CHANGE IN SERUM BLOOD COMPONENTS AS AFFECTED BY BREEDING PERIOD AND PROTECTED PROTEIN.

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SUMMARY

rotein of canola meal was protected by heat (T1) or sodium hydroxide (T2) treatments. Two experiments were carried out in this investigation. A total number of 12 healthy male Sohagi lambs averaged 35.8±1.29 kg body weight were used in the first experiment. Animals were divided randomly into three equal (n=4) groups (control, Tl and T2) to evaluate the effect of protected protein on nutritive value of experimental concentrate rations. Results indicated that protected protein in T1 and T2 resulted significant (P<0.05) increasing total digestible nutrients (TDN) and digestible crude protein (DCP) values. A total of 36 healthy Sohagi ewes at 3-4 years of age and 40.60 ± 1.60 kg body weight were used in the second experiment during breeding period (42 days). Animals were randomly divided into three equal groups as in same as experiment one. Blood samples were collected from all ewes 8 days before mating, estrus (mating day), 8 days and 34 days after mating. Results reveled that serum triiodothyronine recorded significant (P<0.01) differences due to treatment, while thyroxin concentrations were increased insignificantly in T1 and T2. Highest values of triiodothyronine and thyroxin recorded at estrus were 152.72 ng/dl and 7.13 µg/dl, respectively. At the same time, serum progesterone concentrations in different treatments were significant (P<0.01) different and the highest values were observed in T1. Values of progesterone at 8 days before mating, 8 and 34 days after mating resulted significant (P<0.01) increasing, while at estrus there were no significant differences. The effect of treatments or breeding period on total protein, albumen and globulin concentrations were not significant. Blood serum glucose concentrations in different treatments indicated higher values in T1 compared with the other both treatments. Levels of glucose concentrations at 8 days before mating, estrus, at 8 and 34 days after mating showed significant (P<0.01) differences. The results indicated that a highest level of serum glucose (71.69 mg/dl) concentration was coincided with the day of estrus. Urea-N and creatinine concentrations were decreased (P<0.01) as a result of protected protein supplementation in the ration.

Throughout the breeding period differences in urea-N and creatinine concentrations were not significant. The present results indicated that feeding protected protein during breeding period change most studied serum blood components.

Keywards: protected protein, blood components, breeding period, sheep.

INTRODUCTION

Production of meat and wool by sheep play an important role in animal production. The number of lambing per year lead to mainly increasing lambing production. Phenomena of blood components during breeding period can be considered essentially for reproductive performance of animals. Many investigators reported that serum levels of most blood components recorded significant differentces during the breeding period (Korshom *et al.*, 1993; Baumgartner and Parnthaner, 1994; Ranilla *et al.*, 1994; Brzostowski *et al.*, 1996; Abu El-Ella, 2006 and Marai *et al.*, 2006). Also, blood components were affected by presence of protected protein in the diet (Pailan and Kaura, 1996; Aly, 2005 and El- Reweny, 2006). Canola meal, which contains up to 40% crude protein on dry matter, used as a main source of protein in the rations, There are several methods for protecting dietary protein from degradation in the rumen. Protected protein methods can be categorized into chemical (e. g. sodium hydroxide, acetic acid and formaldehyde) and physical (e. g. heat)

The aim of this study was to determine the effect of protection of dietary protein and breeding period on some serum components as indicator for the physiological status of animals.

MATERIALS AND METHODS

The present study was carried out at the Experimental Farm of Department of Animal Production, Faculty of Agriculture, Sohag University in cooperation with Department of Animal Production, Faculty of Agriculture, Minia University.

Two experiments were carried out in this study. In the first experiment a total number of 12 healthy male Sohagi lambs averaged 35.8 ± 1.29 kg of body weight were used. The experimental period lasted for 3 weeks. The animals were divided randomly into three equal (n=4) groups (control, canola meal treated with heat (T1) and canola meal treated with sodium hydroxide (T2). Canola meal, which represent 25% in the concentrate feed mixture, was untreated in control group. Canola meal in T1 was subjected to 135-145 °C in a forced air oven for 4 hours according to Stern *et al.* (1985). While, canola meal in T2 was sprayed by a solution of sodium hydroxide (NaOH) at the rate of 3 gm NaOH/ 100 gm DM of canola meal according to Mir *et al.* (1984). Ewes were offered their requirements of concentrate diet (80%) and wheat straw (20%) according to NRC (1985). Digestibility coefficients of DM, OM, CP, CF, EE and NFE were determined using acid insoluble ash (AIA%) as natural marker according to Van Keulen and Young (1977). Nutritive values as total digestible nutrient (TDN) and digestible crude protein (DCP%) of the experimental rations were calculated.

The second experiment was carried out during the breeding period (42 days). A total number of 36 healthy Sohagi ewes at 3-4 years of age and 40.60 ± 1.60 kg of body weight. The ewes were kept in semi open yard. Animals were divided randomly into three equal (n=12) groups as in experiment one. Blood samples during the breeding period were collected every four days. Thereafter, blood samples were divided into four intervals as follows: 8 days before mating, estrus (mating), 8 days and 34 days after mating per each treatment to study the effect of protected protein and breeding period on blood serum metabolites (total protein, albumin, glucose, creatinine, urea-N, thyroid hormones (Thyroxin(T4) and Triiodothyronine (T3)) and progesterone concentrations). Blood metabolites were determined using commercial Kits purchased by Egyptian company for biotechnology (S.A.E) Blood samples were allowed to clot at room temperature (25° C) and serum was then separated harvested by centrifugation blood samples at 3000 r.p.m for 15 minutes, serum was kept at -20 °C until later analysis.

The results were statistically analyzed using the General Linear Model (SAS, 1998) for complete randomized design. Significant differences among treatments means within the experiment were detected using Duncan multiple range test method (Duncan, 1955).

RESULTS AND DISCUSSION

1-Nutrients digestibility coefficients and nutritive values of the experimental rations:

Data of digestibility trials indicated that protected proteins led to significant (P<0.05) improvement in the digestibility coefficients of different nutrients except crud fiber (CF). Also, protection protein methods in T1 and T2 led to significantly (P<0.05) increase of TDN% and DCP % values, (Table, 1).

Table (1): Effect of (Canola Protein protect	tion on the nutrients	: digestibility c	coefficients and
nutritive va	lues of the experimental	i rations.		

			Digesti	bility coeffic	cients (LSN	1)•	· · · -	
Treatments	DM	OM	CP	EE	CF	NFE	TDN	DCP
Control	66.03 °	68.04°	67.84°	67.69°	61.23	69.39°	65.27°	9.96°
Tl	69.77 [*]	71.42 °	71.87*	71.30 ª	61.11	72.51*	68.01 ª	10.62 ª
T2	67.79 ⁶	69.76 ^ե	70.31 ^b	69.69 ^ь	61.76	71.21 ^b	66.98 ^b	10.46 ^a
±SE	1.44*	1.57*	1.19*	1.38*	1.05 ^{NS}	1.00*	1.32*	0.36*

•Values are least square means (LSM) ± standard error.

a, b, c, d Means with different letters in the same column are significantly different, * (P < 0.05), NS = Not significant

Control=Canola meal without treatment, T1=Canola meal heat treatment, T2= Canola meal sodium hydroxide treatment

2- Serum Biochemical components:

Data of serum Triiodothyronine (ng/dl), Thyroxine (µg/dl) and progesterone (ng/ml) concentrations in ewes fed protected canola proteins during breeding period are presented in Table (2). Differences in blood serum triiodothyronine and thyroxin concentrations (regardless of breeding period) in different the studied treatments recorded in T1 significantly (P<0.01) increased triiodothyronine concentrations, while thyroxin concentrations showed no significant differences. Higher values of thyroid hormones were obtained in T1 compared to the other two treatments. These results may be due to that protected proteins increased energy such as TDN in T1 than T2 (Table, 1). These results are in agreement with those of Shalaby and Shehata (1995). Who reported that thyroid hormones level increased with increased of energy level in ration. Values of triiodothyronine and thyroxine concentration (regardless of treatments) throughout the breeding period were significantly (P < 0.01) different. Thus both thyroid hormones values were higher during estrus. High level of serum thyroid hormones during estrus may be due to the increasing of metabolism activity in ewes during this period. These results are in agreement with Salem et al. (1986), who reported that the secretory activity of thyroid gland was higher during the follicular phase of estrous cycle. Also, El-Medany (2005) indicated that triiodothyronine and thyroxin concentrations in the blood of ewes were higher during estrus (day 0) than 15 days post estrus. On the other hand, Marai etal. (2006) reported that no significant difference in triiodothyronine concentrations was found at estrus in comparison with pre-estrus or post-estrus.

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	Tr	eatments (LS		Effect of	
Item	Control	Tl	T2	- ±SE	breeding period.
Triiodothyronine (ng/dl)					
8 days before mating (-8E)	59.21	61.25	60.55	0.59 ^{N.S}	60.34 ^D
At estrus (E)	151.29 ^b	155.35 ª	151.51 ^b	1.06*	152,72 ^A
8 days after mating (+8E)	143.08 ^b	147.49ª	144.26 ^b	1.41*	144.94 ^B
34 days after mating (+34E)	111,31 ^b	115.08 ^a	112.58 ^ь	0.84*	112.99 ^C
Effect of treatment	116.22 ^B	119. 79 ^A	117.22 ^в	0.89**	± 0.96**
Thyroxine (µg/dl)					
8 days before mating (-8E)	2.63	2.76	2.71	0.12 ^{N.S}	2.70 ^D
At estrus (E)	6.90	7.41	7.07	0.19 ^{N.S}	7.13 ^
8 days after mating (+8E)	5.44	5.72	5.54	0.14 ^{N.S}	5 .57 ^B
34 days after mating (+34E)	4.87	5.23	4.99	0.17 ^{N.S}	5.03 ^{CB}
Effect of treatment	4.96	5.28	5.08	0.10 ^{N.S}	± 0.15**
Progesterone (ng/ml)					
8 days before mating (-8E)	2.25 ^b	2.41 ^a	2.34 ª	0.03**	2 .33 ^C
At estrus (E)	0.31	0.33	0.33	0.01 ^{N.S}	0.32 ^D
8 days after mating (+8E)	3.20 ^b	3.49 °	3.34 ª	0.05**	3.34 ^B
34 days after mating (+34E)	6.22 ^b	6.71 ^a	6.66 ª	0.30*	6.53 ^A
Effect of treatment	2.99 ^B	3.23 ^A	3.18 ^A	0.05**	± 0.04**

Table (2): Effect of feeding Canola protected proteins on serum triiodothyronine ,thyroxine and progesterone concentrations in of ewes during breeding period.

• Values are least square means (LSM) ± standard error.

a, b means with the different letters in same row are significantly different

A, B, C, D Mean with the different letters in the last same row or the last same column in

each parameter are significantly different. * (P < 0.05), ** (P < 0.01), NS= Not significant

With regard to the progesterone (P4) concentrations, the results indicated that protected canola protein (T1 and T2) had positive significant effect on progesterone concentrations at days 8 before mating, and after mating 8 day and 34 days, while there is no significant effect of treatments on

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progesterone concentrations at estrus (Table, 2). Differences in serum progesterone concentrations due to treatments were significant (P<0.01). Progesterone concentrations increased by 8.03% and 6.35% for ewes in T1 and T2, respectively compared with those fed control ration, higher level of P4 value was observed in T1 treatment. These results may be due to that protected protein resulted significant positive effect on TDN and DCP (Table, 1) and significant effect on thyroid hormones (Table, 2). These results agreed with those of Abecia et al. (1997), who reported positive effect of protein and energy content in the ration on ovulation rate. High ovulation rate led to increase progesterone concentration as a result of increased number of corpora lutea. Regardless of treatments, values of progesterone concentrations throughout the breeding period were significantly (P<0.01) different. The present study indicated that the lowest level of serum progesterone (0.32 ng/ml) was coincided with the day of estrus. Highest serum progesterone concentration was 6.53 ng/ml at 34 days after mating. Similar results were obtained by Botha and Morgenthal (1982) who reported that at the time of ovulation progesterone concentration was quite negligible. In addition, El-Medany (2005) reported that the lowest level of plasma progesterone (0.33 ng/ml) was coincided with the day of estrus and increased to 3.68 and 6.50 ng/ml at 15 and 30 days after mating. Recently, Marai et al. (2006) found that progesterone concentration was very low, being 0.56 ng/ml at estrus (day 0) compared to that at pre-estrus (2.25, ng/ml) and 21 days after mating (7.11, ng/ml). Decreases of serum progesterone concentrations at time of estrus are mainly due to absent of active corpus luteum (C.L.)

Data of total protein (TP), albumin (AL) and globulin (GL) concentrations in ewes blood during breeding period are presented in Table (3). Values of total protein, albumin and globulin in different treatments recorded no significant difference. The insignificant effects of treatments (T1 and T2) on blood TP and its fractions may be due to the short period of breeding period, which may not induce positive effect of treatments on the concentrations of these parameters and, also, may be due to maturity of the ewes. These results are in agreement with El-Reweny (1999), who reported that the concentrations of TP, AL and GL recorded insignificant changes as a result of using protected protein in the rations of sheep.

	T	reatments (LS		Effect of	
ltem	Control	T1	T2	- ±SE	breeding period
Total protein(g/dl)					
8 days before mating (-8E)	6.19	6.20	6.18	0.03 ^{N.S}	6.19
At estrus (E)	6.26	6.29	6.25	0.04 ^{N.S}	6.27
8 days after mating (+8E)	6.14	6.11	6.16	0.08 ^{N.S}	6.14
34 days after mating (+34E)	6.19	6.14	6.14	0.06 ^{N.S}	6.16
Effect of treatment	6.19	6.19	6.18	0.03 ^{N.S}	±0.05 ^{N.S}
Albumin(g/dl)					
8 days before mating (-8E)	2.98	2.99	3.02	0.04 ^{N.S}	2.99
At estrus (E)	2.97	3.00	3.01	0.07 ^{N.S}	2.99
8 days after mating (+8E)	3.01	2.97	3.02	0.07 ^{N.S}	3.00
34 days after mating (+34E)	2.95	2.99	2.99	0.03 ^{N.S}	2.98
Effect of treatment	2.98	2.99	3.02	0.04 ^{N.S}	±0.06 ^{N.S}
Globulin(g/dl)					
8 days before mating (-8E)	3.22	3.22	3.16	0.05 ^{N.S}	3.20
At estrus (E)	3.28	3.28	3.20	0.06 ^{N.S}	3,25
8 days after mating (+8E)	3.14	3.14	3.14	0.11 ^{N.S}	3.14
34 days after mating (+34E)	3.25	3.15	3.15	0.07 ^{N.S}	3.18
Effect of treatment	3.22	3.20	3.16	0.05 ^{N.S}	±0.05 ^{N.S}

Table (3): Effect of feeding canola protected protein on serum total protein, albumin and globulin concentrations in of ewes during breeding period.

• Values are least square means (LSM) ± standard error, NS= Not significant.

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Also, El-Sherbieny (2000) observed that mean values of TP, AL and GL concentrations were in significantly affected, when bulls were fed dietary protected protein. El-Reweny (2006) found that the concentrations of TP, AL and GL in serum were nearly similar in sheep fed on control diet or protected protein diet. Regardless of treatment, data in Table (3) indicated that the different breeding intervals had no significant effect on the concentrations of TP, AL and GL. Similar results were reported by Marai *et al.* (2006) who found that there were no significant difference in the concentrations of the TP, AL and GL at pre-estrus, at estrus and 21 days after mating.

Data of glucose, urea-N and creatinine concentrations (mg/dl) in serum of ewes during breeding period are presented in Table (4). Values of glucose, 8 days before mating and at estrus and 8 days after mating were significantly (P < 0.01) increase. Higher values of blood serum glucose concentrations were obtained in T1 compared with the other two treatments. These results may be attributed to the increase of nutritive values of TDN and DCP (Table, 1) and high thyroid hormones concentrations of ewes in T1 and T2 (Table, 2). Thyroid hormones increase gluconeogensis and/ or plasma glucose concentrations in lambs (Cole et al., 1994). Also, Ammann (1991) observed that feeding protected soybean meal increased plasma glucose in the sheep. In addition, Krober et al. (2000) found that plasma glucose concentrations in lactating cows were higher (p < 0.01) in ration supplemented with protected methionine. Also, Alv (2005) reported that values of serum glucose in dairy animals increased (p < 0.01) by using protected methionine and lysine in the diet. Differences in blood glucose concentrations throughout the different breeding period were significantly (P < 0.01) different. The present study indicated that the highest level of serum glucose (71.69 mg/dl) was coincided with the day of estrus (Table 4). These results may be due to the increasing activity and metabolism of ewes during this period as a result of increasing thyroid hormones (Table, 2). These results are in agreement with those of Salem et al. (1986). They reported that the secretary activity of thyroid gland was higher during the follicular phase of estrus cycle.

	Tr	eatments (LSM		Effect of	
Item	Control	TI	T2	±SE	breeding period
Glucose (mg/dl)					
8 days before mating (-8E)	68.55 ^b	71.04 ª	70.54 [∎]	0.24**	70.04 ^B
At estrus (E)	70.77 °	72.39 ª	71.90 ^b	0.16**	71.69 ^A
8 days after mating (+8E)	70.0 9 °	71.98 ^a	71.43 ^b	0.18**	71.00 ^A
34 days after mating (+34E)	69.35 °	71.77 ^ª	71.04 ^b	0.21**	70.72 ^B
Effect of treatment	69,69 ^C	71.79 ^A	71.23 ^B	0.18**	±0.22**
Urea-N (mg/dl)					
8 days before mating (-8E)	15.32 ª	14.24 ^b	14.25 ^b	0.14**	14.60
At estrus (E)	15.45 °	14.48 ^b	14.31 ^b	0.14**	14.75
8 days after mating (+8E)	15.06 ª	14.61 ^b	14.44 ^b	0.12**	14.70
34 days after mating (+34E)	15.46 *	14.42 ^b	14.29 ^b	0.10**	14.72
Effect of treatment	15.46 ^A	14.44 ^B	14.32 ^B	0.12**	$\pm 0.11^{NS}$
Creatinine (mg/dl)					
8 days before mating (-8E)	1.20 ª	1.09 ^b	1.09 ^ь	0.03**	1.13
At estrus (E)	1.20 ª	1.09 ^ь	1.10 ^b	0.01**	1.13
8 days after mating (+8E)	1.15 °	1.09 ^ь	1.07 ^b	0.01**	1,10
34 days after mating (+34E)	1.18 ^a	1.06 ^b	1.09 ^b	0.02**	1,11
Effect of treatment	1,18 ^A	1.08 ^B	1.09 ^B	0.01**	±0.02 ^{NS}

Table (4): Effect of feeding canola protected protein on serum glucose, urea-N and creatinine
concentrations in ewes during breeding period .

• Values are least square means (LSM) ± standard error.

a, b and c means with the different letters in same row are significantly different.

A, B and C Mean with different letters in the last same row or in the last same column in each parameter are significantly different, ** (P < 0.01), NS=Not significant.

Differences of blood serum urea-N and creatinine concentrations in control treatment (regardless of breeding period) were significantly (P<0.01) increase (Table, 4). The values of urea-N and creatinine

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concentrations decreased in T1 and T2 than those untreated (control). The decrease in the concentrations of urea-N in treatments T1 and T2 in comparison with the control group may be due to the reduction of ammonia concentration released through the microbial fermentation in rumen of lambs fed protected protein (El-Ayek, 1999). Decreasing the absorbed ammonia via ruminal wall, which converted into urea in liver, resulted in a lower level of urea in the blood of sheep fed protected protein. In addition, El-Shabrawy (2004) found that the effect of protected protein method led to significantly (P<0.01) reductions in urea-N concentrations as a result of heat or formaldehyde treated diets in comparison with untreated one. El-Shabrawy (2006) reported that lower (P<0.05) values of urea-N were found in plasma of goats receiving diets contain soybean meal treated with heat or formaldehyde than those receiving untreated soybean meal diet.

Creatinine is considered as the major metabolite produced from protein catabolism. Lower creatinine concentrations in serum of ewes fed on protected protein (T1 and T2) may be due to higher utilization of dietary proteins in ewes fed protected protein than those fed the control ration. The present results are in agreement with those of El-Sherbieny (2000), who found that creatinine level decreased in the plasma of bulls fed protected protein in concentrate feed mixture as compared to the control. Differences in the values of urea-N and creatinine concentrations throughout the breeding period were not significant. The present results are disagreement with the results reported by Marai *et al.* (2006), who found that creatinine concentration showed significantly higher value at estrus in comparison with the other breeding period. Insignificant effect of estrus on creatinine concentration in the present study may be due to the lower values of them as a result of using protected protein in the rations.

From the present results it can be concluded that feeding on protected protein in the diet and also time of breeding period led to changes in most serum biochemical components, which can be considered as indicator for the physiological status of animals.

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التغيرات في مكونات سيرم الدم و تأثر ها بفترة التزاوج والبروتين المحمى

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في هذا العمل تم حماية كسب الكانولا بطريقتين باستخدام الحرارة T1 أو بهيدروكسيد الصوديوم T2وهذا العمل تم في تجربتين . التجربة الأولى أجريت باستخدام عدد 12 حمل سوهلجي متوسط وزنها 35.8 \pm 1.29 قسمت إلى ثلاثة مجموعات متساوية هي : الكنترول – المعاملة بالحرارة T1 و المعاملة بهيدروكمبيد الصوديوم T2. وذلك لتقييم تأثير طريقة حماية بروتين الكانولا على القيمة الغذائية لهذا البروتين المحمى مثل المركبات الكلية المهضومة (TDN) و البروتين المهضوم (DCP). والتجربة الثانية تمت على 36 نعجة سوهاجي في عمر 3-4 سنوات متوسط الوزن الحي لما 40.6 ± 1.6 كيلو جرام وذلك أثناء فترة التزاوج التي منتها 42 يوم وقد تم تقسم النعاج بطريقة عشوانية إلى ثلاثة مجاميع الكنترول – المعاملة الحرارة T1 المعاملة بهيدر وكسيد الصوديوم T2 تم اخذ عينات من دم النعاج أقياس التغيرات في مكونات سيرم آلدم لها كمايلي: عينة قبل التلقيح ب 8 أيام وعينة يوم التلقيح (الشياع) وعينة بعد التلقيح ب 8 أيام و عينة بعد التلقيح ب 34 يوم . وقد أظهرت النتائج أن تركيز هرمونات الغدة الدرقية ثلاثي اليود T3 والثيروكسين T4 قد سجلت اختلافات عالية المعنوية (p<0.01) بين المعاملات وكانت القيم أعلى في المعاملة بالحرارة والمعاملة بهيدروكسيد الصوديوم عن الكنترول وقد سجلت أعلى قيم في المجموعة المعاملة بالحرارة للهرمون ثلاثي اليود T3 و الثيروكسين T4 و كانت القيم 152.72 ng/dl و 7.13 µg/dl على التوالي. وفى نفس الوقت سجل مستوى هرمون البروجستيرون في سيبرم الدم اختلافات عاليه المعنوية (P<0.01) وكانتُ اعلى قيمة مسجلةً لمجموعة المعاملة بالحرارة . وقد سجلت مستويات عالية من هرمون البروجستيرون عالية المعذوية في 8 أيام قبل التلقيح 🛛 و 8 أيام بعد التلقيح و 34 يوم بعد التلقيح ولم تسجل اختلافات في فترة الشياع بين المجموعات والكنترول في مستوى البروجستيرون لم تسجل اختلافات معنوية بين جميع المعاملات في مستوى بروتين الكلي بالدم والالبيومين والجلبيولين سجل مستوى جلوكوز الدم معتنوى مرتفع في مجموعة المعاملة بالحرارة عن باقي المعاملات ، سجلت اختلافات معنوية(P<0.01) بين معمتوي الجلوكوز في الدم في 8 أيام قبل التلقيح و 8 أيام بعد الثلقيح و عند الشياع و 34 بعد التلقيح بدرجة معنوية (P<0.01) . وقد سجل اعلى معتوى من الجلوكوز (71.69 mg/dl) عند الثنياع . سجلت مستويات اليوريا والكرياتنين في الدم مستويات منخفضة بدرجة عالية المعنوية (P<0.01) في المجموعات التي تم تغذيتها على علائق تحتوى على البروتين المحمى ، خارج فترة التزاوج الاختلافات غير معنوية بين المجموعات في تركيز اليوريا و الكرياتينين . ومن خلال هذه النتائج فقد أوضحت الدراسة إن التغذية على البروتين المحمى أثناء فترة التزاوج في الأغنام تحدث تغيرات في معظم مكونات مبيرم الدم في الأغنام السو هاجي .