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Effects of different levels of protein treated with formaldehyde on nutrients digestibility and some rumen and blood parameters in Awassi sheep

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Abstract

This study was conducted on 2 stages using 18 Awassi lambs with average body weight (45kg). Animals were divided into 3 groups of six lambs each. During the first stage, the groups of lambs were individually fed on one of the three rations, which consisted mainly of barley, wheat bran and soybean meal, and contain three graded levels of crude protein 14, 16 and 18% in addition to urea and wheat straw. During the second stage lambs were fed on the same ration after treating barely and wheat bran with formaldehyde. Lambs were fed for 10 days as preperiod and then five days for fecal collection, rumen liquor and blood samples. The result indicated that there was no significant effects of formaldehyde treatment under different levels of protein on digestibility coefficient of Dry matter (DDM), Organic matter (DOM), crude protein (DCP), Nutrient Detergent Fiber (NDF) and Acid Detergent Fiber (ADF). While formaldehyde treatments significantly ($P<0.05$) affect the digestibility of ether extract (DEE) which were ranged between 51.61- 33.39% for untreated rations vs. 73.90-76.62% for formaldehyde treated rations. The results, also indicated that formaldehyde treatments significantly ($P<0.01$) affect the rumen liquor pH which was between (5.63- 5.82) in group fed untreated rations vs. (5.87- 6.14) in group fed treated rations. Ammonia concentration in rumen liquor was significantly decreased in group fed formaldehyde treated rations. While the treatments showed no significant effect on microbial protein synthesis in the rumen. Statistical analysis of blood samples showed that formalin treatments had no significant effect on glucose, urea, total protein and globulin serum concentration, but there was a significant effect on triglyceride and albumin concentration.

Keywords: Nutrient Digestibility, Level of Protein, Formaldehyde Treatments.
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تأثير استخدام مستويات مختلفة من البروتين المعامل بالفورمالديهايد في معامل هضم المركبات الغذائية وبعض صفات الكرش والدم في الاغنام العواسية

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المقدمة

هذه الدراسة نفذت على مرحلتين باستخدام 18 حمل عواسي كان متوسط اوزانها (45 كغم)، قسمت الى ثلاثة مجاميع كل مجموعة تضم 6 حملان. خلال المرحلة الاولى، مجاميع الحملان غذيت بصورة فردية على احد العلائق الثلاث التي من الشعير، نخالة الحنطة، كسبة فول الصويا واحتوت على 14 ، 16 ، 18 % بروتين خام على التوالي. خلال المرحلة الثانية غذيت مجاميع الحملان على نفس العلائق بعد معاملة كل من الشعير ونخالة الحنطة بالفورمالديهايدز جميع الحملان غذيت لمدة 10 ايام فترة تمهيدية، ثم 3 ايام لجمع الروث واخذت عينات من سائل الكرش والدم. اوضحت النتائج ان المعاملة بالفورمالديهايد وضمن النسب المختلفة من البروتين ليس

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لها تاثير معنوي على كل من معامل هضم المادة والمادة العضوية والبروتين الخام والياف الغسل المتعادل. بينما كان للمعاملة بالفورمالديهايد تاثير معنوي (أ < 0.05) في معامل هضم مستخلص الايثر الذي تراوحت قيمة بين (51.61 ، 55.39 %) في العلائق غير المعاملة مقابل (76.62 ، 73.90 %) في العلائق التي عوملت بالفورماليهايد. اوضحت النتائج كذلك ان المعاملة بالفورماليدهايد كان لها تأثير معنوي (أ < 0.01) في درجة الـــ pH لسائل الكرش حيث مابين (5.63 – 5.82) في الحيوانات التي غذيت على العلائق غير المعاملة مقابل (5.87 – 6.14) في الحيوانات التي غذيت على العلائق المعاملة بالفورمالديهايد. هذا ولم يكن للمعاملة بالفورمالديهايد أي تأثير معنوي في بناء البروتين الميكروبي في الكرش. التحليل الاحصائي لعينات الدم اوضح ان المعاملة بالفورمالديهايد لم يكن لها تأثير معنوي في تركيز كل من الكلوكوز، اليوريا، البروتين الكلي والكلوبين ولكن لها تأثير معنوي في تركيز الترايكليسيراد و الالبومين.

Introduction

For optimal performance in high-production ruminants, enough soluble redily hydrolise protein should be available to support microbial growth, plus a source of less fermentable protein, which can pass directly to abomasums and small intestine for normal digestion and absorption process. (1). However, to meet the protein demands of high producing ruminants, the flow of microbial protein should be maximized prior to supplementing the bypass protein. On the other hand, the efficiency of protein utilization by rumen microbes involves a strong interaction between the carbohydrate and protein fraction in the diet. The rate of carbohydrate digestion in the rumen is the major factor controlling the energy available for microbial growth, and an adequate supply of nitrogenous sources in the rumen will increase microbial growth. Therefore the substitution of rumen undegradeble protein (RUP) for rumen degradable protein (RDP) can reduce the microbial protein entering the host of intestine (2). However, there are many studies investigate the effect of suppling RUP on performance of ruminants (3,4), but there is a few information about the effect of the ratio between RDP and RUP on nutrients digestibility. The aim of this study was to investigate the influence of the ratio between RDP and RUP and its interaction with carbohydrate digestion on nutrients digestibility on sheep.

Materials and Methods

This study was conducted on two stages using 18 awassi lambs with average body weight (45kg). Animals were divided into 3 groups of six lambs each. They were held in individual pens and allocated to dietary treatments according to complete random design (CRD).

During the first stage, the groups of lambs were individually fed on one of the three rations, which consist mainly of barley, wheat bran and soybean meal, in addition to urea and wheat straw, which contain either 14, 16 or 18% crude protein (table 1). Experimental rations were offered at 8.00 and 16.00 hrs. Samples of rations were weekly taken for chemical analysis.

During the second stage, lambs were fed on the same rations after treating barley and wheat bran with formaldehyde (6L formalin HcHo 37% and 3L acetic acid Ch3COOH/ ton barley or wheat bran).

During the first and second stages, lambs were fed for 15 days experimental periods, the first 10 days was regarded as adaptation period and within the last 5d, feces were collected, weighted and sub-samples were obtained, bulked and dried in oven for later chemical analysis.

Rumen liquor samples were obtained on the day 15 before and 4 hrs. after feeding using 10 cm stainless steel tube fixed to plastic tube which was insert through the esophagus under vacuum. Rumen liquor pH was measured immediately after sampling using portable pH meter. Then the rumen liquor samples were strained through 4 layers of muslin. 5 ml of rumen liquor was diluted with 45ml tungastic acid and kept in plastic bottles under- 20°c for ammonia determination. Blood samples (10 ml) from jugular vein were obtained on day 15 2hrs after feeding. The samples were centrifuged for 10 min. (3000r/min.) for serum separation and kept in glass tubes under -20°c for chemical analysis.

Feed and feces samples were chemically analyzed for dry matter (DM), organic matter (OM), crude protein (CP) and ether extract (EE) according (5), and acid detergent fiber (ADF) and nutral detergent fiber (NDF) (6). Metabolisable energy was calculated according to (7).

Ammonia concentration in rumen liquor (RL) samples were determined according the method used by (8). Blood samples were analyzed for glucose, urea, total protein, albumin and triglyceride by using standard kits supplied by syrbio company. Globulin was calculated by difference between total protein and albumin. Microbial protein was calculated according to the equation given by (9). Statistical analysis: date were analyzed using (CRD) in two factors, formaldehyde treatment (i) and protein levels (j) according to the following model: 1/ijk= the value of observation that affected by formaldehyde treatment (i) and protein level (j) in the replicate (k). μ= all over mean that samples were taken, Ti = effect of formaldehyde treatment, Pj = effect of protein levels, eijk= the value for the experimental error. Test of significant differences between the means was done

using multiple range Duncan test (10) using statistical program SAS (11).

Table (1) Ingredients total mixed rations and their chemical composition %.

Ingredients	Ration (1)*	Ration (2)	Ration (3)
Ground barley	50	50	50
Wheat bran	33.25	32.40	31.20
Soya bean meal	7.25	8.40	10
Urea	0.5	0.95	1.30
Wheat straw	8.5	7.75	7
Nacl	0.5	0.5	0.5
Chemical Composition %			
Dry matter	91.85	91.91	91.87
Organic matter	94.75	94.82	94.61
Crude protein	13.82	15.98	17.93
Ether extract	4.75	4.73	4.59
Natural detergent fiber	33.11	31.38	30.88
Acid detergent fiber	12.63	12.65	13.46
Metabolized energy µcal/kg	2.412	2.410	2.412
RUDP :RDP in untreated ration	24: 76	21.5 : 78.5	20.80
RUDP : RDP in treated ration	63: 37	58: 42	55: 45

* all the relations used in the second stage of the experiment have the same ingredients after treating barley and wheat bran with formaldehyde.

Results and discussion

The feed ingredients and their chemical composition are shown in table (1). It is clear that the ratio between rumen degradable protein (RDP) and rumen undegradable protein (RUDP) are varied between formaldehyde treated ration (FTR) which was (55.45- 63.37) and untreated ration (UTR) (20.80- 24.76b).

Results present in table (2) indicated that the levels of protein and FTR had no significant effect on digestibility coefficient of DM which was ranged between (74.12-77.69%), OM (75.15- 78.21%) ADF (36.68- 49.14) and NDF (51.26- 58.66%). These results are in agreement with those found by (12) and (13). In contrast, (14) reported that FTR had significant effect on fiber digestion which is varied with the results in our study. The reasons of these variations are not clear, but, it may be due to the variation in feed ingredients and formaldehyde concentration that used in these two studies, which may cause a variation in protein and energy degradation in the rumen which may effect the rumen environment and then microorganism activity. Crud protein digestibility was not significantly affected by treatment within each levels of protein, but it is noted that the digestibility of CP in UTR contain 14% cp was significantly ($P<0.05$) decreased as compared with the other treatments (FTR 16%cp and UTR and FTR 18% cp) (table 2). On other hand, ether extract digestibility was significantly ($P<0.01$) improved by FTR, but not by the levels of protein.

Statistical analysis of the data according to the levels of protein only (table 3) showed that CP and ADF digestibility were significantly ($P<0.01$ and $P<0.05$ respectively) improved by increasing the levels of protein in the rations, however there was a numerical improvement in DM, OM, EE and NDF digestibility but not statistically associated with increasing the levels of protein in the rations. Similar results on sheep were observed by (15) and on cattle by (16).

Table (2) Effects of protein level and formaldehyde treatment on nutrient digestibility %.

Treatments*		Dry matter	Organic matter	Crude protein	Ether extract	Acid detergent fiber	Neutral detergent fiber
14% crude protein	UTR	74.85±2.49	75.61±2.44	68.55±3.25 b•	55.39± 3.75 b	38.99±1.62	53.63±4.12
	FTR	74.12±1.26	75.15±1.30	72.06±1.92 ab	73.90±2.91 a	36.68± 1.82	51.26±2.10
16% crude protein	UTR	77.69±1.24	78.17 ±1.32	74.06±1.19 ab	56.67± 2.18 b	46.29± 5.90	58.67±3.39
	FTR	76.67±0.85	78.21±0.99	75.38±1.14 a	79.04±1.21 a	42.91± 4.37	56.72±2.16
18% crude protein	UTR	76.92±1.57	77.26±1.66	78.30±2.08 a	51.61±4.62b	49.14±5.28	56.91±4.09
	FTR	77.69±0.62	78.21±1.02	76.15± 0.83 a	79.04±2.05 a	45.91±3.54	58.66±2.24

* Similar letters mean no significant differences between treatments. UTR: Untreated ration, FTR: formaldehyde treated ration. •Significant at ($P< 0.05$)

Rumen liquor pH was not affected by either the level of protein or FTR before feeding, while there were a significant ($P<0.01$) differences between treated and untreated rations 4 hrs after feeding (Table 4), however, statistical analysis of the data according to the level of protein only indicated that both the levels of protein and

time of sampling were significantly alter the rumen liquor pH values (table 5). These results were expected, since, FTR may cause a depression in starch fermentation in the rumen then decreased volatile fatty acids formation which are highly correlated with rumen liquor pH (17).

Ammonia concentration in the rumen was highly (P<0.01) affected by FTR before feeding, but not at 4 hrs after feeding except in group of lambs that fed on ration contain 18% protein (table 4). However, statistical analysis of the data according to level of protein showed that levels of protein were significantly (P<0.05) affected ammonia concentration before and 4 hrs after feeding (P<0.01). These results were expected since there were high proportion of RDP (76-80% of total protein) in untreated rations as compared with FT rations which contain about 55-63% RUDP (table1). These results were in agreement with those mentioned by (18,19).

Microbial protein synthesis was not affected by treatments and it was ranged from (93.59- 109.45 g/d) (table 4) usually microbial protein synthesis is highly correlated with OM digestibility. The calculation of microbial protein synthesis in this study was according to OM digestibility which was not significantly differs between treatments (table 2), so this may lead to similar quantity of microbial protein synthesis in this study.

The biochemical analysis of blood samples showed that, both the level of protein and FTR had no significant on blood glucose, total protein, globulin and urea concentrations (table 6). These results were similar to those mentioned by (4, 19 and 21). On the other hand, blood triglyceride and albumin were significantly (P<0.01) increased in FTR as compared with untreated rations (table 6). These results were in agreement with those found by (4 and 20). The reasons for these improvement in triglyceride concentration may be in part due to the protection of fat front microbial degradation which may lead to increase rumen fat bypass which hydrolyzed and absorbed in the intestine (22).

Table (3) Effects of protein level on nutrient digestibility %.

Protein Level *	DM	OM	EE	CP	ADF	NDF
14%	74.49±1.34	75.38±1.31	64.64±3.59	70.31±1.87 b••	37.83±1.21 b•	52.95±2.20
16%	77.18±0.67	78.19±0.78	67.86±3.57	74.72±0.81 a	44.60±3.53 ab	57.69±1.94
18%	76.79±0.80	77.41±0.93	64.11±4.47	76.23±1.07 a	47.53±3.06 a	56.57±2.23

* Similar letters mean no significant difference between treatments, • Significant at (p<0.05) •• Significant at (p< 0.01)

Table (4) Effects of protein level and formaldehyde treatment on some rumen parameters.

Protein level	Treatment *	pH of rumen liquor		Ammonia concentration in rumen liquor		Microbial protein synthesis g/d
		Before feeding	4hrs. After feeding	Before feeding	4hrs. After feeding	
14%	UTR	6.46±0.08	6.14±0.07 a	4.29±0.36 b	10.41± 0.89 a	109.03± 2.77
	FTR	6.42±0.07	5.82±0.04 b•	9.23±0.54 a	11.33±0.50 a	109.45±2.99
16%	UTR	6.35±0.08	6.01± 0.08 a	5.05± 0.88 b	11.92± 1.09 a	108.82± 2.82
	FTR	6.24±0.07	0.63±0.08 b	11.62±1.18 a	13.97±1.17 a	102.90±6.22
18%	UTR	6.92±0.05	5.87± 0.07 a	14.69± 0.72 a	14.69±1.14 b	100.82± 4.70
	FTR	6.19±0.05	5.64±0.07 b	18.59±0.72 a	18.59±1.14 a	93.59±4.69

* Similar letters mean no significant difference between treatments, • Significant at (P<0.01).

Table (5) Effects of protein level on some rumen parameters.

Protein level*	pH of rumen liquor		Ammonia concentration in rumen liquor		Microbial protein synthesis g/d
	Before feeding	4hrs. After feeding	Before feeding	4hrs. After feeding	
14%	6.44±0.05 a-c	5.98±0.06 a	6.76±0.80 b•	10.87±0.51 b••	109.24±0.94 a
16%	6.29±0.05 b	5.82±0.08 ab	8.33± 1.21 ab	12.94±0.82 b	105.86±3.37 a
18%	6.24±0.03 b	5.76±0.06 b	10.79± 1.34 a	16.64±0.86 a	97.20±2.81 b

* Similar letters mean no significant difference between treatments, • Significant at (P<0.05) •• Significant at (P< 0.01).

Table (6) Effects of protein level and formaldehyde treatment on blood biochemical analysis.

Treatments*		Glucose Mg/100ml	Triglyceride Mg/100ml	Urea Mg/100ml	Total protein g/100ml	Albumin g/100ml	Globulin g/100ml
14% CP	UTR	57.84±3.10	21.45±2.37 b•	47.21±2.25	6.74±0.54 a	3.36±0.07 b	3.38±0.59
	FTR	63.33±4.23	31.56±3.28 a	50.53±2.17	6.54±0.02 a	4.03±0.08 a	2.34±0.29
16% CP	UTR	56.40±3.63	21.88±1.28 b	48.82±2.78	7.03±0.57 a	3.17±0.11 b	3.87±0.56
	FTR	55.16±2.90	32.45±2.95 a	47.08±2.97	7.19±0.49 a	4.07±0.09 a	2.95±0.53
18% CP	UTR	67.91±5.30	25.65±1.55 b	48.68±3.39	6.62±0.49 a	3.11±0.19 b	3.34±0.48
	FTR	55.25±2.42	35.10± 2.64 a	51.19±3.37	6.85±0.55 a	3.90±0.12 a	2.95±0.48

* Similar letters mean no significant differences between treatments, • Significant at (P< 0.01).

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