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# تأثير بروتين الغذاء على الاستفادة من النيتروجين في الأغنام

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## الملخص العربي

أجريت تجربه لدراسة تأثير مصدر ومستوى بروتين الغذاء على ثمانية حملان رحمانى وأختير لاجراء هذه التجربة تصميمى مربع لاتينى (٤×٤) حيث تم من خلال هذه التجربة دراسة مصدرين مختلفين من البروتين (كسب فول الصويا وكسب القطن غير المقشور) مع مستوين مختلفين من البروتين ، منخفض (١٠% بروتين خام) وعالى (١٤% بروتين خام) وكانت الاربع علائق متساويه تقريبا فى محتواها من الطاقه. أدى ارتفاع مستوى البروتين من ١٠% الى ١٤% الى تحسن معاملات الهضم معنويا لكل العناصر الغذائيه فيما عدا الكربوهيدرات الذائبه بينما كان لمصدر البروتين تاثيرا معنويا فقط على كلا من الماده الجافه والماده العضويه والبروتين الخام والدهن الخام وكانت العليقه الثانيه (كسب فول الصويا ١٤% بروتين) ذات القيم الأعلى لمعاملات الهضم لمعظم العناصر خاصة البروتين الخام. أدى ذلك إلى زيادة القيمة الغذائية لهذه العليقة بصورة معنوية عن باقى العلائق. سجلت العليقه الثانيه (كسب فول الصويا ١٤% بروتين) اعلى ميزان نيتروجين وكانت الفروق معنويه عن باقى علائق التجربه. تفوقت العليقه الثانيه فى انتاج الأحماض الدهنية الطيارة والأمونيا فى سائل كرش الأغنام عند معظم الفترات الزمنيه عن باقى العلائق فى حين لم يكن لمصدر البروتين أى تأثير معنوى. انخفض حامض الخليك ونسبته الى البروبيونيك معنويا بزيادة مستوى البرتين ومصدر البروتين وزادت أحماض البروبيونك والبيوتريك معنويا بزيادة مستوى البروتين ولم تتأثير معنوبا بمصدر البروتين وكانت العليقه الثانيه (كسب فول الصويا ١٤%بروتين ) ذات القيم الاعلى لحامض البربيونيك. أرتفع كل من تركيز البروتين الكلى والالبيومين والجلوبيولين معنويا فى بلازما الدم لحيوانات العليقه الثانية نتيجة ارتفاع مستوى البروتين بينما كان تأثير مصدر البروتين غير معنوى فى معظم الاوقات وكانت الفروق معنوية لصالح العليقه الثانية. لم يتأثر نشاط انزيمى ALTو AST بدرجة معنوية فى سيرم الاغنام التى تغذت على العلائق المختلفه فى مصادر ومستويات البروتين. لم يتأثر تركيز الكرياتينين فى الدم معنويا بمصدر ومستوى البروتين بالعلائق المختلفه وتراوحت قيمته من ١.٠٢–١.٢٥مجم. كان تأثير مصدر البروتين معنويا على تركيز يوريا الدم حتى ساعتين بعد الأكل فى حين امتد التأثير المعنوى لمستوى البروتين الى أربع ساعات بعد الأكل. حيث سجلت العليقه الثانيه (كسب فول الصويا ١٤%بروتين) أعلى قيما ليوريا الدم عند ساعتين بعد الأكل وبفارق معنوي عن باقى علائق التجربه.

# EFFECT OF DIETARY PROTEIN ON NITROGEN UTILIZATION IN SHEEP

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**ABSTRACT:** *An experiment was conducted to study the effect of protein source and level on nitrogen metabolism using eight Rahmani rams (average live body weight of 30 kg) in two 4×4 Latin square design and fed four rations i.e., ration (1) soybean meal (SBM) 10% CP, ration (2) SBM 14% CP, ration (3) cotton seed meal (CSM) 10% CP and ration (4) CSM 14% CP. The results revealed that increasing protein level from 10% to 14% improved all nutrients digestibility. Protein source has significant effect on DM, OM, CP and EE digestibility. Nutritive value (TDN and DCP) was improved for ration 2 (SBM 14% CP). Nitrogen balance was significantly higher with ration 2 (11.46 g/d) than the other three rations. VFA production and ammonia-N in the rumen of sheep fed ration 2 was significantly higher than the other rations as affected by protein level. Sheep fed ration 2 had significantly higher level of plasma total protein, albumin and globulin than other rations as affected by protein level. The concentration of AST and ALT were insignificantly affected by protein source and level. The concentration of urea in blood serum of sheep on ration 2 at 2- hrs post feeding (32.15 mg/dl) was significantly (P<0.01) higher than other rations being, 28.35, 26.55 and 29.75 mg/dl, respectively.*

***Key words:*** *dietary protein, digestibility, nitrogen metabolism, microbial activity*, sheep.

## INTRODUCTION

As reported by many investigators (Oliveira Junior *et al*, 2006; Kiran and Krishnamoorthy, 2007; Chandrasekharaiah, *et al*, 2010 and Tatsapong *et al*, 2010) different factors are affecting protein metabolism in ruminants. They studied the differences in protein degradability among feeds. The composition of feed protein has been suggested to be the main reason for the differences in the protein degradation among the feeds. They demonstrated that the N solubility in buffer and detergent solutions and fermentation kinetics may be an indicator of variability in the degradation of protein and energy. The dietary protein need to be provided to the rumen in the proper source and level to improve microbial efficiency and growth performance. Any imbalances in this system will lead to negative effects on digestibility, growth and animal health due to over-production of short-chain fatty acids and $NH_3$. It is recommended for ruminants to formulate diets balanced in both protein and energy needed for microbial growth based on estimated microbial efficiency and requirements. Lapierre and Lobley (2001) indicated that the form of N in the diet, particularly ruminaly-degraded-protein (RDP) and/or protein solubility are important and determine how much of the dietary protein is directed towards ruminal $NH_3$-N.

The present study was carried out in order to investigate the effect of dietary protein source (soybean meal and cotton seed meal) and protein level (low level, 10% and high level, 14% CP) on nutrients digestibility, nutritive values, nitrogen balance, ruminal microbial activity. The effects on plasma total protein and protein fractions, as well as renal and hepatic functions were evaluated.

## MATERIALS AND METHODS

This study was conducted at the Experimental Station and Nutrition Laboratory, Animal Production Department, Faculty of Agriculture, Minoufiya University (Shebin El-Kom). Eight healthy Rahmani lambs (with average live body weight of 30 kg) were used in two 4x4 Latin square designs. The animals were divided into four

comparable groups according to their live body weight, each group contained two animals. Daily feed allowance was changed quantitatively according to the change in body weight. Each lamb was placed in a separate metabolism cage as described by Maynard *et al*. (1979) for three weeks as a preliminary period followed by one week as a collection period. Feed was offered twice daily at 8:00 and 16.00 hr. Fresh water was available at all times.

Feed was sampled daily during the collection period and subsequently composited into 1-kg samples and preserved in sealed plastic bags kept at room temperature until analysis. Feces were collected at 8:00 hr in the morning. Samples were weighed and a 10% subsample was dried in the forced air oven at 60-65 °C until constant weight was reached. Samples were kept for laboratory analysis. Urine was collected daily; the urine volume was measured and a 10% subsample was stored in plastic containers and frozen for later analysis. Fifty milliliters of diluted sulfuric acid (10%) was added daily to urine collection buckets to prevent ammonia volatilization.

Sheep were fed to meet their requirements of DM according to (NRC, 1985). Four iso-caloric rations were prepared using two sources of protein (Soybean meal and Cottonseed meal; both solvent extracted) with two levels of dietary protein (lower and higher than NRC, 1985) and interaction between them as shown in Table (1) to investigate their effects on nitrogen metabolism in sheep. Experimental rations were R1, low level (10%CP) of SBM; R2, high level (14% CP) of SBM; R3, low level (10% of CSM); R4, high level (14% of CSM).

Samples of rumen fluid were obtained at 0, 2, 4 and 6 hrs after feeding for each metabolism trial. The rumen samples were collected using rubber stomach tube inserted into the rumen via the esophagus. The fluid was strained through 4 layers of cheese cloth and homogenized and *p*H was immediately determined using the *p*H meter (Jenway *p*H meter 3510). Then a preservative was added to keep ammonia nitrogen unchanged and stored in deep freezer at (-20$^{o}$C) until chemically analyzed. Free ammonia-N in the rumen samples was determined by the Van Slyke method as described by Ahmed (1976). After acidification of rumen liquor samples using concentrated ortho-phosphoric acid and 0.1N hydrochloric acid, total volatile fatty acids (VFA) were determined in the rumen fluids according to AOAC (2000). The proportions of VFA were determined by HPLC according to Supelco Inc., 1975).

**Table (1): The formulation of the experimental rations and their calculated feeding value.**

| Ingredients | Soybean meal | | Cottonseed meal | |
|---|---|---|---|---|
| | 10% | 14% | 10% | 14% |
| | ----------%---------- | | | |
| Barley | 19 | 29 | 28 | 3 |
| Yellow corn | 28 | 8 | 19 | 27 |
| Soybean meal | 5 | 15 | ---- | ---- |
| Cottonseed meal | ---- | ---- | 5 | 32 |
| Clover hay (CH) | 26 | 32 | 26 | 31 |
| Rice straw | 14 | 8 | 14 | 2 |
| Wheat bran | 5 | 5 | 5 | 2 |
| Limestone | 1.0 | 1.0 | 1.0 | 1.0 |
| NaCl | 0.5 | 0.5 | 0.5 | 0.5 |
| Min. &Vit Mix | 1.5 | 1.5 | 1.5 | 1.5 |
| Crude protein | 10.08 | 14.06 | 10.19 | 13.92 |
| TDN | 60.88 | 60.66 | 60.43 | 59.87 |

The chemical composition of rations and feces were carried out according to AOAC (2000).The proximate analysis of the experimental rations is presented in Table (2). At the end of the experimental period, blood samples were collected in dried clean heparinized tubes by jugular vein puncture from all rams and immediately centrifuged at 4000 rpm for 15 minutes. Plasma samples were frozen at -20ºC for determination of plasma parameters.

Total plasma protein was determined spectro-photo-metrically at wave length 546 nm according to Babolok *et al.* (1988). Albumin concentration according to Doumas *et al*. (1971) and globulin concentration was calculated by the difference between total protein and albumin concentrations. Albumin / globulin ratio was also calculated. Plasma urea was determined using the colorimetric methods described by Tietz (1990) using commercial kits. Creatinine was measured using the colorimetric method at wavelength 550 nm, according to Young (1995). Liver function was assessed by measuring the activities of aspartate transaminase, AST and alanine transaminase, ALT spectro-photo-metrically at wave length 365 nm according to Reitman and Frankel (1957) using commercial Kites.

The obtained results were statistically analyzed according to SPSS (Statistical Package for Social Science) program version 19, (2010). Differences among means were evaluated using Duncan's Multiple Range Test.

**Model:-** $Y_{ijk} = \mu + T_i + P_j + TP_{ij} + e_{ijk}$

**where:** $Y_{ijk}$ = the parameters under analysis, μ= overall mean, $T_i$ = the fixed effect of the $i^{th}$ protein source, (i = 1, 2), $P_j$ = the fixed effect of the $i^{th}$ protein level, (i = 1, 2), $TP_{ij}$ = the interaction between source and level, $e_{ijk}$ = Random error assumed to be independent normally distributed mean and variance.

## RESULTS AND DISCUSSION

Results of the digestion coefficient as affected by dietary CP sources and levels are presented in Table (3). Digestibility of almost all nutrients (DM, OM, CP, EE and CF) increased as dietary CP level increased. Differences were significant (P<0.01). It was interesting to note that Ration 2 (SBM 14% CP) and ration 4 (CSM 14% CP) showed highly significant (P<0.01) effect on the digestion coefficient than the other studied rations. SBM significantly improved the digestion coefficient of all nutrients. These results are in agreement with the results obtained by others (Holter *et al*., 1982; Olmos Colmnero and Broderick, 2006; Broderick *et al*., 2009).

**Table 2: Chemical composition of the experimental rations.**

| Item | Soybean meal | | | | Cottonseed meal | | | |
|---|---|---|---|---|---|---|---|---|
| | LLP | | HLP | | LLP | | HLP | |
| | As Fed | DM Basis | As Fed | DM Basis | As Fed | DM Basis | As Fed | DM Basis |
| | ----------%---------- | | | | | | | |
| DM | 89.44 | 100 | 90.35 | 100 | 89.18 | 100 | 90.55 | 100 |
| OM | 81.07 | 90.63 | 80.32 | 88.91 | 80.51 | 90.28 | 80.80 | 89.23 |
| CP | 9.02 | 10.08 | 12.70 | 14.06 | 9.09 | 10.19 | 12.60 | 13.92 |
| EE | 2.49 | 2.78 | 2.42 | 2.68 | 3.34 | 3.75 | 3.33 | 3.68 |
| NFE | 53.53 | 59.85 | 53.08 | 58.75 | 50.62 | 56.76 | 48.58 | 53.65 |
| CF | 16.03 | 17.92 | 12.12 | 13.42 | 17.46 | 19.58 | 16.28 | 17.98 |
| Ash | 8.38 | 9.37 | 10.03 | 11.09 | 8.67 | 9.72 | 9.75 | 10.77 |

*DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NFE, nitrogen-free extract; CF, crude fiber.

Generally digestion of DM, OM, CP, EE and CF were relatively higher for rams fed higher dietary CP levels as compared with those fed lower dietary CP levels. Protein source (SBM) had significant effect on DM, OM, CP and EE. The interaction between protein source and level only had significant effect on DM and CP digestibility. The highest digestion obtained for high protein diets may have been due to the higher content of protein and lower fiber content. Huber and Kung (1981) explained such differences on the basis that when nitrogen needs of rumen microorganisms are not met because of low dietary protein, digestibility of fiber and other fractions is reduced. In this concern, Oldham (1984) indicated that increasing the amount of CP in the diets fed to dairy cows would increase the ruminal digestibility of the diet. This increase in ruminal digestibility could be the result of an increased availability of $NH_3$, amino acids or peptides for growth of the ruminal microbes. Moreover, Olmos Colmenero and Broderick (2006) showed that the linear increases in the concentration of both isobutyrate and isovalerate might have contributed to the linear responses in ruminal fiber digestion when dietary CP increased from 13.5 to 16.5%.

It is well known that digestibility of nutrients is considered as one of the basic measurement to determine feeding value. Data in Table (3) show that protein source and level significant increased (P<0.01) the values of TDN and DCP; the preference in TDN was to ration 2 (SBM 14%CP) and ration 4 (CSM 14%CP) followed by ration 1 (SBM 10%CP); but ration 2 showed higher (P<0.01) DCP for protein source and at (P<0.05) for protein level; however, TDN did not differ significantly between rations 1, 2 and 4 being 63.91, 64.54 and 63.71%, respectively.

**Table (3): Effect of dietary protein source and level on nutrients digestibility and nutritive value of the experimental rations**

| Item | Soybean meal | | Cottonseed meal | | Sig | | |
|---|---|---|---|---|---|---|---|
| | 10% | 14% | 10% | 14% | S | L | I |
| DM | 68.32 $^{a}$ ±0.35 | 69.16 $^{a}$ ±0.52 | 65.16 $^{b}$ ±0.33 | 69.36 $^{a}$ ±0.58 | 0.01 | 0.01 | 0.01 |
| OM | 68.13 $^{b}$ ±0.28 | 69.92 $^{a}$ ±0.82 | 65.77 $^{c}$ ±0.57 | 67.9 $^{b}$ ±0.47 | 0.01 | 0.01 | NS |
| CP | 61.56 $^{d}$ ±0.46 | 69.94 $^{a}$ ±0.57 | 63.2 $^{c}$ ±0.39 | 66.18 $^{b}$ ±0.45 | 0.05 | 0.01 | 0.01 |
| EE | 61.00 $^{b}$ ±0.71 | 69.03 $^{a}$ ±0.39 | 58.15 $^{c}$ ±0.63 | 67.47 $^{a}$ ±0.58 | 0.01 | 0.01 | NS |
| NFE | 73.56 $^{a}$ ±0.34 | 72.75 $^{ab}$ ±1.04 | 70.68 $^{b}$ ±0.85 | 72.2 $^{ab}$ ±1.02 | NS | NS | NS |
| CF | 55.04 $^{b}$ ±0.95 | 58.16 $^{a}$ ±0.82 | 54.35 $^{b}$ ±1.03 | 56.56 $^{ab}$ ±1.05 | NS | 0.01 | NS |
| TDN% | 63.91 $^{a}$ ±0.24 | 64.54 $^{a}$ ±0.65 | 62.11 $^{b}$ ±0.50 | 63.71 $^{a}$ ±0.41 | 0.01 | 0.01 | 0.01 |
| DCP% | 6.21 $^{d}$ ±0.05 | 9.83 $^{a}$ ±0.08 | 6.44 $^{c}$ ±0.04 | 9.21 $^{b}$ ±0.06 | 0.01 | 0.05 | NS |

$^{a,b,c,}$ Values having different superscript within each raw are significantly different.*S, source; L, level; I, interaction; LLP, low level of protein; HLP, high level of protein; NS, not significant; DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NFE, nitrogen-free extract; CF, crude fiber.

Data on N-balance are presented in Table (4). Nitrogen intakes were higher with higher protein levels (33.38 and 32.93g/d) than that of the lower protein levels (23.69 and 23.88g/d). The higher N intake was due to the higher CP content of the high CP ration along with higher CP digestibility. Sheep given SBM 14% CP and CSM 14%CP diets secreted more (P<0.01) N in feces and in urine. It was obvious that all sheep fed on the four tested experimental rations had positive nitrogen balances. Protein source, level and interaction between them showed significant effect on nitrogen balance. Ration 2 (SBM 14% CP) had the highest NB value followed by ration 4 (CSM 14% CP) (11.46g/d and 9.36g/d, respectively). Ration 1 (SBM 10% CP) did not differ significantly than ration 3 (CSM 14% CP). It could be observed that the dietary nitrogen intake was slightly lower by animals fed low levels protein rations as compared with those fed high levels protein rations. Differences among protein sources in the N- intake of the four rations were not significant. Nitrogen excreted in feces was 9.12, 10.41, 8.79 and 11.11 g/d for SBM 10%CP, SBM 14%CP, CSM 10%CP and CSM 14%CP, respectively. The differences due to source of protein were not significant (Table 4). Nitrogen excreted in urine was higher in lambs fed high levels protein rations (SBM 14%CP and CSM 14%CP) being 11.51 and 12.46 g/d, respectively as compared with those fed low levels protein rations (SBM 10%CP and CSM 10%CP) being 9.69 and 10.94 g/d, respectively. Protein level had significant effect on nitrogen intake, fecal nitrogen, urinary nitrogen and nitrogen balance. Ration 2 (SBM 14% CP) showed highly significant (P<0.01) effect on nitrogen balance (11.46 g/d) than the rations 1,3 and 4 being, 4.89, 4.15 and 9.36 g/d, respectively. The statistical analysis indicated that protein source had no significant effect on nitrogen intake, fecal nitrogen and urinary nitrogen. Significant differences in nitrogen balance values for nitrogen source may be due to differences in amino acid composition of protein sources and its digestibility. Harvey (1970) indicated that the higher percentage of dietary nitrogen retained with SFM supplemented diets compared with SBM diets may be due to differences in the Methionine content of the two protein supplements.

**Table (4): Effect of dietary protein source and level on nitrogen balance (g/d)**

| Sampling time (hr) | Soybean meal | | Cottonseed meal | | Sig | | |
|---|---|---|---|---|---|---|---|
| | 10% | 14% | 10% | 14% | S | L | I |
| NI, g/d | 23.69 | 33.38 | 23.88 | 32.93 | NS | 0.01 | NS |
| FN, g/d | 9.12 $^{bc}$ ±0.36 | 10.41 $^{ab}$ ±0.75 | 8.79 $^{c}$ ±0.32 | 11.11 $^{a}$ ±0.31 | NS | 0.01 | NS |
| UN, g/d | 9.69 $^{b}$ ±0.39 | 11.51 $^{ab}$ ±0.79 | 10.94 $^{ab}$ ±0.55 | 12.46 $^{a}$ ±0.99 | NS | 0.05 | NS |
| NB, g/d | 4.89 $^{c}$ ±0.21 | 11.46 $^{a}$ ±0.42 | 4.15 $^{c}$ ±0.10 | 9.36 $^{b}$ ±0.31 | 0.01 | 0.01 | 0.05 |

*a,b,c Values having different superscript within each raw are significantly different S, source; L, level; I, interaction; LLP, low level of protein; HLP, high level of protein; NS, not significant

As shown in Table (5) ruminal *p*H values were lower for SBM 14%CP and CSM 14%CP groups before morning feeding, being 7.03 and 6.99, respectively compared with SBM 10%CP and CSM 10%CP being 7.31and 7.04, respectively. Rumen pH decreased, after that, steadily to reach their lowest values at 2hr post morning feeding for SBM 10%CP and CSM 10%CP groups and reach their lowest values at 4hr post morning feeding for SBM 14%CP and CSM 14%CP, then steadily increased for all groups. The differences due to source of protein and interaction between protein source and level were not significant at all times, but protein level had significant effect on rumen pH at 4, 6 hours post feeding. These results agree will with the results obtained by EL-shakankery (2004) who concluded that the pH value of rumen contents from sheep fed SFM, CSM, SBM was not significant as affected by protein source.

**Table (5): Effect of dietary protein source and level on rumen activity.**

| Sampling time (hr) | Soybean meal | | Cottonseed meal | | Sig | | |
|---|---|---|---|---|---|---|---|
| | 10% | 14% | 10% | 14% | S | L | I |
| Rumen *p*H | | | | | | | |
| 0 | 7.31 ±0.13 | 7.03 ±0.21 | 7.04 ±0.15 | 6.99 ±0.29 | NS | NS | NS |
| 2 | 6.21 ±0.27 | 6.24 ±0.32 | 6.15 ±0.35 | 5.85 ±0.20 | NS | NS | NS |
| 4 | 6.29 $^{a}$ ±0.61 | 6.18 $^{ab}$ ±0.88 | 6.25 $^{ab}$ ±0.74 | 6.05 $^{b}$ ±0.27 | NS | 0.05 | NS |
| 6 | 6.49$^{a}$ ±0.02 | 6.34$^{c}$ ±0.01 | 6.45$^{b}$ ±0.01 | 6.34$^{c}$ ±0.01 | NS | 0.01 | NS |
| Ammonia-N (mg/100ml) | | | | | | | |
| 0 | 27.15 ±0.83 | 28.55 ±0.84 | 27.78 ±0.75 | 28.35 ±0.60 | NS | NS | NS |
| 2 | 30.55$^{b}$ ±0.83 | 44.45$^{a}$ ±0.76 | 29.98$^{b}$ ±0.79 | 44.05$^{a}$ ±0.73 | NS | 0.01 | NS |
| 4 | 27.05$^{b}$ ±0.70 | 42.98$^{a}$ ±0.67 | 26.75$^{b}$ ±0.35 | 41.20$^{a}$ ±0.91 | NS | 0.01 | NS |
| 6 | 26.00$^{b}$ ±0.60 | 38.75$^{a}$ ±0.77 | 25.55$^{b}$ ±0.73 | 36.99$^{a}$ ±0.88 | NS | 0.01 | NS |
| Total VFA concentration (meq/100ml) | | | | | | | |
| 0 | 8.75 | 9.25 | 9.45 | 9.00 | NS | NS | NS |
| 2 | 12.26$^{b}$ ±0.27 | 17.15$^{a}$ ±0.36 | 12.05$^{b}$ ±0.40 | 16.45$^{a}$ ±0.36 | NS | 0.01 | NS |
| 4 | 12.35$^{b}$ ±0.30 | 16.85$^{a}$ ±0.25 | 12.12$^{b}$ ±0.33 | 16.31$^{a}$ ±0.25 | NS | 0.01 | NS |
| 6 | 11.98$^{ab}$ ±0.27 | 12.68$^{a}$ ±0.36 | 10.92$^{b}$ ±0.35 | 12.54$^{a}$ ±0.51 | NS | 0.01 | NS |
| Molar proportion of VFA (mol/100mol) | | | | | | | |
| Acetic | 59.45 a ±0.62 | 49.48 b ±0.62 | 60.00 a ±0.64 | 59.66 a ±0.73 | 0.01 | 0.01 | 0.01 |
| Propionic | 17.59 b ±0.47 | 19.12 a ±0.47 | 17.00 c ±0.12 | 18.95 a ±0.15 | NS | 0.01 | NS |
| Butyric | 7.85 b ±0.45 | 10.69 a ±0.41 | 8.08 b ±0.60 | 11.00 a ±0.53 | NS | 0.01 | NS |
| A / P ratio | 3.38 a ±0.39 | 2.59 c ±0.03 | 3.53 a ±0.04 | 3.15 b ±0.05 | 0.01 | 0.01 | 0.01 |

$^{a, b, c, d}$ Values having different superscripts within each raw are significantly different.*S, source; L, level; I, interaction; LLP, low level of protein; HLP, high level of protein; NS, not significant.

There were significant effect (P<0.01) among different rations on rumen $NH_3$-N (Table 5) at all time post feeding; as response to increase protein level. $NH_3$-N concentration at 0 hr was not significant for both protein source and level; ammonia concentration in rations 2 and 4 were 44.45 and 44.05, respectively with no significant difference between them, but significantly higher than that of rations 1 and 3. It is obvious that all $NH_3$-N concentration within all the experimental rations increased post feeding to reach the highest level at 2h and decreased thereafter. Source of protein and the interaction between protein source and level had no significant effect on rumen $NH_3$-N concentration at all times.

Total VFA was significantly higher (*P*<0.01; Table 5) at 2, 4, and 6 hr post feeding for ration 2 (SBM 14%CP) being 17.15, 16.85and 12.68, respectively than rations 1 and 3 with no significant difference than ration 4 (CSM 14%CP). The lowest values were obtained with the rations 1 and 3 being 12.26, 12.35, and 11.98 meq/100 ml- 12.05, 12.12 and 10.92 respectively at 2, 4 and 6h post-feeding. The change in total volatile fatty acids (VFA) concentration in response to source of protein was not significant at all times post feeding.

At zero time (just before morning feeding) all animals had the lowest ruminal VFA concentration being 8.75, 9.25, 9.45 and 9.00 meq/100 ml for SBM 10%CP, SBM 14%CP, CSM 10%CP and CSM 14%CP groups, respectively. The differences were not significant.

Generally with rations 2 and 4, the highest values of VFA were obtained at 2h post feeding but the highest VFA values of rations 1 and 3 were obtained at 4h post feeding It is obvious that high protein level improved the microbial activity in the rumen of the experimental sheep and this is clear in higher significant VFA values of rations 2 and 4 at 2,4 and 6h post feeding. Table (5) also shows the effect of protein source and level on molar proportion of acetate, propionate, butyrate and acetate: propionate (A: P) ratio in the rumen of sheep fed the experimental different protein sources and levels rations at 4hr post-feeding. The molar proportion of acetic acid in rations 1, 3 and 4 were 59.45, 60.00 and 59.99, respectively with no significant difference between them, but significantly higher than that of rations 2. The molar proportion of propionic acid took the opposite trend being higher for ration 2 (19.12) than for rations 1,3 and 4 being, 17.59, 17.00 and 18.95,respectively.Ration 2 (SBM 14%CP) showed higher significant effect than ration 1 (SBM 10%CP) and ration 3 (CSM 10%CP), but the differences than ration 4 (CSM 14%CP) were not significant. This explained that CF content in ration 2 was lower than other rations (table 2). The molar proportion of butyric acid took same trend of propionic acid being higher on ration 2 (10.69) than rations 1,3 and 4 being, 7.85, 8.08 and 11.00, respectively. Ration 2 (SBM 14%CP) showed higher significant effect than ration 1 (SBM 10%CP) and ration 3 (CSM 10%CP), but the differences than ration 4 (CSM 14%CP) were not significant.

The A:P ratio was 3.38:1 for ration 1, 2.59:1 for ration 2, 3.53;1for ration 3 and 3.15:1 for ration 4; differences were significant (P<0.01). In general, sheep fed higher protein level had more molar proportion of propionic and butyric acid and less acetic than those fed lower protein level. Source of protein and the interaction between protein source and level had significant effect on rumen molar proportion of acetic acid and A:P ratio, but took opposite trend with molar proportion of propionic and butyric acid.

Table (6) illustrate the effect of protein source and level on blood protein parameters (TP, albumin (A), globulin (G) and A/G ratio) of Rahmani lambs. It is clearly evident that protein level significantly affected such respective parameters.

In this concern, the average of TP ranged from 6.05 to 8.65 g/dl versus 3.05 to 3.95 g/dl for albumin and 3.00 to 4.7 for globulin. The obtained results are within the values that recorded by Holter *et al*. (1982), Hossein Yazdi *et al*. (2009) and Law *et al*. (2009).

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As dietary protein level was increased in the ration, the concentration of TP significantly increased. Lambs fed higher levels of protein showed higher TP concentrations in their plasma than those fed lower levels of protein. Ration 2 (SBM 14%CP) showed higher significant in plasma TP at 4 hours post feeding than the others rations. The differences due to source of protein at 4, 6 hours post feeding were significant. Plasma albumin took the same trend of TP. The concentration of plasma albumin at 4 hours post feeding was significantly higher for lambs fed the higher level of CP in their rations; i.e rations 2, 4 respectively than for those fed the low level of CP in their rations; i.e., rations 1, 3 . The differences due to source of protein at 4, 6 hours post feeding were significant. The same trend was also noted by Holter *et al*. (1982) who reported that serum albumin was low at dietary low CP. However, Hossein Yazdi *et al*. (2009) illustrated that neither plasma concentration of TP, nor albumin was affected by dietary protein. The concentration of plasma globulin, as affected by dietary CP source and level, follows the same trend of TP and albumin. Plasma globulin concentration at 4 hours post feeding significantly increased in plasma of lambs fed SBM rations as dietary protein level increased from 10%CP to 14%CP. Also Plasma globulin concentration significantly increased in plasma of lambs fed CSM rations as dietary protein level increased from 10%CP to 14%. Ration 2 (SBM 14%CP) showed higher significant globulin concentration at 4 hours post feeding than the other rations. In contrast the differences in plasma globulin concentration due to source of protein were not significant (M'Hamed *et al*. 2001). A/G ratio was insignificantly affected by dietary protein source and level, meanwhile, it decreased as the level of protein was increased, the difference in this concern was not significant. The obtained estimates were found to be within the normal values available in the literature (Holter *et al*., 1982; Law *et al*., 2009; Hossein Yazdi *et al*., 2009).

Table (7) shows that values of plasma AST was insignificant at all times (0, 2, 4 and 6 hr) post feeding and ALT was only significant ($P<0.05$) affected by protein source and level at 4 hours post feeding. In contrast ALT was insignificantly affected by protein source and level at (0, 2, 6 hours) post feeding. In this regard, the activity of AST ranged between 24.69, 26.12 U/L. So the present result indicated that there was no significant effect on liver functions. The activity of plasma ALT was higher in lambs fed higher protein levels rations (14%CP) as compared to those fed lower protein levels rations (10% CP) .The differences were not significant at 0, 2, 6 hours post feeding, but it was significant at 4 hours post feeding. All plasma AST and ALT activity was within the normal range.

**Table (7): Effect of protein source and level on plasma transaminases (u/l)**

| Sampling time (hr) | AST | | | | ALT | | | |
|---|---|---|---|---|---|---|---|---|
| | Soybean meal | | Cotton seed meal | | Soybean meal | | Cotton seed meal | |
| | LLP | HLP | LLP | HLP | LLP | HLP | LLP | HLP |
| 0 | $24.85^{a} \pm 0.63$ | $25.05^{a} \pm 0.55$ | $25.10^{a} \pm 0.55$ | $25.00^{a} \pm 0.65$ | $38.95^{a} \pm 0.87$ | $39.78^{a} \pm 0.79$ | $39.12^{a} \pm 0.69$ | $39.45^{a} \pm 0.66$ |
| 2 | $25.45^{a} \pm 0.74$ | $26.05^{a} \pm 0.63$ | $26.00^{a} \pm 0.62$ | $25.82^{a} \pm 0.64$ | $40.25^{a} \pm 0.94$ | $41.25^{a} \pm 0.94$ | $40.05^{a} \pm 0.67$ | $41.10^{a} \pm 0.87$ |
| 4 | $24.78^{a} \pm 0.82$ | $25.90^{a} \pm 0.65$ | $25.85^{a} \pm 0.69$ | $26.12^{a} \pm 0.65$ | $38.88^{b} \pm 0.73$ | $41.10^{ab} \pm 0.95$ | $40.87^{ab} \pm 0.88$ | $42.35^{b} \pm 0.71$ |
| 6 | $24.65^{a} \pm 0.71$ | $24.95^{a} \pm 0.73$ | $24.95^{a} \pm 0.69$ | $24.69^{a} \pm 0.66$ | $38.75^{a} \pm 0.79$ | $40.68^{a} \pm 0.93$ | $38.55^{a} \pm 0.80$ | $39.55^{a} \pm 0.78$ |

[a, b, c,] Values having different superscripts within each raw are significantly different. *S, source; L, level; I, interaction; LLP, low level of protein; HLP, high level of protein; AST, Aspartate transaminase; ALT, Alanine transaminase.

Table (8) indicated that, the concentration of blood urea in Rahmani lambs was significantly affected by source and level of dietary protein. These results are in agreement with Putnam and Varga (1998); McCormick *et al*. (1999); Law *et al*. (2009) and disagree with Bargo *et al*. (2001); Hossein Yazdi *et al*. (2009) who did not record significant changes in blood urea concentration due to various levels of dietary protein. Data also indicated that feeding lambs ration 2 (SBM 14% CP) significantly (P<0.01) resulted in increasing plasma blood urea concentration (32.15 mg/dl) at 2 hr post feeding as compared to that recorded for lambs fed ration 1 (SBM 10% CP), ration 3 (CSM 10% CP) and ration 4 (CSM 14% CP), being 28.35, 26.55 and 29.75 mg/dl, respectively, the difference was significant. Protein source had significant effect on plasma blood urea concentration at 0 and 2 hr post feeding, moreover significant effect of protein level being until 4 hr post feeding. These findings were consistent with those reports of Olmos Colmenero and Broderick, (2006); Law *et al*. (2009). McCormick *et al*. (1999) reported that average PUN concentrations were elevated (P<0.01) from 20.1 mg/dl in Holstein cows fed 17.7% CP and 5.0% RUP diets to 25.0 mg/dl in receiving 23.1% CP and 5.8% RUP diets, which suggested that PUN affected by level of protein and rumen undegraded protein. Moreover, Law *et al*. (2009) proved that increased dietary CP concentration significantly increased plasma urea (P<0.001) concentration.

The variation in PUN concentration in response to treatment Rahmani lambs with dietary proteins could be due to amount or level of protein intake (McCormick *et al*., 1999; Abeni *et al*., 2000; Olmos Colmenero and Broderick, 2006; Law *et al*., 2009), protein source and degradability (McCormick *et al*., 1999), feeding strategy and methodology of urea analysis (Elrod and Butler, 1993).

As shown in Table (8), the concentration of creatinine in the blood plasma of the experimental groups did not show significant difference due to different dietary proteins, neither source nor level. In agreement with results of Bal and Yarar (2006) who reported that there was no effect of CP level (12 and 16%) on serum creatinine concentration of heifers and steers (1.5 and 1.2 mg/dl for 12% CP of heifers and steers respectively, and 1.0 and 1.2 mg/dl for 16% CP of heifers and steers respectively).

In general, the values of plasma creatinine concentrations ranged from 1.01 to 1.25 mg/dl, and did not affected significantly by neither protein source nor level.

**Table (8): Effect of protein source and level on plasma urea (mg/dl) and Creatinine (mg/dl)**

| Sampling time (hr) | Urea | | | | Creatinine | | | |
|---|---|---|---|---|---|---|---|---|
| | Soybean meal | | Cotton seed meal | | Soybean meal | | Cotton seed meal | |
| | LLP | HLP | LLP | HLP | LLP | HLP | LLP | HLP |
| 0 | $25.95^{c}$ ± 0.19 | $30.25^{a}$ ± 0.43 | $25.48^{c}$ ± 0.29 | $28.85^{b}$ ± 0.20 | $1.18^{a}$ ± 0.09 | $1.20^{a}$ ± 0.08 | $1.25^{a}$ ± 0.08 | $1.22^{a}$ ± 0.11 |
| 2 | $28.35^{bc}$ ± 0.27 | $32.15^{a}$ ± 0.74 | $26.55^{c}$ ± 0.90 | $29.75^{b}$ ± 0.75 | $1.12^{a}$ ± 0.10 | $1.16^{a}$ ± 0.10 | $1.16^{a}$ ± 0.10 | $1.10^{a}$ ± 0.07 |
| 4 | $27.25^{c}$ ± 0.86 | $30.68^{ab}$ ± 0.82 | $28.87^{bc}$ ± 0.87 | $31.74^{a}$ ± 0.86 | $1.08^{a}$ ± .05 | $1.06^{a}$ ± 0.05 | $1.08^{bc}$ ± 0.05 | $1.01^{a}$ ± 0.07 |
| 6 | $26.05^{a}$ ± 0.90 | $26.80^{a}$ ± 0.87 | $25.58^{a}$ ± 0.73 | $26.28^{a}$ ± 0.91 | $1.06^{a}$ ± 0.05 | $1.05^{a}$ ± 0.05 | $1.05^{a}$ ± 0.05 | $1.02^{a}$ ± 0.07 |

[a, b, c,] Values having different superscripts within each raw are significantly different. *S, source; L, level; I, interaction; LLP, low level of protein; HLP, high level of protein.

## REFERENCES


Abeni, F.L., Calaman, L. Stefanini and G. Pirlo.(2000). Effect of daily gain in pre- and postpubertal replacement dairy heifers on body condition score, body size, metabolic profile and future milk production. J. Dairy Sci. 83:1468:1478.

Ahmed, B. M. (1976). The use of non-protein nitrogenous compounds in rabbit ration. M. Sc. Thesis. Tanta univ.

Babolok, W. H. Passing, R. Bender and B. Sehneider. (1988). A General Regression Proceduer for Method transformation. J. Clin. Chem. Clin. Biochem; 26:783-790.

A.O.A.C. (2000). Official methods of analysis association of official analytical chemists international. 17 th ed. AOAC Int.,Gaithersburg. MD.

Bal, M.A. and H. Yarar (2006). Effect of dieatary crude protein level on growth performance and blood parameters of Holstein heifers and steers fed ad libitum. J. Animal and Veterinary Advances. 75: 215-219.

Bargo, F., D. H. Rearte, F.J. Santin and L.D. Muller. (2001). Ruminal digestion by dairy cows grazing winter oats pasture supplemented with different levels and sources of protein. J. Dairy Sci. 84 : 2260-2272.

Broderick, G.A., M.J. Stevenson and R.A. Patton (2009). Effect of dietary protein concentration and degradability on response to rumen-protected Methionine in lactating dairy cow. J. Dairy Sci. 92:2719-2728.

Chandrasekharaiah, M., A. Thulasi and K.T. Sampat (2010). Effect of variable rumen degradable nitrogen levels on microbial protein synthesis estimated with purine. Indian Journal of Animal Sciences. 80: 7, 674-678. derivatives in sheep urine and PDC index.

Doumas, B., W. Wabson and H. Biggs (1971). Albumin standards and measurement of serum with bromochresool green. Clin. Chem. Acta., 31: 87.

Elrod, C.C and W.R. Butler (1993). Reduction of fertility and alterations of uterine pH in hifer fed excess ruminally degradable protein. J. Anim. Sci. 71:694-701.

EL-shakankery, Gada. A. (2004). Evaluation of sunflower plant as feedstuff for sheep M.Sc. Thesis, Fac. Of Agric., Minoufiya Univ., Egypt.

Harvey, D. (1970). Tabels of amino acids in foods and feeding-stuffs. Commonwealth Agr. Bureau Farnham Royal Bucks, England.

Holter, J. B., J. A Byrne and C. G. Schwab (1982). Crude protein for high milk production. J. Dairy Sci . 65 :1175-1188.

Hossein Yazdi, M ., H. Amanlou and E. Mahjoubi (2009). Increasing prepartum dietary crude protein using poultry by-product meal dose not influence performance of multiparous Holistin dairy cows. Pakistan J . Biological Sci. 75: 1448-1454.

Huber, J.T. and L. Jr. Kung (1981). Protein and non-protein nitrogen utilization in dairy cattle. J. Dairy Sci . 64:1170.

Kiran, D. and U. Krishnamoorthy (2007). Rumen fermentation kinetics and nitrogen degradability of commonly used ruminant feedstuffs in vitro. Animal Nutrition and Feed Technology. 7: (1) 63-71.

Lapierre, H. and G.E. Lobley (2001). Nitrogen recycling in the ruminant: A review. J. Dairy Sci. 84(E suppl.):223-236.

Law, R.A., F.J. Young, D.C. Patterson, D. J. Kilpatrick, A. R. G . Wylie and C.S. Mayne (2009). Effect of dietary protein content on animal production and blood metabolites of dairy cows during lactation . J .Dairy Sci . 92:1001-1012.

M'Hamed, D.L., P. Faverdin and R. Verite (2001). Effect of the level and source of dietary protein on intake and milk yield in dairy cows. Anim. Res. 50:205-211.

Maynard, L. A., J.K. Loosli, H.S. Hintz and R.G. Warner (1979). Animal Nutrition. McGraw-Hill book Co. Inc.NY.

McCormick, M. E., D.D. French, T.F. Brown, G. J. Cuomo, A.M. Chapa, J.M. Fernandez, J.F. Beatty and D.C. Bloun (1999). Crude protein and rumen undegraded protein effects on reproduction and lactation performance

of Holstein cows. J. Dairy Sci. 82: 2697-2708.

NRC (1985). National Research Council. Nutrient requirements of domestic animals. Nutrient requirements of sheep. National Academy of science. Washington DC.

Oldham, J.D. (1984). Protein-energy interrelationships in dairy cows. J. Dairy Sci. 67:1090-1114.

Oliveira Junior, R. C. de; A.V. Pires, J.J. Fernandes, de R.,F.A.P. Santos, I. Susin and R.C. Araujo (2006). Effects of nitrogen sources, in high concentrate diets for beef cattle, on dry matter intake, digestibility and nutrient degradability.

Olmos Colmenero, J. J. and G. A. Broderick (2006). Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cow. J. Dairy Sci . 89 :1704-1712.

Putnam, D.E. and G.A. Varga (1998). Protein density and its influence on metabolite concentration and nitrogen retention by Holstein cows in late gestation. J. Dairy Sci. 81: 1608-1618.

Reitman, S. and S. Frankel (1957). Colorimetric method for the dctermination of serum glutamic – Oxloaeetie and glutamic pyruvic trans-aminase. Am. J. Clin .Path .28:56.

SPSS (Statistical Package for Social Science) program version 19.0, (2010).

SupelcoInc., (1975).G.C.Separation of VFAC25.Bull. No.7498. supelco. Inc., Bellefonte, PA.

Tatsapong, P., P. Peangkoum, O. Pimpa and M. D. Hare (2010). Effects of dietary protein on nitrogen metabolism and protein requirements for maintenance of growing Thai swamp buffalo (Bubalus bubalis) calves .Journal of Animal and Veterinary Advances. 9: 8, 1216-1222.

Tietz, N.W. (1990). Clinical guide to laboratory testes. 2nd ed. W.B. Saunders Co.

Young, D.S. (1995). Effect of drugs on Clinical Lab. Tests, 4th ed. AACC

# تأثير بروتين الغذاء على الاستفادة من النيتروجين في الأغنام

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## الملخص العربي

أجريت تجربه لدراسة تأثير مصدر ومستوى بروتين الغذاء على ثمانية حملان رحمانى وأختير لاجراء هذه التجربة تصميمى مربع لاتينى (٤×٤) حيث تم من خلال هذه التجربة دراسة مصدرين مختلفين من البروتين (كسب فول الصويا وكسب القطن غير المقشور) مع مستوين مختلفين من البروتين ، منخفض (١٠% بروتين خام) وعالى (١٤% بروتين خام) وكانت الاربع علائق متساويه تقريبا فى محتواها من الطاقه. أدى ارتفاع مستوى البروتين من ١٠% الى ١٤% الى تحسن معاملات الهضم معنويا لكل العناصر الغذائيه فيما عدا الكربوهيدرات الذائبه بينما كان لمصدر البروتين تاثيرا معنويا فقط على كلا من الماده الجافه والماده العضويه والبروتين الخام والدهن الخام وكانت العليقه الثانيه (كسب فول الصويا ١٤% بروتين) ذات القيم الأعلى لمعاملات الهضم لمعظم العناصر خاصة البروتين الخام. أدى ذلك إلى زيادة القيمة الغذائية لهذه العليقة بصورة معنوية عن باقى العلائق. سجلت العليقه الثانيه (كسب فول الصويا ١٤% بروتين) اعلى ميزان نيتروجين وكانت الفروق معنويه عن باقى علائق التجربه. تفوقت العليقه الثانيه فى انتاج الأحماض الدهنية الطيارة والأمونيا فى سائل كرش الأغنام عند معظم الفترات الزمنيه عن باقى العلائق فى حين لم يكن لمصدر البروتين أى تأثير معنوى. انخفض حامض الخليك ونسبته الى البروبيونيك معنويا بزيادة مستوى البرتين ومصدر البروتين وزادت أحماض البروبيونك والبيوتريك معنويا بزيادة مستوى البروتين ولم تتأثير معنوبا بمصدر البروتين وكانت العليقه الثانيه (كسب فول الصويا ١٤%بروتين ) ذات القيم الاعلى لحامض البربيونيك. أرتفع كل من تركيز البروتين الكلى والالبيومين والجلوبيولين معنويا فى بلازما الدم لحيوانات العليقه الثانية نتيجة ارتفاع مستوى البروتين بينما كان تأثير مصدر البروتين غير معنوى فى معظم الاوقات وكانت الفروق معنوية لصالح العليقه الثانية. لم يتأثر نشاط انزيمى ALTو AST بدرجة معنوية فى سيرم الاغنام التى تغذت على العلائق المختلفه فى مصادر ومستويات البروتين. لم يتأثر تركيز الكرياتينين فى الدم معنويا بمصدر ومستوى البروتين بالعلائق المختلفه وتراوحت قيمته من ١.٠٢–١.٢٥مجم. كان تأثير مصدر البروتين معنويا على تركيز يوريا الدم حتى ساعتين بعد الأكل فى حين امتد التأثير المعنوى لمستوى البروتين الى أربع ساعات بعد الأكل. حيث سجلت العليقه الثانيه (كسب فول الصويا ١٤%بروتين) أعلى قيما ليوريا الدم عند ساعتين بعد الأكل وبفارق معنوي عن باقى علائق التجربه.

**Table(6):Effect of dietary protein source and level on plasma total protein (g/dl) and protein fractions.**

| Sampling time (hr) | Total protein | | | | Albumin(A) | | | | Globulin(G) | | | | A/G ratio | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Soybean meal | | Cottonseed meal | | Soybean meal | | Cottonseed meal | | Soybean meal | | Cottonseed meal | | Soybean meal | | Cottonseed meal | |
| | LLP | HLP | LLP | HLP | LLP | LLP | LLP | HLP | LLP | HLP | LLP | HLP | LLP | HLP | LLP | HLP |
| 0 | 6.05$^{a}$ ± 0.25 | 6.38$^{a}$ ± 0.19 | 6.20$^{a}$ ± 0.13 | 6.33$^{a}$ ± 0.13 | 3.05$^{a}$ ± 0.11 | 3.15$^{a}$ ± 0.03 | 3.15$^{a}$ ± 0.02 | 3.18$^{a}$ ± 0.10 | 3.00$^{a}$ ± 0.25 | 3.23$^{a}$ ± 0.19 | 3.05$^{a}$ ± 0.13 | 3.15$^{a}$ ± 0.19 | 1.02$^{a}$ ± 0.12 | 0.98$^{a}$ ± 0.06 | 1.03$^{a}$ ± 0.04 | 1.01$^{a}$ ± 0.11 |
| 2 | 6.42$^{b}$ ± 0.11 | 7.11$^{a}$ ± 0.16 | 6.40$^{b}$ ± 0.10 | 7.10$^{a}$ ± 0.15 | 3.15$^{a}$ ± 0.08 | 3.40$^{a}$ ± 0.11 | 3.15$^{a}$ ± 0.10 | 3.38$^{a}$ ± 0.07 | 3.27$^{b}$ ± 0.13 | 3.71$^{a}$ ± 0.13 | 3.25$^{b}$ ± 0.18 | 3.72$^{a}$ ± 0.21 | 0.96$^{a}$ ± 0.07 | 0.92$^{a}$ ± 0.05 | 0.97$^{a}$ ± 0.12 | 0.91$^{a}$ ± 0.07 |
| 4 | 6.85$^{c}$ ± 0.11 | 8.65$^{a}$ ± 0.15 | 6.70$^{c}$ ± 0.09 | 8.15$^{b}$ ± 0.24 | 3.35$^{c}$ ± 0.10 | 3.95$^{a}$ ± 0.03 | 3.20$^{c}$ ± 0.08 | 3.65$^{b}$ ± 0.05 | 3.50$^{c}$ ± 0.19 | 4.7$^{a}$ ± 0.14 | 3.50$^{c}$ ± 0.09 | 4.5$^{b}$ ± 0.26 | 0.96$^{a}$ ± 0.9 | 0.84$^{a}$ ± 0.02 | 0.91$^{a}$ ± .04 | 0.81$^{a}$ ± 0.07 |
| 6 | 7.20$^{b}$ ± 0.17 | 8.45$^{a}$ ± 0.18 | 6.91$^{b}$ ± 0.19 | 8.05$^{a}$ ± 0.21 | 3.28$^{a}$ ± 0.13 | 3.65$^{b}$ ± 0.10 | 3.05$^{b}$ ± 0.03 | 3.27$^{b}$ ± 0.07 | 3.92$^{b}$ ± 0.15 | 4.8$^{a}$ ± 0.23 | 3.86$^{b}$ ± 0.19 | 4.78$^{a}$ ± 0.25 | 0.84$^{a}$ ± 0.05 | 0.76$^{a}$ ± 0.06 | 0.79$^{a}$ ± 0.04 | 0.68$^{a}$ ± 0.04 |

$^{a, b, c, d}$ Values having different superscripts within each raw for each protein fraction are significantly different.; LLP, low level of protein; HLP, high level of protein.