IMPACT OF NON-CONVENTIONAL ENERGY SOURCES IN RATION ON PRODUTIVE AND REPRODUTIVE PERFORMANCE OF EWES

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ABSTRACT

The present work aimed to define the influence of the non-conventional energy sources added to the ration on productive and reproductive performance of ewes. The variety of energy sources such as basal conventional energy (BCE) and admixture of unconventional energy (UCE) protected fat (PF) and corn steep liquor (CSL) were investigated. Ten healthy and mature Rahmani ewes were divided into two groups (n=5). Ewes in 1st group fed BCE contained gross energy (GE) at a level of 300.21 cal/100 gm diets as 60% concentrate feed mixture (CFM) + 40% rice straw (RS). While, 2nd group nourished UCE which given ewes GE at a level of 332.46 cal/100 gm diets (55% CFM + 40% RS + 2.5% PF + 2.5% CSL). All ewes were received both BCE and UCE at 21 days pre-mating season and continued until lambing. The changes in body weight, fertility rate and parity pattern of ewes fed BCE and UCE were measured. Progesterone (P₄) profile was also determined during gestation and number of fetuses. Blood metabolites were investigated during different stages of gestation.

The obtained results showed that ewes given UCE had slight (P>0.05) improvement in body weight but at prepartum they had significantly (P<0.05) higher live body weight compared with ewes received BCE energy. Ewes fed UCE showed improvement prolificacy (100%) and parity patterns as twins rate (40.00%) compared to ewes received BCE that have feeble prolificacy (83.33%) and twins rate (20.00%). Progesterone (P₄) in ewes fed UCE had higher (P<0.05) levels than ewes fed BCE during different stages of gestation. Fetus numbers emphasized that the level of P₄ in ewes carrying two fetuses increased (P<0.05) compared to ewes carrying just one fetus throughout gestation. The blood metabolites of ewes fed UCE showed higher (P<0.05) levels of total proteins, albumin, globulin, cholesterol, glucose, calcium and phosphorus than ewes fed BCE. However, serum creatinine, triglycerides and urea-N in ewes fed UCE were insignificantly higher than those of BCE ewes. This study concluded that unconventional energy is recommended to be included in farm animal diets at moderate amounts. Maintaining normal range for most commonly used serum biochemical parameters during different reproductive stages should considered in farm management strategy.

Keywords: Ewes, non-conventional energy, number of lambs born, blood components.

INTRODUCTION

One of the ways to increase mutton production is to increase the number of lambs born of ewe. This can achieve by inducing multiple births or acquiring two lamb crops every year. For this reason, foetal growth is a complex process that involves the interaction of maternal, placenta and fetus environments. The growth and development of fetus depends on nutrients. The primary nutrient consideration for optimum reproductive performance of sheep is energy. Freetly and Leymaster (2004) concluded that approximately 60% of energy dissipated during pregnancy can be contributed to the gravid uterus and the remaining 40% invested to maternal metabolism. Elegant studies on ewes had demonstrated that energy affects directly and indirectly both physiological and reproductive performances. Energy strategy may be used to enhance ovulation rate and prolificacy. Ewes in good body condition at pregnancy stages and lambing

time can tolerate minimal body weight changes before and after lambing. Moreover, ewes during gestation period require energy for maintenance of the developing fetuses. supporting tissues of pregnancy (placenta, fluids and udder development, etc.) and increasing ewes' body tissues for the upcoming lactation. Otherwise, the effects of overfeeding with high-energy diets are well known to stimulate follicular development and increase ovulation rate (Saunders *et al.*, 2010). Nevertheless, ewes' welfare using short-term of energy "termed as flushing" can improve ovulation rate and litter size mainly in small ruminants. Many authors have demonstrated that blood progesterone (P₄) concentration can be influenced by energy feed intake (Dorniak et al., 2012). In the research of Inskeep et al. (2009)they concluded that blood concentration showed negative fluctuations on day 20 of gestation which was an indicator of embryo loss. Complete losses were greater between 25th and 85th days of pregnancy of ewes. In this context, Ataman et al. (2013) revealed that blood P₄ concentration tended to be higher in sheep carrying two fetuses than those conceived one fetus. Therefore, selection of feeding is based on the mean embryonic survival that provides an effective way to improve fetus growth. Manthou et al. (2014) indicated that basic nutrition provides benefit of health and presence of physiologically active Generally, many components. authors proclaimed that addition of unconventional energy sources to feedstuffs caused deficiency in amount of concentrate feed (Hutchinson, et al., 2012), dependable for animals nutrition (Mostafa, et al., 2012) and improvement of productive and reproductive performance (Behery et al., 2014).

The aim of the present work was to study the possible improvement of productive and reproductive performances of Rahmani ewes via unconventional energy sources.

MATERIALS AND METHODS Experimental animals

The investigation was carried out at El-Serw Experimental Research Station belonging to Animal Production Research Institute (APRI), Ministry of Agriculture, Egypt. Ten Rahmani ewes had 47.00±5.70 months of age and 46.90±2.18 kg live body weight were used in the present work. Ewes were raised in semiopen pens and consumed the maintenance requirements of ewes according to NRC (2007). Furthermore, before breeding season ewes were treated against internal and external parasites and dipped against ticks and fleas.

Experimental design

Ten ewes were randomly assigned into two groups (n=5). The first group was nourished basal conventional energy (BCE), while second group was received unconventional energy (UCE). The BCE contained 60% concentrate feed mixture (CFM) + 40% rice straws (RS). While, UCE consisted of 55% CFM + 40% RS +2.5% protected fat (PF) +2.5% corn steep liquor (CSL). Both BCE and UCE were presented gross energy levels of 300.21 and 332.46 cal/100 gm diets. They offered 21 days pre-mating season up to parturition. Ewes displayed estrous cycle from either BCE or UCE groups tupped by the same fertile ram at 12 hours of heating onset. The experimental diets were prepared and adjusted daily by mixing basal diet with non-conventional energy materials and presented to ewes twice a day. Fresh water and salt templates presented free all experimental period to all ewe groups. The chemical compositions of BCE and UCE energy were analysed according to AOAC (2007) and results shown in Table 1.

Productive and reproductive performance: Productive performance:

Body weight (wt) was recorded at start of the experiment, pre- and post-tupping seasons, pre- and post-partum, birth weight and gestation length (days).

Items -	Experimental energy		
	BCE	UCE	
Organic matter (OM)	87.08	90.53	
Crude protein (CP)	14.46	14.58	
Crude fiber (CF)	15.59	12.74	
Ether extract (EE)	2.85	4.26	
Nitrogen free extract (NFE)	54.18	58.95	
Ash	12.92	9.47	
*GE	300.21	332.46	

Table 1. Chemical composition of energy diet.

*GE= Gross energy (cal/ 100 gm diets), it determined by the formula according to Ramin *et al.* (2010) as CP% $\times 4 + EE\% \times 9 + NFE\% \times 4$.

Reproductive performance:

After tupping, ewe that passed another oestrous cycle without showing heating (using scout ram) accounted gravid. Then, reproductive performance was calculated as conception rate (number of maternal conceived / tupping maternal), fertility (number of maternal lambed / tupping maternal), fecundity (number of lambs born / tupping maternal) and prolificacy (number of a live lambs born / lambing maternal).

Parity patterns were calculated as single birth rate (number of maternal lambing single/ number of maternal lambed) and twins birth rate (number of maternal lambing twins/ number of maternal lambed).

Progesterone (P₄) profiles and fetus numbers:

Blood samples were obtained from the jugular vein and drawn into sterile test tube at ovulation time (24 hours) with the start of estrus (day 0). Afterwards, P₄ was checked at 20, 40, 60, 80 and 100 days of gestation. The blood serum was separated by centrifuging at 3000 g for 20 min and then serum was harvested by pipette and stored at -20° C until assayed for progesterone.

The P₄ concentration accomplished during pregnancy period was used to deduce fetus numbers of ewes exhibited either single or twin lambing after parturition proven. The blood P₄ concentration was estimated by RIA using ELISA kit (Human Ge-sellschaft, Biochemical Diagnostica GmbH and Germany).

Blood components:

In the morning, before feeding, blood samples were collected (5 samples / energy group) at three stages of gestation. Thereafter, blood was sampled at 5, 11 and 20 weeks of pregnancy which represent early, mid and late stages, respectively. Blood samples were collected without anticoagulant and centrifuged at 3500 g for 15 min and the serum was stored at -20°C for further chemical analysis. Then, there have been found the immunity function (total protein and albumin then, globulin calculated as total protein - albumin), lipid profiles (cholesterol and triglyceride), pancreatic function (glucose), kidney function urea-N) (creatinine and and minerals concentrations (calcium and phosphorus). The levels of blood biochemical were assayed using commercial kits (Diamond chemical Diagnostics, Egypt).

Statistical analysis:

Date was statistically analysed using Social Sciences (SPSS software, 2011, Version 20) program. Differences among means were tested using Duncan's New multiple-range tests of the same later program. All statements of significance based on a probability of P<0.05.

RESULTS AND DISCUSSION *Productive activity:*

The productive parameters are summarized in Table (2). Differences between body weight of ewes fed BCE or UCE diets were insignificant during start work (kg), pre-tupping

season (kg), post-tupping season (kg), postpartum (kg), at birth (kg) and gestation length (days), while significantly (P<0.05) higher for ewes fed UCE (59.40 kg) than BCE (55.80 kg) during per-partum (kg). It has been observed that deficient energy nutrition during pregnancy induces low weight of offspring at birth, as well as, lower body weight gain for females. Ramirez-Vera et al. (2011) reported that insufficient energy nutrition during pregnancy induces emaciated offspring weight, little colostrum and suckling milk production. Accordingly, Ólafsdóttir et al. (2012) noticed that improving body weight of ewes in this critical period (prepartum) not only affected the growth of the developing fetus, but also the ability of the ewe to supply lamb with adequate amount of colostrum and milk postpartum. On the other hand, several authors have been reported that energy ameliorate feed intake of small ruminants (Hassan et al. 2012 and Khalifa et al., 2013). Furthermore, UCE has been given better (P>0.05) lambing weight than those lambed with feeding BCE which attributed to energy supplemented to ration. This mentioned study agree with that carried out by Radunz et al. (2011) who observed that ewes generally lost weight during pregnancy, lactation or gain weight at a very low rate depending on their plane of energy in nutrition. Further, these authors reported that energy intake can affect fetal growth and stimulates release of glucose, thereby potentially affecting nutrient supply to the gravid uterus. Moreover, non-significant differences were observed on gestation length (151.00 and 151.60 days) when ewes supplied BCE or UCE, respectively. Thus, duration length in this experiment is in agreement with duration range (144 - 155 days) of most sheep breeds (Gootwine, 2013).

The reproductive parameters are shown in Table (3). The reproductive performance of ewes received UCE or BCE diet were insignificantly differed. In addition, the number of dams born single and single rate (%) of ewes fed UCE diet was insignificantly lower than those ewes nourished BCE diet. Ewes received UCE could regulate sexual hormonal secretion and appeared positive reproductive activity. It is obvious from previous studies that mechanism of secretion of a major sexual hormone such as Luteinizing hormone (LH), that stimulate ovary and occurred ova ovulation, regulated partially by indirect way from the pituitary and hypothalamic gland. These findings confirmed by Radunz (2009) who explained that fat supplementation might increase glucose production through increasing propionate Consequently, the increase in production. glucose may have a positive effect on LH release. Furthermore, the same later author elucidated that glucose provide great signal to the hypothalamic pituitary that control system secrete more LH. Otherwise, the to development of nutrition programs emphasize that energy supplementation might improve ration composition to maintain or enhance reproductive efficiency (Pescara et al., 2010). In addition, energy balance in diet has a significant influence on multiple reproductive functions (Evans and Anderson, 2012). In this context, most studies about the influence of positive balance (PEB) reproductive energy on efficiency have been performed on goats (Khalifa et al., 2013) and ewes (Alkass et al., Generally, 2014). dietary energy supplementation (by glucose and fructose) causes an array for the metabolic effects on reproductive activity where increases in number of carbohydrates have been identified in cervical mucus. These compounds appear to be the major free sugars in uterine flushed from the ewes. Futhermore, insulin concentration which play an essential role in mediating increased follicular growth, by insulin-growth factor I (IGF-I) and increased concentration of growth hormone (GH), during the postpartum which develop an increase in the accumulation of follicular fluid (Rahbar et al., 2014).

Energy types		
BCE	UCE	
46.80±1.07	47.00±1.00	
48.80 ± 1.06	49.40±1.08	
50.40±1.67	52.00±1.05	
$55.80{\pm}1.16^{b}$	59.40±1.03ª	
50.80±1.83	51.00±1.70	
3.00 ± 0.55	3.90±0.56	
151.00±2.03	151.60±0.75	
	BCE 46.80±1.07 48.80±1.06 50.40±1.67 55.80±1.16 ^b 50.80±1.83 3.00±0.55	

Table 2. Productive performance of ewes fed either BCE or UCE diets.

a, b : Means in the same row with different superscripts are significantly different (P<0.05). Reproductive performance of ewes:

Itoma	Energy types	
Items —	BCE	UCE
No. of maternal tupping	5.00	5.00
No. of maternal conceived	5.00	5.00
Conception rate (%)	100	100
No. of maternal lambing	5.00	5.00
Total no. of lambing	6.00	7.00
No. of a live lambs at birth	5.00	7.00
Fertility rate (%)	100	100
Fecundity (%)	100	100
Prolificacy (%)	83.33	100
Parity patterns:		
No. of maternal born single	4.00	3.00
Single rate (%)	80.00	60.00
No. of maternal born twins	1.00	2.00
Twins rate (%)	20.00	40.00

Table 3. Reproductive performance of ewes fed either BCE or UCE diets.

Progesterone (P₄) profiles

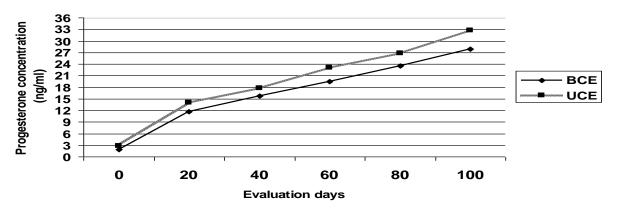
The following graph (Fig. 1) show that ewes fed UCE significantly (P<0.05) had higher progesterone in pregnant ewes than those nourished BCE. At the start, on 0 day after tupping, the P₄ concentration did not show any significant difference between ewes fed BCE and UCE energy. UCE kept a little, but significant, elevation in P4 level compared to BCE ewes by progress of gestation. Meanwhile, the curve lines show a clear positive relationship between P4 levels and advancing stage of pregnancy. Hence, energy material addition had interpreted positively the reproductive function at several important tissues specially ovary and uterus. Moreover, supplementation dietary energy increases circulating concentrations of cholesterol, progesterone and increased lifespan of corpora lutea (CL). These observations are supported by the results of Lopes et al. (2011) who noticed that cholesterol serves as a precursor for the synthesis of progesterone by ovarian luteal cells; also it could be decided that P₄ prepares the uterus for implantation of the embryo and helps maintaining pregnancy. In addition, Reis

et al. (2012) reported that adding protected fat (PF) increased serum P₄ concentration and tended to increase serum insulin concentration. Subsequently, the same later authors found that longer periods of PF supplementation was required to potentially increase circulating concentrations of P₄. On the other hand, Alizadeh et al. (2012) suggested that synthesis of steroids in luteal tissues depends on the availability of cholesterol, so increasing of cholesterol augment progesterone can production in follicular and luteal cellules by increasing the availability of sterol precursor. At all events, blood progesterone is one of the most important factors to be observed for its importance in maintaining pregnancy. Researchers recognized that the physiological mechanism responsible for increasing progesterone concentration in blood serum is the increase of its pioneer source likely cholesterol (Moriel et al., 2014).

Pregnancy diagnosis:

Progesterone concentration was higher for ewes lambing twins than single (P<0.05) (Fig. 2). In spite of the belated prediction of fetal number by using P₄ concentration method, it seems to be cheap and practical and it is an important criterion for better reproductive management in livestock before trimester of gestation. Accordingly, Yotov (2007) reported that measuring number of fetuses in sheep breeds through progesterone method could performed later (60th day) than that by ultrasonography method (40th day). He reported that early pregnancy diagnosis is crucial to shorten the lambing interval through enabling the farmer to identify open animals to treat and/or rebreed them at the earliest opportunity. On the other hand, Kalkan et al. (1996) confirmed that there was a positive relationship between the number of fetuses and the mean progesterone concentrations. Also, Karen et al. (2001) reported that progesterone concentration was used to indicate the presence or absence of functional corpora lutea and fetal number predictions, so, progesterone concentration was significantly higher in ewes carrying two fetuses (19.2 ng/mL) and three fetuses (29.9 ng/ml) than those carrying one (9.2 ng/mL) fetus. Furthermore, Nessim et al. (2009) summarized that P₄ level significantly increase in ewes with single lamb than those with twin lambs at days 10 and 20 of pregnancy then, higher P₄ level was detected in ewes carrying twins. The diameter of corpus luteum (CL) and P₄ concentration of pregnant ewes bearing a single fetus or twin fetuses were higher than that found in non-pregnant ewes. This notion was defined by Gür et al. (2011) who indicated that P₄ concentration in pregnant ewes bearing twin fetuses was higher than that found in bearing a single pregnant ewes fetus. Additionally, El-Tarabany (2012) found that comparison between ewes conceived twin fetuses showed that most blood P₄ values were related with survivability of lambs and the diameter of corpus luteum (CL) on ovaries thence, progesterone concentration was lower (9.34 ng/mL) in single lamb than twin lambs (14.22)ng/mL).

Fig. 1. Progesterone (P4) profile during gestation of ewes fed either BCE or UCE diets.



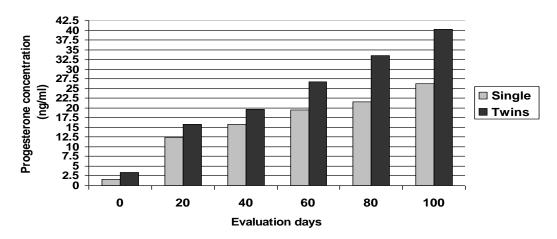


Fig. 2. Progesterone (P4) concentration in single or twins pergnancy ewes.

Blood metabolites:

The tabulated data in Table (4) indicate that total protein, albumin and globulin (that displayed immunity function) significantly (P <0.05) affected by gestation stages and energy types. The variation of total protein amount during pregnancy stages might related to a decrease in globulin level in blood stream. Accordingly, El-Sherif and Assad (2001) manifested significant increase in serum total protein in early and mid pregnancy stages compared to late gestation which could be due to detract in serum globulin. In the current study, the total protein, albumine and globulin obtained similar diminishing trends to those levels obtained with forwarded gestation stages. Similarly, Hefnawy et al. (2010) reported lower immune function in pregnant dams than nonpregnant dams, therefore, pregnancy results in a change in number and function of immune cells in utero that potentially affects fetal survival and uterine defense mechanisms postpartum. In the present study, ewes fed UCE could achieve positive immunity function compared to dams fed BCE energy. In addition, similar trend was observed with the results reported by Ritz and Gardner (2014) who defined that energy diet acknowledged to delay the development of immunity and maintain its function later in lifespan. In addition, energy produced the most convincing evidence of an increased immune response. In the present study, decreasing total protein may be due to increasing foetal growth especially utilization of amino acids in maternal

circulation for protein synthesis in the foetal muscles (Lloyd *et al.*, 2012).

Table (5) highlited the mean values of lipid levels and pancreatic function of ewes fed either BCE or UCE energy during different pregnancy weeks. The data indicate that cholesterol and glucose appeared significantly (P<0.05) higher in ewes received UCE than ewes fed BCE energy. While, triglyceride concentration was insignificantly higher in ewes received UCE than those fed BCE diet. Furthermore, similar significant (P<0.05) variation trend was reported among different stages of pregnancy. In this study, total cholesterol had heightened during early gestation, which might related to responsiveness of the target tissue to low insulin during early pregnancy hence predisposes the ewes to increase cholesterol concentration. This observation is supported by other studies that reported high cholesterol levels at early gestation (El-Tarabany, 2012). Contrariwise, the lowest cholesterol in end of gestation may be associated with reducing glucose level and increasing insulin that elevated cholesterol level. This define is consistent with those of Piccione et al. (2009) who reported that total cholesterol concentration were 1.73, 1.60 and 1.45 mmol/l throughout early, mid and late gestation, respectively. However other studies explained that reduction in cholesterol level is probably attributed to the role of ovarian steroidogenesis on declining food intake which is associated with alteration in lipid metabolism and marked reduction in lipogenesis during

pregnancy (Papacleovoulou et al., 2013). The decrease in serum triglycerides found in this study during late pregnancy is in accordance increased concentration of with these compounds in the ewes' liver as reported by Mazur et al. (2009). Also, the quantity of mobilized fatty acids from adipose tissue depends on the energy balance and has a direct relationship with the severity of the fatty liver. For this reason, fatty acids metabolized by the liver have to be imported via the blood. Therefore, triglyceride secretion is limited from the liver in ruminants when free fatty acid uptake which could be responsible for their sensitivity to developing particular liver steatosis (Piccione et al., 2010). Increasing in triglyceride levels at mid gestation time may be due to insulin, which plays a direct role in adipose tissue metabolism during pregnancy (Deghnouche et al., 2013). Decreasing blood sugar during late pregnancy weeks can be explained by increasing; maternal glucose permeability, consumption by the fetus, physiological stage and diseases (Duehlmeier et al., 2013). The present results indicated that serum glucose concentrations had gradually descended during prepartum period. This observation is supported by Taghipour et al. (2010) who found that glucose level changed at

day's 45th and 30th prepartum, being 2.45 and 2.14 mmol/l, respectively. Also, the same later authors reported that negative energy balance appears to be related to the glucose demands of the fetal-placental unit in pregnant ewes thus, energy needs of the fetus and placenta are met almost entirely by glucose. However, others indicated that rising glucose level during early pregnancy is associated with stress and increase in secretion of glucocorticoids and epinephrine which stimulate glycogenolysis in the liver (Alameen and Abdelatif, 2012).

Table (6) illustrates the effect of feeding BCE or UCE energy and weeks of pregnancy on the kidney function and some mineral levels. The renal function principally represented by urea and creatinine concentrations, which affected during the different physiological phases of pregnancy in ewes. The serum creatinine level was significantly (P<0.05) lower during the 5th and 11th weeks than 20th week of gestation. These results agree with those of El-Tarabany (2012) who stated that creatinine level was 1.27, 1.33 and 1.52 mg/dl during early, mid and late stages of pregnancy, respectively. Thus, it is recognized that during late gestation the foetal maternal circulation, assumes the load of organic waste of the newborn so, the increase in serum creatinine

	ergy types in ewe			
Items	Gestation	Energy types		0 "
stages	stages	BCE	UCE	 Overall mean
Total protein (g/dl)	Early	5.70±0.11	6.04±0.27	$5.87{\pm}0.18^{a}$
	Mid	5.61±0.09	5.88 ± 0.08	$5.75 {\pm} 0.10^{b}$
	Late	5.41±0.07	5.64 ± 0.06	$5.53 \pm 0.06^{\circ}$
Overall	mean	$5.57{\pm}0.06^{b}$	$5.85{\pm}0.09^{a}$	5.71
Albumin (g/dl)	Early	3.85 ± 0.06	4.02 ± 0.03	$3.94{\pm}0.04^{a}$
	Mid	3.72 ± 0.07	3.92 ± 0.08	$3.82{\pm}0.08^{b}$
	Late	3.55±0.06	3.84 ± 0.05	$3.70 \pm 0.05^{\circ}$
Overall	mean	3.71 ± 0.01^{b}	$3.93{\pm}0.04^{a}$	3.82
Globulin (g/dl)	Early	1.85 ± 0.07	2.02 ± 0.04	1.94±0.06 ^a
	Mid	1.89 ± 0.07	1.96 ± 0.01	$1.93{\pm}0.05^{a}$
	Late	1.86 ± 0.02	181±0.04	$1.84{\pm}0.02^{b}$
Overall	mean	$1.87{\pm}0.01^{b}$	$1.93{\pm}0.05^{a}$	1.90

 Table 4: Total protein, albumine and globulin in blood serum as affected by gestation stages and energy types in ewes.

a, b, c : Means in the same rows and columns with different superscripts are significantly different (P<0.05).

Items	Costation stages	Energ	y types	- Overall mean
items	Gestation stages –	BCE	UCE	
	Early	122.03±0.35	123.74±0.38	122.89±0.35 ^a
Cholesterol (mg/dl)	Mid	106.68±0.91	110.93±0.95	$108.81 \pm 0.92^{\circ}$
(ing/ui)	Late	107.39 ± 0.64	113.23±0.78	110.31 ± 0.71^{b}
Over	all mean	112.03 ± 0.62^{b}	$115.97{\pm}0.71^{a}$	114.00
	Early	28.36 ± 0.88	31.69 ± 1.84	$30.03 \pm 0.52^{\circ}$
Triglyceride (mg/dl)	Mid	42.99±0.32	44.02±0.29	43.51 ± 0.31^{a}
	Late	37.26±0.36	37.84±0.13	37.55 ± 0.41^{b}
Over	all mean	36.20±0.53	37.85±0.74	37.03
Glucose (mg/dl)	Early	72.32±0.27	74.95±0.19	73.64±0.11 ^a
	Mid	61.90±0.29	65.28 ± 0.70	63.59 ± 0.51^{b}
	Late	57.85 ± 0.48	59.79±0.18	58.82±0.34 °
Over	all mean	64.02 ± 0.33^{b}	66.67 ± 0.36^{a}	65.35

Table 5: Cholesterol, triglyceride and	glucose in blood serum	as affected by gestation stages
and energy types in ewes.		

a, b, c : Means in the same rows and columns with different superscripts are significantly different (P<0.05).

levels could be attributed to the development of the foetal musculature (Piccione et al., 2012). Urea is nitrogen-based product secreted from the kidneys, and created by the breakdown of proteins and may be utilized by rumen microbial population to synthesize protein (Kioumarsi et al., 2011). In fact, serum urea concentrations reached little higher (P>0.05) level at early of gestation stages than ewes in mid or late pregnancy status. Contrariwise, the decrease of serum urea-N in mid and late gestation may be associated with either the decline of feed intake due to stress or hormonal changes and it is strictly dependent on the dietary intake of proteins (Taghipour et al., 2010). The slight elevated values of urea during late gestation compared to mid gestation could be ascribed to the high thyroid activity in pregnant dams, which induces an increased protein catabolism (Piccione et al., 2009). In this study, the high requirement for energy by pregnant sheep during their second half of pregnancy led to an increase in urea level which is evident during late pregnancy. Thus, the trivial high values of blood urea-N in the last trimester of pregnancy was observed also by Alameen and Abdelatif (2012). The current results revealed the elevation of urea level measured during early and late gestation, which

could be related to enhancement of metabolic activities that induce an increase in protein catabolism. El-Tarabany (2012) revealed that urea-N level in early, mid and late stages of pregnancy were 31.05, 30.10 and 31.50 mg/dl, respectively. Also, in this study the presented results of urea levels were corroborated in dairy cow by Alameen and Abdelatif (2012) who declared that urea concentration varied between 32.13, 30.10 and 31.44 mg/dl in the early, mid and late pregnancy stages, respectively. On the other hand, glucose availability for oxidation is supported by increased catabolism of amino acids at the expense of protein synthesis, thus increasing urea production (Deghnouche et al., 2013).

Serum calcium (Ca) level had significantly (P<0.05) changed in ewes fed BCE or UCE during pregnancy weeks. The decline in serum Ca level in late gestation is related to increase in movement of Ca out of blood, which is not balanced by increase in rate of absorption from gut or mobilization from bone (Khan *et al.*, 2011). Furthermore, Takaya *et al.* (2013) suggested that the decrease in Ca level in late pregnancy might also be associated with haemodilution (an increase in fluid content of blood, resulting in diminuting concentration of formed elements especially in pregnant rats).

Items	Costation stages	Energy types		 Overall mean
Items	Gestation stages –	BCE	UCE	Over all mean
	Early	1.28±0.03	1.29 ± 0.04	1.29±0.03 ^c
Creatinine (mg/dl)	Mid	1.37 ± 0.01	1.41 ± 0.02	1.39 ± 0.01^{b}
(IIIg/uI)	Late	1.54 ± 0.02	1.57 ± 0.01	1.56 ± 0.02^{a}
Over	all mean	1.40 ± 0.04	1.42 ± 0.03	1.41
	Early	32.33±0.33	32.63±0.35	32.48±0.35
Urea (mg/dl)	Mid	30.40±0.34	30.62±0.37	30.51±0.34
(IIIg/uI)	Late	31.08±0.41	31.40 ± 0.45	31.24±0.42
Over	all mean	31.27±0.36	31.55±0.38	31.41
C 1 1	Early	8.61±0.12	$8.97 {\pm} 0.02$	$8.79{\pm}0.08^{\mathrm{a}}$
Calcium	Mid	8.46±0.13	$8.77 {\pm} 0.09$	8.62 ± 0.07^{b}
(mg/dl)	Late	7.74 ± 0.06	$7.95 {\pm} 0.08$	$7.85 \pm 0.05^{\circ}$
Over	all mean	$8.27 {\pm} 0.11^{b}$	8.56 ± 0.07^{a}	8.42
Phosphorus (mg/dl)	Early	3.83±0.07	4.04 ± 0.05	3.94±0.06 ^c
	Mid	4.29 ± 0.04	4.92 ± 0.05	4.61 ± 0.05^{b}
	Late	4.57±0.10	5.02 ± 0.04	$4.80{\pm}0.06^{a}$
Over	all mean	4.23 ± 0.06^{b}	4.66±0.05 ^a	4.45

 Table 6: Kidney function and some mineral levels in blood serum as affected by gestation stages and energy types in ewes.

a, b, c : Means in the same rows and columns with different superscripts are significantly different (P<0.05).

Also, these authors suggested that relative stability of Ca is controlled mainly by a sensitive hormonal mechanism involving hydroxylated metabolites of vitamin D, parathyroid hormone and thyrocalcitonin. Indeed, serum Ca level may be affected by physiological status of animal body's response to environment, as well as, its nutritional conduction (Poppenga et al., 2012). Also, phosphorus ion (Pi) level serum was significantly (P<0.05) lower in early pregnancy than either mid or late pregnancy states. The fluctuation in levels of Pi among gestation weeks may be attributed to an increasing rate of mobilization of phosphorus ion (Pi) out of maternal circulation into the fetus, which was not balanced by increasing the rate of absorption of Pi from the gut or the rate of resorption of Pi from the bone of dams (Adriana et al., 2013). At all events, unconventional energy sources that used in rations of small ruminants could have positive effect on body function and could conform normal range of blood metabolites (Behery et al., 2014).

CONCLUSION

This study helps to recommend the following. Firstly, supporting addition of nonconventional energy in the ration formula of ewes. Secondly, feeding non-conventional energy could offer cheap energy source, which led to decrease feeding cost and safe tool in preventing deficiencies constrain high production of ruminants. Thirdly, if we give consideration for ewes' fertility, conception rate, parity rate and blood metabolites as important reproduction criteria we will advise feeding CFM + RS + non- conventional energysources rather than feeding CFM+ RS only.

REFERENCES

- Adriana, A., Violeta, E. S. and Monica, P. (2013). Research on phosphocalcic status in sows reared in semi- intensive system in different periods of gestation. Animal Science and Biotechnologies. 46 (1): 1-4.
- Alameen, A. O. and Abdelatif, A. M. (2012). Metabolic and endocrine responses of crossbred dairy cows in relation to pregnancy

and season under tropical conditions. American-Eurasian J. Agric. & Environ. Sci., 12 (8): 1065-1074.

- Alizadeh, A., Azizi, F., Karkoodi, K., Jalali, S. and Ghoreishi, M. (2012). Effects of calcium salts of fatty acids (Megalac) on reproductive performance and blood parameters of Kalkohi ewes. Journal of Animal and Poultry Sciences. 1 (1): 6-12.
- Alkass, E. J., Darwesh, A. K. and Merkhan, Y. K., (2014). Performance of Docked vs. Undocked fat-tailed Sheep: A review. Advanced Journal of Agricultural Research. 2 (003): 029-037.
- AOAC (2007). Association of Official Analytical Chemists. Official Methods of Analysis. 19th Edition. Washington, DC: AOAC. USA.
- Ataman, M. B., Akoz, M., Saribay, M. K., Erdem, H. and Bucak, M. N. (2013). Prevention of embryonic death using different hormonal treatments in ewes. Turkish Journal of Veterinary and Animal Sciences. 37:6:8.
- Behery, H. R., Khalifa, E. I. and Mahrous, A.
 A. (2014). Influence of feeding nonconventional energy sources on pubertal phases, blood metabolites and fattening of Zaraibi male kids. Journal of Animal and Poultry Production Mansoura Univ., 5 (3), 127–141.
- Deghnouche, K., Tlidjane, M., Meziane, T. and Touabt. A. (2013). Influence of physiological stage and parity on energy, nitrogen and mineral metabolism parameters in the Ouled Djellal sheep in the Algerian Southeast arid area. African Journal of Agricultural Research. 8(18): 1920-1924.
- **Dorniak, P., Bazer, F. W. and Spencer, T. E.** (2012). Biological role of interferon tau in endometrial function and conceptus elongation. J. ANIM. SCI., 24(2012): jas.2012-5845.
- **Duehlmeier, R., Fluegge, I., Schwert, B. and Ganter, M. (2013).** Post-glucose load changes of plasma key metabolite and insulin concentrations during pregnancy and lactation in ewes with different susceptibility to pregnancy toxaemia. Journal of Animal Physiology and Animal Nutrition. 97: (5): 971-985.

- EL-Sherif, M. M. A. and Assad, F. (2001). Changes in some blood constituents of Barki ewes during pregnancy and lactation under semi arid conditions. Small Ruminant Research. 40: 269-277.
- **El-Tarabany, A. A. (2012).** Physiological changes in ewes conceived single or twins fetuses related with survivability of lambs. Arab Journal of Nuclear Science and Applications, 45 (3): 223-235.
- **Evans, J. J. and Anderson, G. M. (2012).** Balancing ovulation and an ovulation: integration of the reproductive and energy balance axes by neuropeptides. Human Reproduction Update.18 (3): 313–332.
- Freetly, H. C. and Leymaster, K. A. (2004). Relationship between litter birth weight and litter size in six breeds of sheep. J. Anim. Sci., 82 (2): 612-618.
- Gootwine, E. (2013). Meta-analysis of morphometric parameters of late-gestation fetal sheep developed under natural and artificial constraints. J. Anim. Sci., 91:111-119.
- Gür, S., Türk, G., Demirci, E., Yüce, A., Sönmez, M., Ozer, S. and Aksu, E. (2011). Effect of pregnancy and foetal number on diameter of corpus luteum, maternal progesterone concentration and oxidant/antioxidant balance in ewes. Reprod. Domest. Anim., 46 (2): 289-95.
- Hassan, T. M., Ibrahim, M., Itman, K. and Abdel-Hai, I. (2012). Productive and reproductive performance of Zaraibi goats fed different types of protected fat. The 3rd Scientific Conference for Animal Nutrition, Sharm El-Sheikh, 14-17 Feb., 24 (2):312-219.
- Hefnawy, A. E., Youssef, S. and Shousha, S. (2010). Some immunohormonal changes in experimentally pregnant toxemic goats. Veterinary Medicine International. 2010 (2010): 1-5.
- Hutchinson, I. A., Hennessy, A. A., Waters, S. M., Dewhurst, R. J., Evans, A. C., Lonergan, P. and Butler, S. T. (2012). Effect of supplementation with different fat sources on the mechanisms involved in reproductive performance in lactating dairy cattle. Theriogenology, 78(1):12-27.

- Inskeep, K., Holler, T., Dean, M., Hare, S., Smith, B. and Johnson, E. (2009). Increasing lamb crop by decreasing embryonic and fetal mortality. Proceedings of U.S. Sheep Research Programs. American Sheep Industry Association Convention.p:35-38.
- Kalkan, C., Cetin, H., Kaygusuzoglu, E., Yilmaz, B., Ciftci, M., Yildiz, H., Yildiz, A., Deveci, H., Apaydin, A. M. and Ocal, H. (1996). An investigation on plasma progesterone levels during pregnancy and parturition in the Ivesi sheep. Acta. Vet. Hung., 44: 335-340.
- Karen, A., Kovacs, P., Beckers, J. F. and Szenci, O. (2001). Pregnancy diagnosis in sheep: review of the most practical methods. Acta. Vet. Brno., 70: 115–126.
- Khalifa, E. I., Behery, H. R., Hafez, Y. H., Mahrous, A. A., Amal, A. Fayed, and Hanan, A. M. Hassanien. (2013). Supplementing non-conventional energy sources to rations for improving production and reproduction performances of dairy Zaraibi nanny goats. Egyptian Journal of Sheep & Goat Sciences. 8 (2): 69-83.
- Khan, H. M., Mohanty, T. K., Bhakat, M., Raina, V. S. and Gupta, A. K. (2011). Relationship of blood metabolites with reproductive parameters during various seasons in Murrah buffaloes. Asian-Austr J. Anim. Sci., 24: 1192-1198.
- **Kioumarsi, H., Yahaya, Z. S. and Rahman, A. W. (2011).** The effect of molasses/mineral feed blocks along with use of medical blocks on haematological and biochemical blood parameter in Boer goats. Asian Journal of Animal and Veterinary Advanced. 2011: 1-7.
- Lloyd, L. J., Foster, T., Rhodes, P., Rhind, S. M. and Gardner, D. S. (2012). Proteinenergy malnutrition during early gestation in sheep blunts fetal renal vascular and nephron development and compromises adult renal function. J. Physiol., 590 (2012): 377–393.
- Lopes, C. N., Cooke, R. F., Reis, M. M., Peres, R. F. G. and Vasconcelos, J. L. M. (2011). Strategic supplementation of Ca salts of polyunsaturated fatty acids to enhance reproductive performance of *Bos indicus* beef cows. Journal of Animal Science. 89: 3116-3124.

- Manthou, E., Kanaki, M., Georgakouli,
 K., Chariklia K. D., Kouretas, D.,
 Koutedakis, Y. and Athanasios Z. J.
 (2014). Glycemic response of a carbohydrateprotein bar with ewe-goat whey. Nutrients. 6
 (6): 2240–2250.
- Mazur, A. Ozgo, M. and Rayssiguier, Y. (2009). Altered plasma triglyceride-rich lipoproteins and triglyceride secretion in feed-restricted pregnant ewes. Veterinarni Medicina. 54 (9): 412–418.
- Moriel, P., Cappellozza, B., Ferraretto, L. F., Aboin, A. C., Vieira, F. V. R., Rodrigues, R. O., Cooke, R. F. and Vasconcelos, J. L. M. (2014). Effects of supplementation of calcium salts of polyunsaturated fatty acids on serum concentrations of progesterone and insulin of pregnant dairy cows. Revista Brasileira de Zootecnia. 43 (1): 20-26.
- Mostafa, T. H., Etman, K. E. I., Abd El-Hamid, A. A. and Ahmed, M. I.(2012). Studies on nutritional and economical models under different production system: 1. Effect of fatty acids of calcium soap on productive performance of dairy goats. Egyptian J. Nutrition and Feeds, 15 (1): 225-235.
- Nessim, M. Z., Kottb, M. K. I. and Mustafa, M. M. (2009). Progesterone blood level for pregnancy diagnosis and prediction of twinning in Egyptian ewes. Isotope and Radiation Research. 41 (4s1): 1335-1345.
- NRC (2007). Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids. National Research Council of the National Academies, National Academies Press, Washington, D.C., U.S.A.
- Ólafsdóttir, H. Ó., Sveinbjörnsson, J. and Harðarson, G. H. (2012). Energy and protein in the diet of ewes in late pregnancy: Effect on ewe feed intake, life weight, body condition and concentration of plasma metabolites. MSthesis, Agricultural University of Iceland Department of Land and Animal Resources.
- Papacleovoulou, G., Abu-Hayyeh, S., Nikolopoulou, E., Briz, O., Owen, B. M., Nikolova, V., Ovadia, C., Huang, X., Vaarasmaki, M., Baumann, M., Jansen, E., Albrecht, C., Jarvelin, M., Marin, J. J.G., Knisely, A. S. and Williamson, C. (2013). Maternal cholestasis during pregnancy

programs metabolic disease in offspring. J. Clin. Invest., 123(7): 3172–3181.

- Pescara, J. B., Sá Filho, O. G., Losi, T. C., Cooke, R. F. and Vasconcelos, J. L. M. (2010). Serum progesterone concentration and conception rate of beef cows supplemented with ground corn after a fixed-time artificial insemination protocol. Arq. Bras. Med. Vet. Zootec., 62 (1): 130-135.
- Piccione, G., Caola, G., Giannetto, C., Grasso, F., Runzo, S. C., Zumbo, A. and Pennisi, P. (2009). Selected biochemical serum parameters in ewes during pregnancy, post-parturition, lactation and dry period. Animal Science Papers and Reports. 27 (4):321-330.
- Piccione, G., Casella, S., Lutri, L., Vazzana, I., Ferrantelli, V. and Caola, G. (2010). Reference values for some haematological, haematochemical, and electrophoretic parameters in the Girgentana goat. Turkish Journal of Animal Science. 34: 197–204.
- Piccione, G., Messina, V., Marafioti, S., Casella, S., Giannetto, C. and Fazio, F. (2012). Changes of some haematochemical parameters in dairy cows during late gestation, post partum, lactation and dry periods. Veterinari JA IR Zootechnika (*Vet Med Zoot*) 58 (80): 59-64.
- Poppenga, R. H., Ramsey, J., Gonzales, B. J. and Johnson, C. K. (2012). Reference intervals for mineral concentrations in whole blood and serum of bighorn sheep (*Ovis canadensis*) in California. Journal of Veterinary Diagnostic Investigation. 3: 531-538.
- Radunz, A. E. (2009). Effects of prepartum dam energy source on progeny growth, glucose tolerance, and carcass composition in beef and sheep. PhD Diss. The Ohio State University, Columbus, USA.
- Radunz, A. E., Fluharty, F. L., Zerby, H. N. and Loerch, S. C. (2011). Winter-feeding systems for gestating sheep I. Effects on preand postpartum ewe performance and lamb progeny pre-weaning performance. J. Anim. Sci., 89: 467-477.
- Rahbar, B., Amir, H. A. S.and Nasroallah, M. K. (2014). Mechanisms through which fat supplementation could enhance reproduction

in farm animal. European Journal of Experimental Biology. 4 (1): 340-348.

- Ramírez-Vera, S., Terrazas, A., Delgadillo, J. A., Serafín, N., Flores, J. A. and Hernández, , H. Sometido. (2011). Supplementation with maize during the last 12 days of gestation improves colostrum production and kid's behavior in goats kept under extensive grazing conditions. Journal of Animal Science. 90 (7): 2362-2370.
- Reis, M. M., Cooke, R. F., Ranches, J. and Vasconcelos, J. L. M. (2012). Effects of calcium salts of polyunsaturated fatty acids on productive and reproductive parameters of lactating Holstein cows. Journal of Dairy Science. 95: 7039-7050.
- **Ritz, B. W. and Gardner, E. M. (2014).** Malnutrition and energy restriction differentially affect viral immunity. Journal of Nutrition. 136: 1141-1144.
- Saunders, G. A., Alves, N. G. and Perez, J. R. O. (2010). Efeito da sobrealimentação com fontes de proteína de diferentes degradabilidades sobre a ovulação em ovelhas Santa Inês. Revista Brasileira de Zootecnia.39: 2731-2738.
- **SPSS (2011).** Statistical package for social sciences, IBM[®]SPSS Statistics Data Editor 20 License Authorization Wizard, Munich, Germany.
- Taghipour, B., Seifi, H. A., Mohri, M., Farzaneh, N. and Naserian, A. (2010). Variations of energy related biochemical metabolites during periparturition period in fat-Tailed Baloochi breed sheep. Iranian Journal of Veterinary Science and Technology. 2 (2): 85-92.
- Takaya, J., Iharada, A., Okihana, H. and Kaneko, K. (2013). A calcium-deficient diet in pregnant, nursing rats induces hypomethylation of specific cytosines in the 11β -hydroxysteroid dehydrogenase-1 promoter within pup liver. Nutr. Res., 33: 961–970.
- **Yotov, S. (2007).** Determination of the number of fetuses in sheep by means of blood progesterone assay and ultrasonography. Bulgarian Journal of Veterinary Medicine. 10 (3): 185-193.