ABSTRACT

This investigation included two experiments. Experiment 1 was executed to study the effect of feeding different rations of protected protein of canola meal on digestibility and nutritive values within sheep. Twenty male, healthy sheep were divided into five treatments according to the methods of protein protection (control, heat, sodium hydroxide, formaldehyde, and acetic acid treatments). Experiment 2 was carried out on developing lambs to investigate the effect of protected protein on growth performance and some blood metabolites. Animals in this experiment were also divided into the same treatments as Experiment 1. In the first and second experiment were fed concentrate ration (80%) and wheat straw (20%) to cover the feed requirements. Nutritive values expressed as total digestible nutrients (TDN%) and digestible crude protein (DCP%) of the experimental rations was calculated. In the second experimental all animals were weighed biweekly and the amounts of rations were adjusted throughout the experimental period (120 days) according to their body weight change. Results indicated that in the first experimental protected protein by heat (HE) and sodium hydroxide (NH) had positive \((P < 0.05)\) effects on most of digestibility coefficients of different nutrients. Protein protection methods also improved \((P < 0.05)\) the nutritive values (TDN and DCP) in the HE treatment and NH treatment. In the second experiment body weight increased by 14% and 7% and also daily gain by 27% and 14% in HE and NH, respectively, while FM and AC decreased body weight by 8% and 4.4%. Higher values \((P < 0.01)\) of total protein, albumin, and glucose were observed in HE and NH than other treatments. The control (CTL) group recorded higher concentrations of urea-N and creatinine at different periods of the experiment in comparison with other treatments. Generally, from the present investigation it can be concluded that protected protein of canola meal by heat or sodium hydroxide treatments were more efficient for productive performance and some blood metabolites of sheep.

Keywords: Protected Protein; Performance; Blood Metabolites; Sheep

1. INTRODUCTION

Protein is an expensive component of animal diets, and its content in diets of ruminant animals is very essential for improving the productive performance. Protein content of some feedstuffs with high quality protein can be degraded in the rumen while 80 to 90% of the protein content of some feedstuffs with high quality protein may be degraded in the rumen (Beever, 1984). There are several methods for protected protein which can be categorized into chemical (e.g. sodium hydroxide (Mir et al., 1984), acetic acid (Waltz and Loerch, 1986) formaldehyde (Ferguson et al., 1967) and physical (e.g. heat) treatments (Stern et al., 1985). Incorporation of protected protein in diets is recommended in high producing animals to increase their productivity. Virk et al. (1994) reported that protected protein increased growth rate and nitrogen retention in goats. The beneficial effects of protected protein on body weight and weight gain in lambs were established and documented (El-Ayek et al., 1999 a and b). Economic value of supplemental protein feed is determined largely by the amount that escapes ruminal degradation and is available for digestion and absorption in the small intestine (Beauchemin et al., 1995 and Tomllinson et al., 1997).
2. MATERIALS AND METHODS

2.1. Methods of Protected Protein in Canola Meal

Canola meal used in the different experimental rations in the present study is classified into four treatments:

1) Heat treatment (HE): Two cm layer of canola meal was subjected to 135°C - 145°C in a forced air oven for 4 hrs according to Stern et al (1984). After the heating treatment, canola meal was kept at room temperature (25°C) for 3 days before being mixed with other ingredients to formulate concentrate ration.

2) Sodium hydroxide treatment (NH): Canola meal was treated with a solution of sodium hydroxide at the rate of 3 gm NaOH/100 gm DM of canola meal, according to Mir et al. (1984). Then, treated canola meal was air dried at room temperature (25°C) for one week before being mixed with other ingredients.

3) Formaldehyde treatment (FM): Canola meal was treated with a 40% formaldehyde solution at rate of 1 ml formaldehyde/100 gm crude protein in canola meal according to Ferguson et al (1967). The treated canola meal was stored in light plastic containers to complete reaction of formaldehyde with canola meal for 2 weeks at room temperature (25°C) before being used.

4) Acetic acid treatment (AC): A solution of acetic acid with a concentration rate of 30 ml acetic acid /1kg DM of canola meal was spread according to Waltz and Loerch (1986). Then, treated canola meal was air dried at room temperature (25°C) for one week before being used.

2.2. Experimental Design

2.2.1. Experiment 1

The first experiment was designed to evaluate the effect of feeding protected protein on digestibility coefficients of nutrients and nutritive values of different tested rations. Twenty healthy male Sohagi lambs with an averaged body weight of 35.8 ± 1.29 kg were used in this experiment. The experimental period lasted 3 weeks. Animals were divided randomly into five equal (n = 4) treatments according to method of protein protections (CTL, HE, NH, FM, and AC). Animals were fed a concentrate diet (80%) and wheat straw (20%) according to NRC (1985) requirements. Animals in each treatment were fed individually during the whole experimental period. Formulation of the experimental concentrate rations are shown in Table 1.

Fecal from each animal was collected at the last week of the experimental period twice daily. Fecal samples and rations were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), and ether extract (EE) according to A.O.A.C. (1995). Digestibility coefficients of DM, OM, CP, CF, EE and nitrogen free extract (NFE) were determined using acid insoluble ash (AIA%) as the natural marker according to Van Keulen and Young (1977). Nutritive values (TDN and DCP%) of the experimental rations were also calculated.

| Treatments          | Items                  | CTL | HE | NH | FM | AC |
|---------------------|------------------------|-----|----|----|----|----|
| Canola meal         | Untreated              | 25  |    |    |    |    |
|                     | Heat                   |    | 25 |    |    |    |
| Sodium hydroxide    |                        |    |    | 25 |    |    |
| Formaldehyde        |                        |    |    |    | 25 |    |
| Acetic acid         |                        |    |    |    |    | 25 |
| Maize grain         |                        | 42  | 42 | 42 | 42 | 42 |
| Wheat bran          |                        | 30  | 30 | 30 | 30 | 30 |
| Premix*             |                        | 0.5 | 0.5| 0.5| 0.5| 0.5|
| Sodium chloride     |                        | 0.5 | 0.5| 0.5| 0.5| 0.5|
| Limestone           |                        | 2.0 | 2.0| 2.0| 2.0| 2.0|

*Premix contents per 3 kg are of vit. A. 1200000 IU, vit. D 3, 2200000 IU, vit. E, 10 gm, vit. K 3, 2 gm, copper, 10 gm, zinc, 50 gm, Manganese, 55 gm, Iodine, 1 gm, Selenium, 0.1 gm, Carrier (CaCO₃), up to 3000 gm. CTL – Canola meal without treatment; HE – Canola meal heat treatment; NH – Canola meal sodium hydroxide treatment; FM – Canola meal formaldehyde treatment; AC – Canola meal acetic acid treatment.
16.80 ± 0.23 kg, were used in this study. Animals were ingo to the rations type in the first experiment. The experimental period lasted for 4 months.

Animal fed on concentrate feed mixture rations (80%) and wheat straw (20%) to cover the requirement of DM and TDN for average daily gain (ADG) and body weights (BW) according to NRC (1985). Animals’ body weights were recorded every 15 days, and the amounts of ration were adjusted throughout the experimental period according of the BW changes. Fresh water was available at all times.

Total dry matter intake and total protein intake (from concentrate diets and wheat straw) per animal and treatment were recorded. Daily dry matter, protein intakes, and body weight gains were also calculated. Dry matter and protein efficiency were also calculated.

Blood samples (about 8 ml/animal) were collected at the beginning of the experiment and then at monthly intervals. Blood samples were allowed to clot at room temperature, and serum was then separated by centrifugation at 3000 r.p.m for 15 minutes. Serum samples were divided into two parts and then transferred into dry glass vials and stored at -20°C until subsequent analysis. In the first part of the serum, the concentration values of total protein (g/dl), albumin (g/dl), glucose (mg/dl), creatinine (mg/dl) and urea-N (mg/dl) were determined by spectrophotometer using commercial kits produced by the Stanbio Company. Globulin (g/dl) values were determined by subtracting albumin values from total protein values. The second part of the serum was used to determine the concentrations of triiodothyronine (FM) and thyroxin (AC) hormones using radioimmunoassay of serum techniques. The coat-A count T3 kits produced by Diagnostic Products Corporation (USA) were used for the determination of serum triiodothyronine concentration according to Bates (1994). The coat-A count kits produced by Diagnostic Products Corporation (USA) were used for the determination of serum thyroxine concentration according to Albertini (1982).

2.3. Statistical Analysis

Results were statistically analyzed using the General Linear Model (SAS, 1998) for Complete Randomized Design (CRD). Productive performance and blood parameters were performed by methods of analysis of variance. Significant differences among treatments means were tested using Duncan, (1955).

3. RESULTS AND DISCUSSION

3.1. Digestibility Coefficients and Nutritive Values

Data presented in Table 2 illustrates that the different

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Table 2. Effect of treatment on the nutrients digestibility coefficients and nutritive value of the experimental rations.

| Treatments | Digestibility coefficients (LSM) | ±SE | Sig. |
|------------|---------------------------------|-----|------|
|            | DM  | OM  | CP  | EE  | CF  | NFE | TDN | DCP |
| CTL        | 66.03 | 68.04 | 67.84 | 67.69 | 61.23 | 69.39 | 65.27 | 9.96 |
| HE         | 69.77 | 71.42 | 71.87 | 71.30 | 61.11 | 72.51 | 68.01 | 10.62 |
| NH         | 67.79 | 69.76 | 70.31 | 69.69 | 61.76 | 71.21 | 66.98 | 10.46 |
| FM         | 62.69 | 65.43 | 66.83 | 67.12 | 59.66 | 68.35 | 64.09 | 9.91 |
| AC         | 62.94 | 65.60 | 67.11 | 67.20 | 59.38 | 68.52 | 64.14 | 10.01 |

±SE values are least square means (LSM) ± standard error; a, b, c, d values with different letters in the same column are significantly different, *(P < 0.05), NS = Not significant.

Table 3. Effect of protected protein methods on some productive performance of lambs during the experimental periods.

| Items                        | Treatments (LSM)* | ±SE | Sig. |
|------------------------------|-------------------|-----|------|
| Initial weight               | 16.80             | 17.00 | 16.80 | 16.60 | 0.23 | NS |
| Final weight                 | 34.20             | 39.00 | 36.60 | 31.50 | 32.70 | 0.29 | ** |
| Total gain (Kg)              | 17.40             | 22.00 | 19.80 | 14.70 | 16.10 | 0.24 | ** |
| Daily gain (g/d)             | 144.40            | 183.00 | 164.40 | 122.00 | 133.80 | 1.97 | ** |
| Total dry matter intake (g/d)| 800.62            | 810.72 | 811.87 | 798.45 | 806.89 | 3.54 | NS |
| Total protein intake (g/d)   | 117.61            | 119.74 | 120.81 | 118.48 | 120.38 | 0.67 | NS |
| Dry matter efficiency        | 0.18              | 0.20 | 0.20 | 0.15 | 0.17 | 0.01 | ** |
| Protein efficiency           | 1.23              | 1.53 | 1.36 | 1.03 | 1.10 | 0.02 | ** |

±Values are least square means (LSM) ± standard error; a, b, c, d values with different letters in the same row are significantly different; **(P < 0.01), NS = Not significant; Total gain calculated by subtracts initial body weight from final body weight; Daily gain calculated by divided total gain on 120 days.

The values of dry matter and protein efficiency were significantly different (P < 0.01). The highest values were obtained with HE followed by NH and CTL, while the lowest values were recorded in FM and AC. Protected protein supplementation increased dry matter efficiency for lambs fed HE and NH rations by 27.8 and 11.1%, respectively, as compared with CTL group. Conversely, protected protein by FM treatment and AC treatment decreased dry matter efficiency for lambs fed FM and AC rations by 16.7 and 5.6% respectively, compared with those fed CTL ration. The improvement of dry matter efficiency and protein efficiency values in HE and NH may be due to the positive effects of protected protein methods on digestibility coefficients of most nutrients, feeding values, and/or the significant, positive effects of these treatments on daily gain. At the same time there are no significant effects of treatment on total dry matter intake or total protein intake. These results are in agreement with Abd El- Maksoud (1990) and Virk et al (1994) who found no improvement in feed conversion when were goats fed protein treated diet compared with the CTL. Recently however, El- Reweny (2006) observed that lambs fed protected protein diets revealed significantly high (P < 0.05) feed conversion compared with those fed CTL diet.

3.3. Blood Metabolites

3.3.1 Thyroid Hormones

Blood serum triiodothyronine and thyroxin concentrations at experimental periods in ram lambs groups are presented in Table 4. Thyroxin values differed significantly...
(P < 0.01) at the 2nd and 4th month stages of testing. Thyroid hormones concentrations recorded were significantly different (P < 0.01) concerning the effect of treatments (regardless of age) on serum.

Triiodothyronine concentration increased by 7.94% and 2.33% for HE and NH compared with CTL. Also, the corresponding increase for thyroxin was 9.97% and 2.67%, respectively. Improvement in the secretion of thyroid hormone concentrations in HE and NH may be due to the increase of carbohydrate, fat, and protein metabolism, as reflected by a positive effect on digestibility coefficient of carbohydrate, fat, and protein (Table 2). Also, the increase in the secretion of thyroid hormones may be due to the increase of TDN values (Table 2) in HE and NH in comparison with the other treatments. There was a positive relationship between energy intake and the concentration of the thyroid hormones as reported by Tiirates (1997) and Ahmed (2003). Thyroid hormones are necessary for normal growth and development of mammals as reported by Shalaby and Shehata (1995) and Abdel-Hafez (1997). Concerning the effect of age (regardless of treatments) on serum triiodothyronine and thyroxin concentrations (Table 4), it can be observed that the lowest values of triiodothyronine and thyroxin concentrations were recorded at the beginning of experiment, while the highest values are recorded at the end of experimental period (after 4 months).

Results indicate that the values of triiodothyronine and thyroxin concentrations increased gradually with the advancement of age. Similar trends were observed by Hussein (1991), Yousef (1992), Shaban (2000) and Saleem (2006). They found that the two hormone concentrations were increased by the advancement of age.

3.3.2 Total Protein, Albumin, and Globulin Concentrations

Blood serum total protein, albumin, and globulin (g/dl) concentrations at the beginning and after 2 and 4 months in ram lambs of the experimental groups were presented in Table 5. Total protein and albumin values were significantly different (P < 0.05 or P < 0.01, respectively) at 2 and 4 months. Protected protein by HE or NH led to a significant increase of serum protein and albumin concentrations at 2 and 4 months during the experimental period in comparison with those of the CTL, FM, and AC treatments.

Generally, data in Table 5 indicate that the highest values of total protein and albumin were within HE and NH followed by CTL, FM and AC. The improvement in HE and NH values may be due to the increase of digestibility coefficient of CP and nutritive values expressed as DCP (Table 2) as a result of protected protein methods (HE and NH treatments). A positive correlation between dietary protein and plasma protein concentrations were reported by Yousef and Zaki (2001) and Shabrawy (2006). They reported that values of serum total proteins, albumin, and globulin were increased (P < 0.01) when goats were fed protected protein in the diet. On the other hand, El-Reweny (1999 and 2006) found that the concentration of total protein, albumin, and globulin did not significantly change by using different sources of protein and protected protein treatment methods.

Concerning the effect of age (regardless of treatments) on serum total protein and its fractions, data presented in Table 5, illustrates that there was a significance difference (P < 0.01) among the different ages. The values (overall mean) of total protein increased gradually. From the present results, it is clear that serum total protein, albumin, and globulin tended to increase with advancing age. Similar results were reported with sheep by Hayder (1996 and 2004) and El-Reweny (2006).

3.3.3. Blood Urea Nitrogen, Creatinine, and Glucose

Blood serum urea-N (BUN), creatinine (Cr), and glucose (mg/dl) concentrations at three different intervals, of 0, 2, and 4 months, of the experiment are presented in Table 6. It can be noticed that differences in urea-N and creatinine values were not significant at the beginning of the experiment, but after 2 and 4 months, the values were significantly different (P < 0.01). Concentrations of urea-N and creatinine in CTL group showed the highest values at different testing periods in comparison with the other treatments, while the lowest values (P < 0.05) or (P < 0.01) of urea-N and creatinine were obtained in HE. Data in Table 6 illustrates that blood serum urea-N and creatinine concentrations in treatments groups (regardless of age) recorded were significantly different (P < 0.01). It can be observed that the values of urea-N and creatinine decreased as a result of protein protection more than those with untreated (CTL) feed. These differences may be due to the reduction of ammonia concentration released through the microbial fermentation in rumen of lambs fed protected protein. Subsequently, then decreasing the absorbed ammonia via the ruminal wall, which in turn is converted into urea in liver. So, the decreased level of ammonia in rumen of sheep fed pro-
Table 4. Effect of protected protein methods on the concentrations of Triiodothyronine and thyroxin in serum of lambs during experimental periods.

| Items                  | Treatments (LSM) | ±SE | Age effect |
|------------------------|-----------------|-----|------------|
|                        | CTL  | HE  | NH  | FM  | AC  |      |          |
| Triiodothyronine (µg/dl) | At the beginning |     |     |     |     | 0.66** | 64.40C |
|                        | 2 months       |     |     |     |     | 1.80** | 97.25B |
|                        | 4 months       |     |     |     |     | 2.54** | 105.90A |
| Treatment effect       | 84.56^a        | 91.28^a | 86.53^b | 84.68^c | 82.27^c | 0.80** | ±0.90** |
| Thyroxin (µg/dl)       | At the beginning |     |     |     |     | 0.05** | 2.99E |
|                        | 2 months       |     |     |     |     | 0.18** | 4.81C |
|                        | 4 months       |     |     |     |     | 0.22** | 5.36E |
| Treatment effect       | 4.11^d         | 4.52^a | 4.22^b | 3.95^c | 4.04^c | 0.07** | ±0.09** |

*Values are least square means (LSM) ± standard error; a, b, c, d means with the same letters in same row are significantly different. A, B, C, D, E means with same letters in the same row or the same column in each parameter are significantly different, *(P<0.01), NS= Not significant.

Table 5. Effect of protected protein methods on total protein, albumin, and globulin concentrations in serum of lambs during the experimental periods.

| Items          | Treatments (LSM) | ±SE | Age effect |
|----------------|-----------------|-----|------------|
|                | CTL  | HE  | NH  | FM  | AC  |      |          |
| Total protein (g/dl) | At the beginning |     |     |     |     | 2.22** | 4.86E |
|                | 2 months       |     |     |     |     | 0.06** | 5.31B |
|                | 4 months       |     |     |     |     | 0.09** | 6.35A |
| Treatment effect | 5.43^a         | 5.66^a | 5.63^a | 5.39^b | 5.38^b | 0.05** | ±0.18** |
| Albumin (g/dl) | At the beginning |     |     |     |     | 0.06** | 2.75B |
|                | 2 months       |     |     |     |     | 0.04** | 3.16C |
|                | 4 months       |     |     |     |     | 0.05** | 3.80A |
| Treatment effect | 3.23^a         | 3.36^a | 3.36^a | 3.23^b | 3.20^b | 0.02** | ±0.03** |
| Globulin (g/dl) | At the beginning |     |     |     |     | 0.25** | 2.11^EP |
|                | 2 months       |     |     |     |     | 0.06** | 2.15E |
|                | 4 months       |     |     |     |     | 0.11** | 2.58^A |
| Treatment effect | 2.20          | 2.27 | 2.27 | 2.17 | 2.19 | 0.06** | ±0.02** |

*Values are least square means (LSM) ± standard error; a, b, c means with the same letters in same row are significantly different. A, B, C, D, E means with same letters in the same row or the same column in each parameter are significantly different, *(P<0.05), **(P<0.01), NS= Not significant.

Recently, El-Shabrawy (2006) found lower (P < 0.05) values of urea-N in plasma of goats receiving formaldehyde soybean meal and heat soybean seed diets than those receiving untreated soybean meal diets. However, Guillaume et al. (1991), Bruckental et al. (1996), and Rodriguez et al. (1997) found a non-significant effect of protected dietary protein on the blood level of urea-N with dairy cows.

Concerning the effect of age (regardless of treatments) on urea-N and creatinine concentrations, data in Table 6, illustrates that the values of urea-N increased gradually.
This result indicates that serum urea-N and creatinine increased with advancing age (Table 6). Consistent results were reported by Rezaei-Roodbari and Zamiri (2003), and Hayder (2004).

The effect of protected protein on glucose concentrations are presented in Table 6. The values of glucose after 2 months were significantly different \((P < 0.01)\), while the differences at the beginning and after 4 months were not significant. Results of blood serum glucose concentrations (regardless of age) recorded the highest value in HE and NH in comparison with the other treatments. Ammann (1991) and Krober et al. (2000) observed that feeding protected protein increased plasma glucose in the blood. In addition, Aly (2005) reported that values of serum glucose were increased \((P < 0.01)\) by using protected methionine and lysine in the diet. The improvement of glucose values in HE and NH compared with the other treatments may be due to the positive effect of protein protection methods on the nutritive values expressed as TDN (Table 2). These results are in agreement with those of Hadley (1984). He reported that the high intake of energy supply may increase serum glucose concentration. Also, results obtained by Abd El-Latif (2003) indicated that values of blood glucose concentration in growing Friesian calves were correlated with energy in the diets. Moreover, an increase of glucose concentrations in HE and NH compared with the other treatments may be due to the higher carbohydrate metabolism as a result of higher thyroid hormones secretion (Table 6). The increase in blood glucose in response to thyroid hormones may also be attributed to the increase of carbohydrate metabolism (Haper et al.1979). Thyroid hormones increase gluconeogenesis and/or plasma glucose concentration in blood (Cole et al. 1994).

Concerning the effect of age (regardless of treatments) on glucose concentrations (Table 6), it can be observed that the glucose concentration started at a high level at the beginning of the experiment then decreased progressively by the advancing of age of lambs. The decrease of glucose levels by the advancement of age may be due to high metabolic rates of young animals resulted from the high rates of cellular reactions, but this may also be partly attributed to the rapid synthesis of cellular reaction materials and growth of the body, which require moderate quantities of energy (Abd-El-Fattah 1993). Similar results were obtained by Yousef (1992) and Abd-El-Fattah (1993). They recorded that blood glucose levels significantly declined with an increase of age in calves (from 1 to 9 to 12 month of age).

From the present results it can be concluded that protected protein by HE or NH treatments were more efficient than the other treatments (CTL, FM, and AC). Both methods improved the digestibility coefficients and nutritive values of tested rations and also improved the productive performance of ram lambs (body weights, daily gains, total gains, feed efficiency, and protein effi-

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### Table 6. Effect of protected protein methods on urea-N, creatinine, and glucose concentrations in serum of lambs during the experimental periods.

| Items        | CTL     | HE      | NH      | FM      | AC      | ±SE  | Age effect |
|--------------|---------|---------|---------|---------|---------|------|------------|
| **Urea-N (mg/dl)** |         |         |         |         |         |      |            |
| At the beginning | 8.49    | 8.49    | 8.49    | 8.49    | 8.49    | 0.08NS| 8.49p     |
| 2 months      | 12.67a  | 9.83b   | 9.70b   | 9.56b   | 9.49b   | 0.16**| 10.25b    |
| 4 months      | 12.43a  | 9.79b   | 9.93b   | 10.38b  | 10.52b  | 0.25**| 10.61b    |
| Treatment effect | 11.33b  | 9.40b   | 9.42b   | 9.49b   | 9.49b   | 0.10**| ±0.17**   |
| **Creatinine (mg/dl)** |         |         |         |         |         |      |            |
| At the beginning | 0.95    | 0.95    | 0.95    | 0.95    | 0.95    | 0.01NS| 0.95c     |
| 2 months      | 1.01a   | 0.93b   | 0.93b   | 0.93b   | 0.93b   | 0.02**| 0.96b     |
| 4 months      | 1.11a   | 0.94b   | 0.94b   | 0.96b   | 0.98b   | 0.03**| 0.99b     |
| Treatment effect | 1.06A   | 0.94A   | 0.95A   | 0.96A   | 0.98A   | 0.02**| ±0.01**   |
| **Glucose (mg/dl)** |         |         |         |         |         |      |            |
| At the beginning | 80.58   | 80.59   | 80.46   | 80.36   | 80.28   | 0.68NS| 80.45b    |
| 2 months      | 71.85bc | 77.44a  | 73.64b  | 68.45e  | 72.64b  | 1.40**| 72.80b     |
| 4 months      | 70.59   | 71.12   | 71.05   | 70.75   | 70.95   | 0.59NS| 70.89f     |
| Treatment effect | 73.08bc | 76.77a  | 74.90b  | 74.90b  | 73.94c  | 0.55**| ±0.79**   |

*Values are least square means (LSM) ± standard error. a, b, c means with the same letters in same row are significantly different; A, B, C, D means with same letters in the same row or the same column in each parameter are significantly different, \(^{(P < 0.05)}, **(P < 0.01), \text{NS} = \text{Not significant}\)
ciency). In addition, the methods of protected protein had beneficial effects on some blood metabolites.

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