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Effect of Different Levels of Undegradable Protein on Performance, Blood Parameters, Colostrum Composition and Lamb Birth Weight in Pregnant Ewes

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Abstract

The objective of this study was to investigate the effect of different protein sources with different degradability ratios during late gestation of ewes on colostrum composition, and its IgG concentration, body weight change of dams, and birth weight of their lambs. 35 multiparous native crossbred ewes (BW= 59±2.5kg) were randomly allocated to five dietary treatments (7 ewes / treatment) for 2 months prior to lambing. Experimental diets were isonitrogenous (12.27% CP) and isocaloric (2.22 Mcal ME/kg DM). In diet I (the control), solvent extract 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. Results showed no significant effect on feed intake, crude protein (CP), or metabolizable energy (ME), and body condition score (BCS). Ewes fed the 37% RUP diet gained more ($p<0.05$) weight compared with ewes fed the 31% RUP diet (5.62 vs. 2.5kg). Ewes in EESM 60 had the highest levels of fat, protein, total solid, solid not fat, and immunoglobulin and the lowest in urea N content ($P< 0.05$) in colostrum during the first 24hrs after lambing. Protein source and RUP levels in ewes' diets had no significant effect ($P< 0.05$) on lambs' birth weight and ewes blood biochemical parameters. Increasing the RUP content of diet during late gestation resulted in an increase in colostrum constituents and its IgG level but had no effect on ewes' performance and their lambs' outcome.

Keywords

Colostrum, Ewes, Lambs Output, Pregnancy, Undegradable Protein

1. Introduction

Ewe productivity is one of the most important factors that determine the profitability of small stock farming. 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 valuable source of amino acids in the form of rumen undegradable protein (RUP) and a nitrogen supply from rumen degradable protein (RDP) for the synthesis of microbial protein, dietary proteins are essential in ruminant nutrition **(Nocek and Russell, 1988; Kaur and Arora, 1995).**

Consuming rumen degradable protein (RDP) in excess of microbial utilization allowing for pre duodenal nitrogen losses, raises animal energy needs and reduces embryo survival in sheep. Additionally, the excretion of urea N adds to natural contaminants like nitrates in groundwater and atmospheric ammonia (NH3) **(Mikolayunas et al., 2011).** Supplementing diets with rumen undegradable protein (RUP) sources has been shown to lower plasma urea nitrogen and improve reproductive indices **(McCormick et al., 1999).** Therefore, balancing rations for protein degradability may improve animal performance and reduce the environmental impact of livestock production.

An anabolic physiological condition characterizes the first two thirds of gestation **(Vernon et al., 1985),** whereas the latter third is catabolic in terms of maternal metabolism **(Symonds and Clarke, 1996)**. Also, 80% of fetal development occurs in the final two months of pregnancy, which increases ewes' nutrient requirements significantly. In addition, there is a large increase in the ewe's net protein requirements for udder growth and colostrum production in the last 2 weeks of pregnancy **(Wang et al., 2021)**. However, during the last 2 weeks of pregnancy, voluntary feed intake declines **(Ocak et al., 2005),** recommending that extra crude protein (CP) should be supplemented.

The delivery of healthy lambs and the production of adequate colostrum to satisfy their nutritional needs should be assured by an appropriate feeding program for ewes throughout pregnancy. On the other hand, ewe nutrition during last gestation has an impact on birth weight and colostrum intake **(Nash et al., 1997),** which in turn has an impact on lamb survival **(Binns et al., 2002).** Most lamb mortalities (± 80%) occur in the period just before birth until seven days after birth and research has shown that nearly 80% of these mortalities are related to the nutrition of the ewe during the last weeks before lambing and the first weeks after lambing **(Seymour, 1998)**. In order to sustain embryonic and fetal development, maintain animal physiological needs, and promote mammary gland growth, pregnant ewes must be fed enough energy and protein.

Ewes that received insufficient amounts of nitrogen in late gestation, mobilized maternal tissue nitrogen reserves for conceptus and mammary gland development, but at a slower rate **(McNeill et al., 1997)**, which could affect postnatal development, performance and lactation. Increased nitrogen (N) consumption in late gestation, when the basal diet may be restricted, has been shown to be an effective strategy for preserving dam body weight (BW) and body condition as well as improving offspring postnatal performance in beef cows **(Martin et al., 2007)**. When compared with cows that have had a N supplement during late gestation, dams that did not receive one weigh less just prior to calving **(Larson et al., 2009)**. Reduced birth weights are a consequence of these adverse effects on dam performance, which can also have a negative impact on calve performance **(Larson et al., 2009)**. According to the same study, calves born to cows supplemented with crude protein during late gestation generally had higher birth weights.

Research results suggested that the supply of a high level of bypass protein (rumen undegradable protein) is essential to increase the colostrum and milk production of ewes **(Hinch et al., 1996)**. Additionally, ewes given 140% of the CP required during late gestation had higher lamb birth weights, according to **Ocak et al., (2005)**. In dairy ewes, supplementing RUP from expeller soybean meal increased milk yield by 14% in low- and high-milk-yielding ewes **(Mikolayunas-Sandrock et al., 2009)**. Furthermore, **Amanlou et al. (2011)** found a correlation between higher CP supplementation at 114 and 124% of requirement and an increase in colostrum yield. **Amanlou et al. (2011)** concluded that ewes that ingested more metabolizable protein (MP) during late gestation had higher levels of protein, fat, and solids-not-fat in their colostrum. This may have improved the quality of the colostrum. There are no documented requirements for RUP during late gestation period in sheep diets. Therefore, the objectives of the current study were to evaluate the effects of different levels of dietary undegradable protein in the diets of ewes in late pregnancy on some blood biochemical parameters, colostrum composition, and its IgG concentration, body weight change of dams and birth weight of their lambs.

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 3.0, and body weight of 59±2.5kg, were used. All ewes were subjected to 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 60mg of medroxy-progesterone acetate inserted for 14 days. To avoid vaginitis 0.25mL of oxytetracycline was applied in each sponge. From twelve hours after the sponge was removed, the estrous manifestations were monitored at 8:00 and at 17:00 with teaser rams for a minimum of 15min each time. Ewes were naturally mated using mature rams that were introduced 17 days after sponge removal. 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 weeks period before the last gestation period. The last gestation period covered two months before lambing season and the feeding continued for one week after. 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 **Table (1).**

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

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 Lamb Birth Weight

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) of the diets was estimated using **NRC of sheep (1985)**. The higher levels of dietary protein were nominated to cover the needs of ewes having twins if any, and the recommended percentage in the NRC plus the used to be added safety margin. The amount of TMR offered and refused by ewes were collected and weighted weekly before the morning feed to determine the DM intake. Ewes were weighed and (BCS) were also assessed before morning feeding at 6 and 2 weeks pre lambing, and within 24hr of parturition. The BCS was assessed by handling over and around the backbone, in a scale of 0 to 5 according to **Gordon (1997)**. Newly born lambs were weighed at birth to determine the effect of the different sources of RUP on the lamb birth weight and after three and seven days post lambing to determine the same effect on lambs gain.

2.4. Chemical Analysis of Blood and Colostrum

Blood samples were obtained by jugular venipuncture 3hr. post morning feeding at 6, 4 and 2 weeks pre lambing and at lambing date. Serum was separated and stored at -20°C until being used. Thereafter acetone according to **Nadeau (1952)**, glucose, blood urea nitrogen, total protein, and albumin were measured spectrophotometrically using chemical test kits according to the manufacture instructions of each test kit **(Reactivos GPL Barcelona, España)**, globulin was calculated and immunoglobulin (IgG) were determined. The glucose was measured immediately after sampling. Immediately after lambing, an individual colostrum sample was taken from each dam for composition analysis and determination of IgG concentration. Ewes were hand-milked, after 15min of intramuscular injection of oxytocin hormone, and about 40 ml of fresh colostrum were taken from each animal and were analyzed for estimating the percentage of fat, protein, lactose, urea nitrogen (UN), total solids (TS), and solids – not – fat (SNF) by using infrared milk analyzer (Bentley-150) according to **Teh et al. (1994).** Colostrum IgG concentration was estimated by using sheep immunoglobulin G ELISA kit **(Bioassay Technology Laboratory, China)**.

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 computer software (SSPS, 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 and Feed Additives Industrial.

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

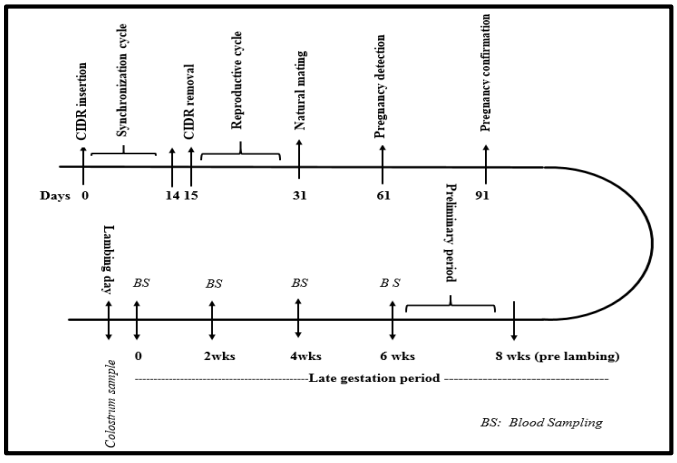

Fig. 1. Schematic figure showing the experimental plan in late gestation period.

3. Results

The daily DMI in the five groups **(Table, 4)** ranges from 1270-1450 g daily consumption and 2.13-2.33% as related to mean body weight. The difference is 180g on maximum over 2 months, an amount not so high for animals of an average mean weight ranging from 58-64 kg. The feed intake, when related to mean body weight, was nearly equal in groups urea and EESM6 60, highest in control and lowest in EESM 40. As to the protein consumption the highest level was in the control (177 g/day) followed by the groups EESM 40, EESM 60, and SRU (161-167g/day) and the least was the FGU group (157g/day). Regarding energy, it was on the same level of consumption, the highest is in the control group followed by EESM 40 and EESM 60 then the groups SRU and FGU.

Regarding body weight and change, the EESM 40 was the highest in mean body weight and change from zero to 2 weeks pre lambing (64 and 5.6kg) followed by the control and EESM 60 which were nearly equal (62 and 5.0 and 4.88kg). The least of the groups were the urea ones (58kg and 3.4 in SRU and 2.5 in FGU). And as to the loss in body weight 2 weeks pre-till 1 day post lambing it ranged from 6.25 to 8.0kg with no significant difference between groups and did not point to any specific meaning by itself. Also it had no relation to twining rate as the most high rate was in EESM 40 (50% triplet) and the loss in weight at lambing was 6.75kg while in SRU 16.67% and loss 8kg. The lamb birth weight was about 3.7kg in EESM 40 and 3.9kg in SRU group. The most prominent indication is that with the control group where twining rate is zero and lamb birth weight 3.5kg however the loss in weight at birth was 6.88kg.

In the body condition scores the least group got affected by gestation was EESM 40 where BCS change is - 0.01 followed by the other four groups which nearly equate with each other as the BCS changes ranged from –0.37 in EESM 60 to –0.50 in the control.

As to the lamb birth weight the FGU, EESM 60, SRU showed the highest weight; they were statistically equal (4106, 3990 and 3943g) and the EESM 40 and control the lowest (3707 and 3518g) respectively. No significant difference of high level of RUP on lambs birth weight especially the EESM 60 showed a weight did not differ so much from the weight of the low or zero level of RUP, the FGU and SRU groups. EESM 40 was the twins and triple- bearing group and this might be reflected on their lamb's birth weight. And as to the control with the lowest lamb weight, there is no interpretation what crime did this group commit to be so although the FGU and SRU diets had 31% RUP of CP clearly less than that of the control (33%). From birth to 7 days post-partum, the differences in weight were less sever with the highest in the control and SRU then EESM 60 and the lowest in the equal two groups EESM 40 and FGU.

Colostrum constituent **(Table, 5),** the fat varied from 6.64 to 10.62% with the highest percentage scored by EESM 60, ESSM 40, and control (10.62, 9.55, 9.16 and 8.91%) and the FGU was the lowest (6.64%). Still we got no indication for the superiority of the diets high in RUP, the control, SRU, EESM 40 and EESM 60 are nearly equal in spite of the differences in protein degradability among them. The five groups follow the same trend in protein % and in lactose; in lactose % all the groups were nearly equal except the control had the highest value (8.99%). For the total solids the groups showed different figures with the equality of EESM 60 and the control (35.62 and 33.48%) followed by EESM 40 (32.11%) then SRU and FGU (28.15 and 25.05%). Solids not fat showed the equality of EESM 60, EESM 40 and control (22.55 – 24.99%) and also that of FGU and SRU (18.41 and 19.23%). In salt all the groups were equal (1.07 – 1.15%) except control had the highest (1.64%). In urea N the FGU and control were the highest (52.28 and 50.97%) followed by SRU and EESM 40 (45.35 and 43.63%) and at the end EESM 60 was the lowest (36.91%). Immunoglobulin was the highest in EESM 60 (86.07%) followed by EESM 40 and SRU (78.75 and 64.51%) and the lowest were FGU (51.18%) followed by the control (42.93%).

Table (6) displays the findings of blood biochemical parameters for all treatments. Acetone was similar in all groups (4.66 – 4.91mg/dl) so it can be concluded that the four supplements of protein have no effect on acetone although the high RUP group EESM 60 tends to decrease. In the overall mean of glucose concentration all the five groups were statistically equal and ranging from 53.18 (in EESM 40) to 62 mg/dl in SRU.

Regarding blood urea N, the overall means; SRU & EESM 40 the highest (23.33 and 22.63mg/dl) and EESM 60 (12.73mg/dl) the lowest. There is no clear reason for the equality of SRU diet (31% RUP) and EESM 40 (37%), while it is of advantage for the EESM 60 to be the lowest all over the days and mean. It has no explanation except on the basis of high undegradable protein (41%) and low degradable one compared with the other diets.

As to the serum total protein the groups were statistically equal in all groups ranging from 6.09 to 6.74g/dl. There is nothing to say about albumin where all the five groups were equal in the concentration of albumin varied from 2.67 to 2.84g/dl in mean, and there is no effect for the diets. Regarding to globulin results, the same was as in albumin, the mean concentration varied from 3.37 to 4.02g/dl with no clear effect for diets.

Regarding to serum IgG **(Table, 6)** at 2 weeks pre–lambing the highest figures were EESM 40, EESM 60 and SRU (34.6, 32.38 and 31.27mg/ml) and the lowest of control and FGU (21.28 and 25.75mg/ml). At the day of lambing the levels decreased to reach 25.55, 24.65 and 27.83mg/ml for EESM 40, EESM 60 and SRU and in respective order 17.18 and 21.31mg/ml in control and FGU, with the highest value in the first three groups and the lowest in the other two especially the control.

Table 4. Feed intake, body weight and body condition score change, and lamb performance in ewes offered different levels of undegradable protein during late gestation.

	Control	FGU	SRU	EESM 40	EESM 60
Feed intake g/animal/day	1450	1270	1300	1370	1350
Intake CP g /animal/day	177.33	156.72	161.20	167.30	164.20
Intake RUP g/animal/day	58.61	48.32	49.46	62.29	67.34
Intake ME Mcal/animal/day	3.23	2.81	2.87	3.06	3.01
Body weight change (kg) Zero – 2 wks. pre-lambing	5.00±0.79^{ab}	2.50±0.34^{c}	3.42±0.2b^{c}	5.62±0.55^{a}	4.88±0.59^{ab}
Body weight change (kg) 2 wks. pre-till 1-day post lambing	- 6.88±0.83^{a}	-7.33±0.68^{a}	- 8.00±0.51^{a}	- 6.75±0.75^{a}	- 6.25±0.63^{a}
BCS change	- 0.50±0.00^{b}	-0.42±0.08^{b}	- 0.42±0.08^{b}	- 0.01±0.00^{a}	- 0.37±0.1^{b}
Lamb birth BW (g)	3517.5±35.26^{d}	4106.43±25.45^{a}	3943.57±30.37^{b}	3707.14±23.01^{c}	3990±29.23^{b}
BW gain from birth to 7days pp (g)	1667.50±34.97^{a}	1233.57±13.95^{c}	1519.29±16.84^{b}	1231.43±14.08^{c}	1260.00±11.54^{c}

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

Table 5. Changes in composition of colostrum produced by ewes offered different levels of rumen undegradable protein.

Parameter (%)	Control	FGU	SRU	EESM 40	EESM 60
Fat	9.16±0.38^{b}	6.64±0.32^{c}	8.91±0.17^{b}	9.55±0.36^{b}	10.62±0.24^{a}
protein	12.22±0.56^{c}	9.52±0.26^{d}	11.76±0.24^{c}	14.09±0.45^{b}	16.14±0.30^{a}
lactose	8.99±0.28^{a}	7.63±0.47^{b}	7.56±0.46^{b}	6.91±0.60^{b}	7.34±0.28^{b}
Total solids	33.48±1.25^{ab}	25.05±0.88^{d}	28.15±0.90^{c}	32.11±0.53^{b}	35.62±0.38^{a}
Solid not fat	24.32±1.14^{ab}	18.41±0.71^{c}	19.23±0.93^{c}	22.55±0.88^{b}	24.99±0.23^{a}
Salt	1.64±0.13^{a}	1.07±0.13^{b}	1.08±0.11^{b}	1.15±0.34^{b}	1.14±0.07^{b}
Milk urea nitrogen	50.97±1.72^{a}	52.28±2.64^{a}	45.35±1.59^{b}	43.63±1.82^{b}	36.91±1.05^{c}
Immunoglobulin G (mg/ml)	42.93±0.72^{e}	51.18±1.07^{d}	64.51±1.07^{c}	78.75±1.07^{b}	86.07±2.31^{a}

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

Table 6. Overall means of blood biochemical parameters of experimental ewes in late gestation period.

Parameters	Control	FGU	SRU	EESM 40	EESM 60
Acetone (mg/dl)	4.66±0.07^{bc}	4.91±0.09^{a}	4.89±0.08^{a}	4.77±0.07^{ab}	4.51±0.07^{c}
Glucose (mg/dl)	59±2.54^{ab}	60.33±1.81^{a}	62±2.75^{a}	53.18±3.48^{b}	58.69±2.53^{ab}
Blood urea nitrogen (mg/dl)	18.64±0.63^{b}	18.36±0.59^{b}	23.33±0.76^{a}	22.63±0.77^{a}	12.73±0.67^{c}
Total protein (g/dl)	6.74±0.18^{a}	6.24±0.23^{ab}	6.69±0.19^{a}	6.09±0.19^{b}	6.73±0.19^{a}
Albumin (g/dl)	2.84±0.06^{a}	2.70±0.06^{ab}	2.67±0.07^{b}	2.72±0.04^{ab}	2.72±0.03^{ab}
Globulin (g/dl)	3.89±0.20^{a}	3.53±0.21^{ab}	4.02±0.21^{a}	3.37±0.18^{b}	4.01±0.19^{a}

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

Table 7. Serum immunoglobulin G concentration (mg/ml) of ewes during late gestation period.

Sampling time	Control	FGU	SRU	EESM 40	EESM 60
2 weeks pre-lambing	21.28±0.85^{d}	25.75±1.16^{c}	31.27±0.69^{b}	34.60±0.49^{a}	32.38±0.81^{ab}
Day of lambing	17.18±0.54^{d}	21.31±0.86^{c}	27.83±0.86^{a}	25.55±0.51^{ab}	24.65±0.72^{b}

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

4. Discussion

The current study showed no clear effect for the different supplements and the levels of feed intake, CP, or ME were nearly equal with the surpassing of the control group, followed by EESM 40 as it is clear in **(Table, 4).** Rather, there is a possibility that the five groups have met sufficiently their needs from RUP even with the feed grade urea group; the rations have sufficient protein and percentage of degradability to satisfy. The intake of CP varies from 157 to 177g and 48 to 67g RUP, it seems that the 157g level of CP of which 48 g RUP is optimal for microbial population. Similar observation were reported by **Dawson et al. (1999), Annett et al. (2005),** and **Amanlou et al. (2011),** where they found that**,** protein source and dietary undegradable protein (DUP) concentration of the concentrate had no significant effect on dry matter intake.

It is clear that**,** during the late pregnancy period, most of ewes acquired weight, EESM 40 group was the highest about (5.6kg) and FGU was the lowest about (2.5kg) in spite diets were nearly equal in CP %. Conversely, **Ocak et al. (2005), Van Emon et al. (2014), and Larson et al. (2009) s**tudied ewes and cows, respectively. According to these studies, the pregnant ewe's live weight was improved at lambing by feeding them 130 or 140% of their CP needs during the last six weeks of pregnancy.

An explanation reported by **Larson et al. (2009),** and **Van Emon et al. (2014)** that increasing CP intake during late gestation enhances dam performance and minimizes the mobilization of dam body reserves to maintain fetal growth. As to RUP **Scholljegerdes et al., (2005),** reported that intestinal supply of essential amino acids can be increased by dietary supplementation with CP feeds selected for ruminal escape. Other studies reported that increasing the dietary undegradable nitrogen improved animal performance **(Chaturvedi and Walli, 2001; Flis and Wattiaux, 2005; Gulati et al., 2005).** These studies agree with our results, so it is not the effect of increasing the CP requirement alone, but

it is also the effect of the bypass protein or protein degradability.

On the contrary, **Dawson et al. (1999), Annett et al.(2005),** and **Amanlou et al. (2011)** found no improvement by digestible undegradable protein of the concentrate or protein source on pregnant dams live weight change.

Neither protein source nor RUP levels had a positive effect on the ewes body condition score; this may be due to the evidence that the ewes were in good condition at the start of the experiment (mean condition score 3.0), therefore, they were less likely to restore their own body's fat with dietary protein and were offered well fermented carbohydrate and good quality hay. Others have shown that late gestation ewe diets with additional CP or RUP help ewes maintain their body condition **(Annett et al., 2005; Amanlou et al., 2011; Van Emon et al., 2014).**Conversely**, Larson et al. (2009)** demonstrated that cows fed with CP during late gestation had higher BCS levels than cows who weren't.

Birth weight of lambs was not affected with increasing levels of RUP and protein source. A fact consistent with the findings of **Amanlou et al. (2011)** and **Rezai et al. (2012).** The likely explanation for these results is that, compared to degradability, dietary protein intake is more closely correlated with lamb birth weight as mentioned by **(Kleemann et al., 1988; McNeill et al., 1997).** Another explanation is that the placenta's ability to transmit additional AA to the fetus is constrained, and the amount of AA that is transported from the mother's circulation to the fetal tissue is highly regulated **(McNeill et al., 1997).** Furthermore, because glucose is the only energy source for the uterus and fetus, it is hypothesized that urea group rations with high RDP content induced higher gluconeogenesis, which in turn led to higher blood glucose levels and lambs' birth weight. Lowered blood glucose levels in groups ESSM 40 and 60 may be the cause of lack of response for high levels of RUP on birth weight. This is in agreement with **Barry and Manley (1985)** and **Ocak et al. (2005)** who mentioned that, the higher level of glucose in pregnant ewe's circulation may have increased the fetal growth, resulting in a higher birth weight.

The higher birth weight in FGU group over the high RUP and control groups was observed by **Hoon et al. (2000),** while the large litter size in EESM 40 group may had a direct effect on lowering lambs' birth weight.

On lambs' growth rate during the first week post lambing, the amount of colostrum available at lambing and the milk production of the ewe after lambing are important factors influencing lamb survival and growth rate. The supply of sufficient protein during late pregnancy and lactation influences the quantity as well as quality of milk produced. Research results suggested that the supply of a high level of bypass protein (rumen undegradable protein) is essential to increase the colostrum and milk production of ewes **(Hinch et al., 1996).** However, our results showed that lamb growth was not enhanced by high level of RUP supplementation as control and SRU groups recorded higher growth rate over the other groups which were statistically equal. Our observation was similar to **Polan et al. (1997)** in cows, and **Liamadis and Milis (1999), Liamadis et al. (2001),** and **Milis et al. (2005)** in ewes, who reported that corn gluten meal (source of RUP) was less effective than soybean meal at increasing milk production. In contrast, work done by **Hall et al. (1992), O'Doherty and Crosby (1996),** and **Amanlou et al. (2011)** in which different CP levels, RUP and period of supplementation were tested, found a considerable increase in colostrum production after providing ewes protein supplements. **Hall et al. (1992a)** concluded that, the increase in colostrum production after feeding supplements of legumes and oleiferous seeds (lupins and sunflower seed meal) is mainly related to their high protein content, especially their ability to supply undegraded protein to the small intestine. Others found that neither protein sources nor levels of their RUP had an effect on colostrum yield **(Dawson et al., 1999; Annett and Carson, 2003; Annett et al., 2005).**

It's intriguing that the EESM 60 group had the highest colostrum components. Only ruminants received highly biologically valuable diets with a high rumen undegradable protein content or diets supplemented with bypass amino acids have shown such effect. **(Milis et al., 2005; Amanlou et al., 2011).** With increasing levels of RUP, first and second phase of colostrum percentage of SNF, protein and first and second phase colostrum fat were increased **(Rezai et al., 2012). Hall et al. (1992)** noticed a considerable increase in the colostrum's components after providing ewes with protein supplements at pasture According to **O'Doherty and Crosby (1997),** this response most likely resulted from the supplemented ewes' higher nutritional intake. Moreover, the increase in colostrum components after feeding supplemented legumes and oleiferous seeds (lupins and sunflower seed meal) is mainly related to their high protein content, especially their ability to supply undegraded protein to small intestine **(Hall et al., 1992a).** These results were in contrast to those observed by **Ocak et al. (2005),** however it agrees with the results of FGU groups. They reported a decrease of colostrum production and contents due to feeding of diets high in digestibility and degradability**.** Other investigations came to the conclusion that colostrum components were unaffected by pre-lambing increases in the dietary CP content **(Hatfield et al., 1995)** or RUP when a steady crude protein level is maintained **(Annett et al., 2005)** and this concurs with **Dawson et al. (1999),** and **Annett and Carson (2003).**

From the data of acetone in **Table (6)** there was no remarkable difference among groups. Two authors reported that protein source and increasing DUP concentration had no significant effect on plasma ketone bodies (ß-hydroxybutyrate (BHB)) **(Dawson et al., 1999; Annett and Carson, 2003).** Regarding the results of EESM 60 in comparison to the control group and urea groups, **Annett et al. (2005)** found similar findings, a decrease in plasma BHB in ewes given supplements of different levels of DUP in comparison to the control one and no significant difference between groups of DUP supplementation. They claimed that the higher BHB of control ewes was driven by their susceptibility to greater

body condition score loss, their lack of significant correlation with concentrate DUP levels, and their failure to use any additional amino acids that might have been provided by the DUP as precursors for hepatic gluconeogenesis **(Bell and Bauman, 1997).** Also, **O'Doherty and Crosby (1998)** noted a decrease in plasma BHB in twin-bearing ewes treated with soya-bean meal; this reaction was connected to higher intakes of protein and calories.

Regarding the overall mean of glucose levels, the lower blood glucose in groups of high RUP (EESM 40 and EESM 60) compared with control and urea groups is in agreement with **Milis et al. (2005).** Their results revealed that a high rumen degradable protein (RDP) content diet increased gluconeogenesis, which in turn raised blood sugar levels. In light of this and the fact that these diets make up a large amount of UP, the lower glucose levels in groups EESM 40 and EESM 60 may be a result of this. **Amanlou et al. (2011)** mentioned that dietary undegradable protein was not intended to stimulate hepatic gluconeogenesis but rather to meet the protein demands of the fetus. In contrast, **McNeill et al. (1997),** and **Amanlou et al. (2011)** reported an increase in blood glucose level in ewes fed diets high in DUP compared to control and medium DUP groups.

Blood urea nitrogen level dependent mostly on dietary crude protein, rumen crude protein degradability, and energy consumption **(Jordan et al., 1983).** In line with our results except for the EESM 60 group, **Amanlou et al. (2011)** reported that increasing the level of DUP diet increased the content of blood urea nitrogen, most likely because the RDP conversion into microbial protein was less effective. In accordance with **Palmquist et al. (1993),** cows fed diets high in RUP (blood meal and feather meal) exhibited elevated BUN levels, but this finding may only be relevant when feeding animal byproducts. In agreement with the groups of high RDP (control, FGU, and SRU), **Milis et al. (2005)** found that a higher RDP content in the SW (soybean meal and wheat bran) ration led to a higher BUN concentration than it did in the GF (corn gluten and corn gluten feed) ration. This high BUN indicates that RDP and carbohydrates in the rumen are not properly synchronized. Consequentially, large amounts of NH3 are exiting the rumen and entering the blood circulation **(Hongerholt and Muller, 1998).** Nevertheless, **Cunningham et al. (1994)** and **Dawson et al. (1999)** observed that the amount of RUP in the diet had no effect on BUN. Contrarily, **Annett et al. (2005)** found that plasma urea concentration tended to be adversely and linearly associated with the increase in DUP level, which is consistent with our experiment's EESM 60 results.

There is no significant effect for the different diets on serum total protein, albumin and globulin, and the equality of the low RUP diets FGU and SRU (31%) with the high RUP EESM 60 (41%) is a strong proof. Similarly, **Dawson et al. (1999), Milis et al. (2005), Annett et al. (2005),** and **Amanlou et al. (2011)** reported that, protein source and RUP levels of ewes' diets had no significant effect on total protein levels.

Regarding serum IgG **(Table, 7),** the reduction of IgG level in the blood at lambing day is due to the transfer of this immunoglobulin to the mammary gland and an increase in their levels in colostrum. The equality of the SRU group with EESM 40 and EESM 60 aborts any defense of the high RUP diets. The present investigation demonstrated that supplementing pregnant ewes with different levels of RUP had no effect on their IgG levels. According to **Rezai et al. (2012),** supplying pregnant ewes with 40 and 60 % RUP of total crude protein increased ewes 'serum IgG level significantly in comparison to control groups without any significant difference among the RUP groups, which contrasts in part with the findings of the current investigation.

5. Conclusion

The current study's findings suggest that for ewes with a good body condition score (BCS 3.0) six weeks preceding lambing, in terms of ewe performance and the weight of their lambs at birth, adding different protein sources or raising the RUP content in the diet had little impact. The parameters measured show superiority in certain groups and a lack in others. Accordingly, it is clear that the highest and low levels of undegradable protein are equal. The level of 33% RUP in the control was sufficient as long as the dietary protein percentage was not less than 12 without being bound by **NRC (1985)** levels.

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7. Conflict of Interest

The authors declare no conflict of interest.

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