058–4069. https://doi.org/10.2527/ jas2014-8299.

Chegeni A, Li YL, Deng KD, Jiang CG, Diao QY (2013). Effect of dietary polymer-coated urea and sodium bentonite on digestibility, rumen fermentation and microbial protein yield in sheep fed high levels of corn stalk. Livest. Sci., 157: 141-150. https://doi.org/10.1016/j.livsci.2013.07.001

Chen KJ, Jan DF, Chiou PWS, Yang DW (2002). Effects of Dietary Heat Extruded Soybean Meal and Protected Fat Supplement on the Production, Blood and Ruminal Characteristics of Holstein Cows. Asian-Aust. J. Anim. Sci.15:821-827. https://doi.org/10.5713/ajas.2002.821

Cherdthong A, Wanapat M (2010). Development of urea products as rumen slow-release feed for ruminant production: a review australian journal of basic and applied sciences, 4(8): 2232-2241.

Cherdthong A, Wanapat M, Wachirapakorn C (2011a). Influence of urea-calcium mixtures as rumen slow-release feed on in vitro fermentation using gas production technique. Arch. Anim. Nutr., 65: 242-254. https://doi.org/ 10.1080/1745039x.2011.568277

Chumpawadee S, Sommart K, Vongpralub T, Pattarajinda V (2006). Effects of synchronizing the rate of dietary energy and nitrogen release on ruminal fermentation, microbial protein synthesis, blood urea nitrogen and nutrient digestibility in beef cattle. Asian-Australasian Journal of Animal Sciences, 19:181-188. https://doi.org/10.5713/ajas.2006.181

Con-way EJ (1948). Micro-diffusion analysis and volumetric errors. 9 th Ed. London Crosby-Cock Wood and Son Ltd. https://doi.org/10.1038/161583a0

Cunningham KD, Cecava MJ, Johnson TR (1994). Flows of nitrogen and amino acids in dairy cows fed diets containing supplemental feather meal and blood meal. J. Dairy Sci. 77: 3666. https://doi.org/10.3168/jds.S0022-0302(94)77311-2

Daghigh Kia H, Ahmad Fazel A, Hossein Khani A (2016). Different sources of protein effect in the flushing rations on some blood parameters and the reproductive performance of Ghezel sheep. Iranian Journal of Applied Animal Science 6(3): 629-638.

Dawuda PM, Scaramuzzi RJ, Leese HJ, Hall CJ, Peters AR, Drew SB, Wathes DC (2002). Effect of timing of urea feeding on the yield and quality of embryos in lactating dairy cows. *Theriogenology*. **58:** 1443-1455. https://doi.org/ 10.1016/s0093-691x(02)00973-1

Dawuda,PM, Wathes DC, Scaramuzzi R (2014). Effect of feeding quickly degradable nitrogen (QDN) on development of pre-ovulatory follicles in lactating dairy cows. Pp. 36 in Proc. Soc. Reprod. Fertil. Ann. Conf. Oxford, UK.

Diego BG, Pires AV, vaneteSusin I, Gouvêa VN, Berndt A, Abdalla AL, Tedeschi LO (2018). Net protein requirements and metabolizable protein use for growing ram lambs fed diets differing in concentrate level and roughage source. Small Ruminant Res, Volume 165: 79-86. https://doi.org/10.1016/j.smallrumres.2018.05.012

Downing JA, Joss J, Scaramuzzi RJ (1995). A mixture of the branched chain amino acids leucine, isoleucine and valine increases ovulation rate in ewes when infused during the luteal phase of the oestrous cycle: an effect that may be mediated by insulin. *J. Endocrinol*. 145: 315-323. https://doi.org/10.1677/joe.0.1450315

Flis SA, Wattiaux MA (2005). Effects of parity and supply of rumen degraded and undegraded protein on production and nitrogen balance in Holsteins. J. Dairy Sci., 88:2096-2106. https://doi.org/10.3168/jds.S0022-0302(05)72886-1

Funston R (2007). Nutrition and Reproduction Interaction, Proceedings of Applied Reproductive Strategies in Beef Cattle. Billings, Montana**.**

Gardinal R, Gandra JR, Calomeni GD, Henrique T, et al. (2016). Effects of polymer coated slow-release urea on ruminal fermentation and nutrient total tract digestion of beef steers. R. Bras. Zootec, 45(2):63-70. https://doi.org/10.1590/S1806-92902016000200004

Giallongo F, Hristov FAN, Oh J, Frederick T, Weeks H, Werner J, Lapierre H, Patton RA (2015). Effects of slow-release urea and rumen-protected methionine and histidine on performance of dairy cows. J. Dairy Sci. 98:3292–3308. https://doi.org/10.3168/jds.2014-8791

Gidlund H, Hetta M, Krizsan S, Lemosquet S, Huhtanen P (2015). Effects of soybean meal or canola meal on milk production and methane emissions in lactating dairy cows fed grass silage based diets. J. Dairy Sci. 98:8093–8106. https://doi.org/10.3168/jds.2015-9757

Gleghorn J (2003). Effect of dietary crude protein level and degradability on performance and carcass characteristics of growing and finishing beef calves. Ph.D Thesis, Texas Tech. University.

Gordon I (1997). Controlled reproduction in sheep & goats. New York: CAB International, V: 2, 450p.

Gulati SK, Garg MR, and Scott TW (2005). Rumen protected protein and fat produced from oil seeds and meals by formaldehyde treatment; their role in ruminant production and product quality: A review. Aust. J. Exp. Agric., 45:1189-1203. https://doi.org/10.1071/EA04131

Haddad, SG, Kirdli RT, Al-Wali DM (2005). Influence of varying levels of dietary undegradable intake protein on nutrient intake, body weight change and reproductive parameters in postpartum Awassi Ewes. *Asian-Aust. J. Anim. Sci.*, 18: 637–642. https://doi.org/10.5713/ajas.2005.637

Hall PF (1994). Testicular steroid synthesis: organization and regulation.In: KNOBIL, E.; NEILL, J.D. (Eds.). The physiology of reproduction. 2.ed. New York: Raven, 2: 363-410.

Hassan AS, Saeed AA (2012). Effect of feeding different levels of dietary protein and rumenal degradability on awassi lambs performance. 2-Rumen fermentation characteristics. Egyptian Journal of Nutrition and Feeds.

Hassan SA, AL-Sultan AA (1996). Awassi lambs responses to dietary supplement of rumen undegradable nitrogen fed toasted or untoasted corn. IPA J. Agric. Res., 6: 118-141.

Heldt J S, Cochran RC, Mathis CP, Woods BC, Olson KC, Titgemeyer EC, Nagaraja TG, Vanzant ES, Johnson DE (1999). Effects of level and source of carbohydrate and level of degradable intake protein on intake and digestion of low-quality tallgrass-prairie hay by beef steers. J. Anim. Sci. 77:2846–2854. https://doi.org/10.2527/1999.77102846x

Hess BW, Lake SL, Scholljegerses EJ, Weston TR, Nayigihugu V, Molle JDC, Moss GE (2005). Nutritional controls of beef cow reproduction. J. Anim. Sci. 83, (Suppl. E): E90–E106, https://doi.org/10.2527/2005.8313_supplE90x

Hinch GN, Roelofs. JHW (1986). Lupin feeding and insulin infusion during late luteal phase can increase ovulation rate in sheep. Proc. Australian Soc. Reprod. Biol. 18: 43-50.

Hoaglund CM, Thomas VM, Peterson MK, Kott RW (1992). Effects of supplemental protein source and metabolizable energy intake on nutritional status of pregnant ewes. J. Anim. Sci. 70:273-280. https://doi.org/10.2527/1992.701273x

Hongerholt D D, Muller LD (1998). Supplementation of rumen undegradable protein to the diets of early lactation Holstein cows on grass pasture. J. Dairy Sci. 81:2204–2214. https://doi.org/10.3168/jds.S0022-0302(98)75799-6

Jabbar MA, Marghazani IB, Pasha TN, Khalique A, Abdullah M(2013). Effect of protein supplements of varying ruminal degradability on nutrients intake, digestibility, nitrogen balance and body condition score in early lactating nili-ravi buffaloes. Journal of animal and plant sciences, 23(1 suppl.): 108-112

Jahani-Moghadam M, Amanlou H, Nikkhah A (2009). Metabolic and productive response to ruminal protein degrad-ability in early lactation cows fed untreated or xylose-treated soybean meal-based diets. *J. Anim. Physiol. Anim. Nutr.* **93:** 777-786. https://doi.org/10.1111/j.1439- 0396.2008.00867.x

Jasim IS (2019). Effect of Substitution protected whey or sunflower meal with blood or Formaldehyde on Awassi lamb's performance. *PhD thesis*, College of Agriculture Engineering Science, University of Baghdad.

Kabir F, Sultana MS, Shahjala M, Khan MJ, Alam MZ (2004). Effect of protein supplementation on growth performance in female goats and sheep under grazing condition. *Pak. J. Nutr*. **3:** 237-239. https://doi.org/10.3923/pjn.2004.237.239

Kaur H, Arora SP (1995). Dietary effects on ruminant livestock reproduction with particular reference to protein. J. Nut. Res. Rev., 8(1): 121-136. https://doi.org/10.1079/NRR19950009

Khalid MF, Sarwar M, Mahr UN, Zia UR (2011). Response of growing lambs fed on different vegetable protein sources with or without probiotics. Int. J. Agric. Biol. 13: 332-338

Kridli RT, Haddad SG, Muwalla MM (2001). The effect of feeding ruminally undegradable protein on postpartum reproduction of Awassi ewes. Asian-Aust. J. Anim. Sci. 14:1125. https://doi.org/10.5713/ajas.2001.1125

Kuhara T, Ikeda S, Aohneda A, Sasaki Y (1991). Effects of intravenous infusion of 17 amino acids on the secretion of GH, glucagon and insulin in sheep. *Am. J. Physiol.* 260: 21-26. https://doi.org/10.1152/ajpendo.1991.260.1.E21

Lascano GJ, Heinrichs AJ (2009). Rumen fermentation pattern of dairy heifers fed restricted amounts of low, medium, and high concentrate diets without and with yeast culture. Livest. Sci. 124, 48–57. https://doi.org/10.1016/j.livsci.2008.12.007.

Laven RA, Biggadike HJ, Allison RD (2002). The effect of pasture nitrate concentration and concentration intake after turnout on embryo growth and viability in the lactating dairy cow. Reprod. Domest. Anim. **37:** 111-115. https://doi.org/10.1046/j.1439-0531.2002.00342.x

Lazarin GB, Alves NG, Perez JRO, Lima RR, et al (2012). Plasma urea nitrogen and progesterone concentrations and follicular dynamics in ewes fed proteins of different degradability R. Bras. Zootec., 41(7):1638-1647. https://doi.org/10.1590/S1516-359820120007 00012

Lee C, Hristov AN, Heyler KS, Cassidy TW, Long M, Corl BA, Karnati SKR (2011a). Effects of dietary protein concentration and coconut oil supplementation on nitrogen utilization and production in dairy cows. J. Dairy Sci. 94:5544–5557. https://doi.org/10.3168/jds.2010-3889

Mandibela OR, Mcevovy TG, Robinson JJ, Findlay PA, Aitken RP, Robinson IS (1995). Excess rumen degradable protein influences the rate of development and glucose metabolism of fertilized sheep ova. Anim Sci. 60: 536-537. https://doi.org/10.1017/S03082296000 28658

Marais WJ (2011). The influence of flush feeding with different nitrogen sources on ovulation and conception rates in Dohne-Merino ewes. MS Thesis. University of Pretoria. South Africa.

Mathis CP, Cochran RC, Heldt JS, Woods BC, Abdelgadir IEO, Olson KC, Titgemeyer EC, Vanzant ES (2000). Effects of supplemental degradable intake protein on utilization of medium to low-quality forages. J. Anim. Sci. 78:224–232. https://doi.org/10.2527/2000.781224x

Merchen NR, Titgemeyer EC (1992). Manipulation of Amino Acid supply to the growing ruminant. *J. Anim. Sci.* 70: 3238-3247. https://doi.org/10.2527/ 1992. 70103238x

Milis C, Liamadis D, Roubies N, Christodoulou V, Giouseljiannis A (2005). Comparison of corn gluten products and a soybean-bran mixture as sources of protein for lactating Chios ewes. Small Ruminant Research 58: 237–244. https://doi.org/10.1016/j.smallrumres.2004.10.006

Mohammed AA, Abdel-Hafez GA, Ziyadah HMS (2012). Effect of dietary urea on ovarian structures in Saidi ewes during follicular and luteal phases. Egyptian J. Anim. Prod. 49(1):29-35. https://doi.org/10.21608/ejap.2012.94345

Molle G, Branca S, Ligios S, Sitzia M, Casu S, Landau S, Zoref. Z (1995). Effect of grazing background and flushing supplementation on reproductive performance in Sarda ewes. *Small Rumin. Res*. 17: 245-254. https://doi.org/10.1016/0921-4488(95)00691-D

Muniz JA, Savian TV, Scalon JD (2008). Parameters estimation in the model for In situ degradability of Mertens and Loften. Ciênc. agrotec., Lavras, 32(5): 1622-1628, set./out. https://doi.org/10.1590/S1413-70542008000500040

Nejad AZA, Ghorbani RG, Kargar S, Sadeghi-Sefidmazgi A, Pezeshki A, Ghaffari MH (2017). Nutrient intake, rumen fermentation and growth performance of dairy calves fed extruded full-fat soybean as a replacement for soybean meal. The Animal Consortium 1-8. https://doi.org/10.1017/S1751731117002154

NRC (1985). Nutrient Requirements of Domestic Animals. Nutrient' Requirements of Sheep. National Academy Press, Washington, DC.

NRC (2007). Nutrient Requirements of Domestic Animals. Nutrient' Requirements of Sheep. National Academy Press, Washington, DC.

Paula EM, Monteiro HF, Silva LG, Benedeti PDB, Daniel JLP, Shenkoru T, Broderick GA, Faciola AP (2017). Effects of replacing soybean meal with canola meal differing in rumen-undegradable protein content on ruminal fermentation and gas production kinetics using 2 in vitro systems. J. Dairy Sci. 100:5281–5292 https://doi.org/10.3168/jds.2016-12301.

Ponnampalam EN, Egan AR, Sinclair AJ, Leury BJ (2005). Feed intake, growth, plasma glucose and urea nitrogen concentration, and carcass traits of lambs fed isoenergetic amounts of canola meal, soybean meal, and fish meal with forage based diet. Small Rumin. Res. 58: 245-252. https://doi.org/10.1016/j.smallrumres.2004.10.007

Puga DC, Galina HM, perez-gil RF, Sangines GL, Aguilera BA, Haenlein GFW, Barajas CR, Herrera HJG (2001). Effect of a controlled-release urea supplementation on feed intake, digestibility, nitrogen balance and ruminal kinetics of sheep fed low quality tropical forage. *Small Rumin. Res*., 41: 9-18. https://doi.org/10.1016/S0921-4488(01)00171-7

Rodrigues M, Silva LM, da Silva CGM, Araújo AA, Nunes-Pinheiro DCS, Rondina D (2015). Reproductive and metabolic responses in ewes to dietary protein supplement during mating period in dry season of northeast brazil. Cienc. anim. bras. 16, (1): 24-36, https://doi.org/10.1590/1089-6891v16i124613.

Roe MB, Sniffen CJ, Chas LE (1990). Techniques for measuring protein fractions in feedstuffs. In Proc. Cornell Nutr. Conf. Feed manuf. pp 81-88. Cornell University Press, Ithaca, NY.

Rufino LM, de A, Detmann E, Gomes DI, Reis WLS, Batista ED, Valadares Filho SC, Paulino MF (2016). Intake, digestibility and nitrogen utilization in cattle fed tropical forage and supplemented with protein in the rumen, abomasum, or both. J. Anim. Sci. Biotechnol. 7 (11). https://doi.org/10.1186/s40104-016-0069-9.

Rusche WC, Cochran RC, Corah LR, evenson JS, Harmon DL, Brandt RT, Minton JE (1993). Influence of source and amount of dietary protein on performance, blood metabolites, and reproductive function of primiparous beef cows. *J. Anim. Sci.* 71: 557-563. https://doi.org/10.2527/1993.713557x

Saeed AA, Latif FA (2008). Effect of ensiling and level of supplementation with concentrate on the voluntary intake and digestibility of wheat straw by Arabi lambs. Alquadisya J. Vet. Med.7 (1).

Saeed AA (2011). Effect of level and degradability of dietary protein fed with or without baker's yeast (*saccharomyces cerevisiae*) on turkish awassi lambs performance. Doctor of philosophy in animal production science, Agriculture University of Baghdad.

Scaramuzzi RJ, Campbell BK, Downing JA, Kendall NR, Khalid M, Muñoz-Gutiérrez M, Somchit A (2006). A review on the effects of supplementary nutrition in the ewe on the concentration of reproductive and metabolic hormones and the mechanisms that regulate folliculogenesis and ovulation rate. *Reprod. Nutr. Dev.* **46,** 339-354. https://doi.org/10.1051/rnd:2006016

Salih AM (2007). Effect of high percentage of low degradability in the rumen on sheep performance. Ph.D. Thesis, University of Mosul.

Saunders, G.D.A., Alves, N.G., Pérez, J.R.O., Souza, J.C.D., Muniz, J.A. and José Neto, A., (2010). Effect of flushing with sources of protein with different degratability on ovulation in Santa Inês ewes. *Revista Brasileira de Zootecnia*, *39*, pp.2731-2738. https://doi.org/10.1590/S1516-35982010001200025

Schroeder G F, Gagliostro GA (2000). Fish meal supplementation to grazing dairy cows in early lactation. J. Dairy Sci., 83: 2899-2906. https://doi.org/10.3168/jds.S0022-0302(00)75190-3

Shain DH, Stock RA, Klopfenstein TJ, Herold DW (1998). Effect of degradable intake protein level on finishing cattle performance and ruminal metabolism. J. Anim. Sci. 76: 242-248. https://doi.org/10.2527/1998.761242x

Shamoon M, Saleh N, Abbo NY (2009). Effects of different levels of protein treated with formaldehyde on nutrients digestibility and some rumen and blood parameters in Awassi sheep. Iraqi J. Vet. Sci., 23, Supplement II, 169-173.

Silva AL, Rennó EDLM, Pedroso AM, et al (2018). Effects of rumen undegradable protein on intake, digestibility and rumen kinetics and fermentation characteristics of dairy heifers. Animal Feed Science and Technology 244: 1–10. https://doi.org/10.1016/j.anifeedsci.2018.07.019

Smith RD, Chase LE (2010). Nutrition and Reproduction, Dairy Integrated Reproductive Management.

SPSS (2007). Statistical package for social sciences. SPSS Inc., Chicago https://doi.org/10.4135/9781412963947.n549

Tagari H, Arieli A, Mabjeesh S, Bruckental I, Zamwell S, Aharoni Y (1995). Assessment of duodenal amino acid profile in dairy cows by the in situ method. Livest. Prod. Sci. 42: 13-22. https://doi.org/10.1016/0301-6226(94)00061-B

Taylor-Edwards CCT, Hibbard G, Kitts SE, McLeod KR, Axe DE, Vanzant ES, Kristensen NB, Harmon DL (2009). Effects of slow-release urea on ruminal digesta characteristics and growth performance in beef steers. J. Anim. Sci. 2009. 87:200–208. https://doi.org/10.2527/jas.2008-0912

Ward A, Tawila GA, Sawsan MA, Gad M, El-Muniary MM (2008). Improving the nutritive value of cottonseed meal by adding iron on growing lambs diets. *World J. Agric. Sci*. **4(5):**533-537.

Wiley JS, Peterson MK, Ansotegui RP, Bellows RA (1991). Production from first-calf heifers fed a maintenance or low level of prepartum nutrition and ruminally undegradable or degradable protein postpartum. J. Anim. Sci. 69:279-4293. https://doi.org/10.2527/1991.69114279x

Xin HS, Schaefer DM, Liu QP, Axe DE, Meng QX (2010). Effects of polyurethane coated urea supplement on *in vitro* ruminal fermentation, ammonia release dynamics and lactating performance of Holstein dairy cows fed a steam-flaked corn-based diet. Journal of Animal Science 23:491-500. https://doi.org/10.5713/ajas.2010.90153

Yokoyama M T, Johnson KA (1988). Microbiology of the rumen and intestine. Pages 125–144. in The Ruminant Animal. D. C. Church, ed. Simon and Shuster, New York.

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