Research article

Effects of bypass proteins on growth performance, kidneys and liver functions, rumen fermentation and meat amino acid analysis in male lambs

Zahid Ismaeel Mohammed Al-Jebory
Department of public Health, College of Veterinary Medicine, University of Diyala, Iraq
Corresponding Author Email: zahidma1977@yahoo.com

(Received 2/9/2017, Accepted 25/11/2017)

Abstract
An experiment was carried out in the College of Veterinary Medicine; University of Baghdad to investigate the effect of feeding formaldehyde treated meal on body weight gain, rumen fermentation, and some blood traits in local lambs. Twenty four lambs with body weight ranged from 20 to 23 kg and aged about 3 to 4 months were selected and divided randomly into two groups of twelve each (body weight was considered). These animals fed daily concentrate diet (3% of their body weight) and 0.5 kg of alfalfa hay, in addition to freely graze for 2-3 hours/ day. Results showed highly (p ≤ 0.05) a significant difference in the body weight gain (24.12 ± 1.01 kg) and weight of wool fleece, testis (298.00± 23.09 g) and kidneys (195.00± 8.66 g) between groups of lambs fed bypass protein compared with control group. Also, animals fed treated meal had higher (p ≤ 0.05) significantly in meat content of crude protein (17.42 ±0.31), digestibility(57.19 ± 2.58 ) and alkaline phosphatase enzyme(ALP) compared with control group, while there are no differences in amino acids in meat between groups of lambs. The results showed significant differences appeared in serum total protein and blood urea. In addition, no significant differences appeared in rumen fermentation parameters except rumen Ammonia-N. In conclusions: the bypass proteins technology may improve the production performance in local lambs. Therefor the bypass meal can be added to the diet of lambs for fattening. It had a positive effect on growth and fat composition of meat.

Keywords: Amino acids, Lambs, Bypass protein, Growth performance, Kidney functions, Rumen fermentation.

Introduction
In the feed, fraction of nutrients, which are non-degradable in the rumen by the microbes and they are digestible and absorbable at lower tract and become available to animal called as bypass nutrient fraction. Bypass nutrient mean that the essential and more important nutrient (high biological value) should be escape from the rumen or face minimum fermentation. In ruminants, the increase of un-degradable protein then increase its utilization in small intestine consequently increased amino acids following enzymatic digestion. Such modification of feeding called Bypass Proteins which resulted in improved animal's productivity Also, bypass protein meal improved animal health and some blood constituents and wool production (1). The treated meal with formaldehyde has no harmful effect on the health of animals. The method is also low cost and feasible; the technology has gone commercial in some countries (2). Protein is important resources for human with essential amino acids as one of the most important nutritional qualities (3). Amino acids are the fundamental units of protein, and amino acid content plays an important role in meat quality by providing nutritive value and flavor characteristics (4). Accordingly, the present experiment is set up
to verify the effect of Bypass protein applied to local male lambs ration on growth performance, kidneys and liver functions, rumen fermentation meat amino acids analyses in male lambs.

Materials and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: 116

Twenty four males local sheep lambs, their age ranged between 3 and 4 months and their body weight 20 to 23 kg, were divided randomly according their body weight into two groups (12 of each) Animals were fed on a concentrate diet to ensure the feed intake would be 3% of their body weight, 0.5kg alfalfa hay. In addition of freely grazed for 2-3 hours/day. The concentrate diet contained 11% yellow corn, 8% Guar, 50% wheat, bran wheat 30% and 1% salts. Its energy was 2000 MJ/Kg DM and crude protein level was 12.5%. The animals were fed concentrate diet two times daily and the experimental period lasted for 90 days. Body weight was recorded weekly and daily gain was calculated. At the end of experimental, rumen fluid was taken from six lambs by stomach tube, rubber stomach tube manually flush manner as described by (5). Blood samples were collected from all animals then blood serum was separated using centrifuge 3000 RPM for 15 minutes. Serum urea was measured according to (6) and total serum protein and alkaline phosphatase enzyme (ALP) was measured according to (7).

Dietary Treatment

Guar as source of protein treated with formaldehyde acid (54 liters / ton) which formed by mixed 3 liters of commercial acetic acid plus 6 liters formalin (concentration of 37%) plus 45 liters of water. Formaldehyde treated - guar preserved in nylon bags and kept it closed for 48 hours, then opened the bags and deployed components 3-5 cm thick on the floor furnished with nylon for 72 hours to expose materials to the atmosphere for the drying and get rid of the smell, then mixed the treated guar with the other components of the diet according to (8).

Amino acids and fatty acids determination:

At the end of the experimental period, six lambs randomly were selected from each group, fasted overnight with free access to water, weighed and slaughtered in the early morning of the next day. Femoral muscle (500gm) was obtained from the right side of each carcass to estimate meat chemical composition, (amino acids) using Diode Array 7200 NIR Analysis System. Fatty acids were determined by using Soxhulate apparatus to extract the fat and individual fatty acids were determined by using gas chromatography. The results were statistically analyzed to find the Least Significant Differences (LSD) between groups, using one-way Analysis of Variance (9). The digestibility coefficients of crude protein was calculated as follows:

\[
crude\text{\ protein\ digestibility}\% = \frac{protein\ intake - protein\ in\ feces}{protein\ intake} \times 100
\]

Results

Feeding of bypass protein meal lead to increased (p≤0.05) growth rate of animals (Table 1). For instance, lambs fed untreated meals gained daily body weight 168.73 ± 7.49 g/d while lambs fed formaldehyde-treated meals gained 201.00 ± 6.83g/d. whereas the total feed intake per animal was insignificantly affected by bypass protein meal.
Table (1): Effect of bypass protein on live weight gain in local male lambs

| Items                              | Treatments                  |
|------------------------------------|-----------------------------|
|                                    | Control                     | Bypass protein               |
|                                    | 0.807 ± 0.009               | 0.815 ± 0.005                |
| DMI/head/Day, kg                   | 0.815 ± 0.005               | 23.23 ± 0.62                 |
| Initial body weight, kg            | 22.99 ± 0.68                | 47.35 ± 1.26                 |
| Final body weight, kg              | 43.23 ± 0.95 b              | 24.12 ± 1.01 a               |
| body weight change in 90 days, kg  | 20.25 ± 0.99 b              | 24.12 ± 1.01 a               |
| body weight gain (g)/day           | 168.73 ± 7.49 b             | 201.00 ± 6.83 a              |

The different small letters refers to significant variations at (p≤0.05)

Table (2) showed higher (p≤0.05) significant differences of liver enzymes ALP and Serum Total protein of male lambs fed diet supplemented with bypass protein at the third month of study in compared with control group. Total Serum protein of bypass group was improved with time and showed a significantly (P≤0.05) increase more than control group during the late studied period. As well as there are significantly (P≤0.05) decrease in Blood urea concentration in bypass protein group compared with control group at third month. Serum concentration of urea and Total protein found in the present study were fall within the normal range reported for male lambs (10, 11).

Table (2): Effect of bypass protein on some blood serum constituents of local male lambs

| Parameters                         | Treatments                  |
|------------------------------------|-----------------------------|
|                                    | 1st month                  | 2nd month                  | 3rd month                  | 1st month                  | 2nd month                  | 3rd month                  |
|                                    | Control                     | Bypass protein             | Control                     | Bypass protein             | Control                     | Bypass protein             |
| Serum Total protein, g/dl          | 5.77±0.11                   | 5.96 ±0.23                 | 5.87±0.14 b                 | 5.62±0.10                  | 6.19±1.17                  | 6.52±0.84 a                |
| Blood urea, mg/dl                  | 21.30±0.60                  | 18.96±0.99                 | 23.13±0.80 a                | 21.38±0.48                 | 19.90±0.43                 | 20.21±0.85 b               |
| Liver enzyme ALP (IU/L)            | 209.36+5.3 4               | 198.08±5.2 b               | 288.93+14.3 b               | 199.23+9.1 6               | 353.00+51.76 a             | 378.66+15.3 0 a            |
| A/G ratio                          | 0.74+0.03                   | 0.81+0.05 b                | 0.76+0.03                   | 0.77+0.3                   | 1.04+0.07 a                | 0.75+0.24                  |

The different small letters refers to significant variations at (p≤0.05)

The weight of kidneys, testes, and Wool fleece of lambs fed bypass protein were heavier (P ≤ 0.05) than those of lambs fed control diet, while no significant differences were found in Weight of head, feet and liver between the lambs fed control diet and those fed bypass protein (Table 3).

Table (3): Effect of bypass protein on weight of organs in local male lambs

| Items                              | Treatments                  |
|------------------------------------|-----------------------------|
|                                    | control                     | Bypass protein              |
|                                    | 2.15 ± 0.13                 | 2.07 ± 0.14                 |
| Weight of head (kg)                | 0.95 ± 0.31                 | 0.93 ±0.37                 |
| Weight of feet (kg)                | 3.99 ± 0.17 b               | 4.20 ± 0.09 a              |
| Weight of Wool fleece (kg)         | 176.00±2.44 b               | 195.00±8.66 a              |
| Weight of kidneys(g)               | 258.00±23.96 b              | 298.00±23.09 a             |
| Weight of testis (g)               | 1.65 ± 0.74                 | 1.72±0.12                 |

The different small letters refers to significant variations at (p≤ 0.05)

Table (4) demonstrates that there is no significant differences in the rumen fermentation parameters such as total volatile fatty acid (TVFA) and (pH) for each of the control group and bypass protein group while rumen ammonia nitrogen had lower value in bypass protein group compared with control group.

Table (4): Effect of bypass protein on rumen fermentation parameters in local male lambs

| Items                              | Treatments                  |
|------------------------------------|-----------------------------|
|                                    | control                     | Bypass protein              |
| Rumen pH                           | 6.53 ±0.15                  | 6.79 ±0.09                  |
| Rumen VFAs mEq/100ml               | 9.8 ± 0.63                  | 10.0 ± 0.70                 |
| Rumen ammonia-N (mg/ 100ml)        | 6.8 ± 0.62 a                | 5.9 ± 0.31 b               |

The different small letters refers to significant variations at (p≤ 0.05)
We found a large amount of Meat crude protein in the LD muscle of local lambs. Analyses of the study amino acids compositions of meat showed that there is no significant differences were present in meat between the control group and Bypass protein group. A significant difference (p≤0.05) in Crude Protein digestibility of Bypass protein group compared with control one showed in found (Table 5).

Table (5): Effect of bypass protein on meat amino acids in local male lambs

| Amino acids      | Treatments | Control    | Bypass protein |
|------------------|------------|------------|---------------|
|                  |            | 7.597± 0.203 | 7.2100± 0.080 |
| Arginine         |            | 1.975± 0.046 | 1.9750± 0.125 |
| Cysteine         |            | 7.027± 0.334 | 6.4450± 0.235 |
| Isoleucine       |            | 7.435± 0.238 | 6.8150± 0.345 |
| Leucine          |            | 2.090 ± 0.161 | 1.9050± 0.075 |
| Lysine           |            | 3.902± 0.097  | 3.5700± 0.150 |
| Threonine        |            | 0.732± 0.026  | 0.6700± 0.010 |
| Tryptophan       |            | 5.483± 0.135  | 5.1650± 0.215 |
| Valine           |            | 15.85 ±0.18b  | 17.42 ±0.31a  |
| Meat crude protein |          | 46.31 ± 2.28b | 57.19 ±2.58a |

The different small letters refers to significant variations at (p≤0.05)

The fatty acid, which the most abundant in the meat from both groups was oleic acid (C18:1). The overall the oleic acid and linoleic acid (C18:2) was significantly (P≤0.05) higher in meat of lambs fed bypass protein compared with meat from control group. There was an insignificant difference in Palmitic acid (C16:0), Stearic acid (C18:0) and Arachidonic acid (C20:4) contents. The lambs of the control group showed the highest levels (P≤0.05) of the Myristic acid (C14:0) and Palmitoleic acid (C16:1) compared to the lambs of bypass group Figure (1).

![Percentage of fatty acids](image_url)

Figure (1): Meat fatty acids in local male lambs of bypass protein and control group
Discussion

The growth performance of lambs was improved by supplement treating protein with formaldehyde. Similar results were found by (12, 13) on sheep and non-lactating dairy cows, respectively. Other researchers found an increase (p≤0.05) in average daily gain (ADG) in animals fed on total mixed ration (TMR) containing rumen treated soya bean meal (SBM) with formaldehyde compared with those fed TMR containing only soya bean meal (SBM). These results indicate that the sheep fed soybean treated with formaldehyde had significantly best daily gain compared to sheep fed untreated soybean (12), and similar results was found by (14). Table (1) refereed to that lambs in both groups consumed same quantity of feed (treated and untreated meals). Similar results were reported by (15) in lactating goats. (16) reported no differences in dry matter intake (DMI) when replaced the soybean meal by rumen undegradable protein (RUP) sources in dairy cow due to increase in quantity of amino acid escaping rumen degradation and reaching the lower gut for absorption in addition to decrease losses of ammonia from the rumen or a combination of these factors (16, 17). Unfortunately, there is limited data regarding the protein requirements of local lambs in our region. The significant increases in the ALP could refereed to the high anabolism process of young animals, which play a vital role in this process either in the bone or muscle anabolism and caused an increase in its level in the body with age. Data concerning ALP excretion of liver enzyme indicator for better metabolism. Results were agreement with (18), whom showed that level of total serum protein a significantly increased in lamb fed dietary treated (basal diet with protected protein) as compared with the group of control lambs. This indicated that, treatment with protected protein was affected protein synthesis in liver and utilization of diet proteins. On the other hand, significantly (P ≤ 0.05) increase in A/G ratio at the second month of study, which refereed to normal status of liver function, because the main organ of albumin synthesis is the liver, as well as A/G ratio has been shown to be a good indicator of nitrogen status in lambs (19). The depression in the rumen ammonia of lambs fed protected meal was reflected significantly in lower level of urea in the blood of lambs (20). In addition, such improvement of wool production may be related to increase of amino acids available for absorption in bypass protein-fed group. In fact nutrition is highly correlated with wool production and improvement in health status (21, 22, 23). In addition, (24) showed high correlation coefficient between body weight and wool growth this means that wool growth can attribute with body growth as a whole. In addition, (25) confirmed that wool growth and its physical traits improved when sheep fed on a diet containing high protein and sulfur, causing an increase in amino acids such as methionine and cysteine which have a vital role for wool growth and its physical traits (26). The lower rumen ammonia production in the animals fed protected protein meal attributed to the low degradation of protein (27). The major source of fat in the feed is meat, which have been implicated in diseases, especially in developed countries. The fatty acid composition plays an important role in the definition of meat quality because it is related to the nutritional value for human consumption (28). Several studies have been shown those animals diet can strongly influence the fatty acid composition of meat (29). Limited information is available upon the effect of bypass meal on fatty acids composition of meat in ruminants. Overall, the data showed significant qualitative and quantitative differences in the lipids composition of lamb’s meat, those attributed to different nutritional conditions might alter fatty acids composition in the muscles of ruminants (30). Similar results found by (28, 31).
Conclusion:
The addition of bypass protein meal to diet of local lambs for fattening improve the production performance by give the significant positivity effect on growth and fat composition of meat.

References
1- Al-Jeboory ZI. Effect of Guar Meal Substitution and Sweet Basil Addition to the Diet on Productive Performance and Some Biochemical Traits of Awassi Lambs. Ph.D. Dissertation, (2014); College of veterinary medicine, University of Baghdad.
2- Kumar S, Kumari R, Kaushalendra K, Walli TK. Roasting and formaldehyde method to make bypass protein for ruminants and its importance: Indian Journal of Animal Sciences,(2015);85 (3), 223–230.
3- Chen DW, Zhang M. Non-volatile taste active compounds in the meat of Chinese mitten crab (Eriochir sinensis). Food Chemistry,(2007); 104(3), 1200-1205.
4- Cai ZW, Zhao XF, Jiang XL, Yao YC, Zhao CJ, Xu NY, Wu CX. Comparison of muscle amino acid and fatty acid composition of castrated and uncastrated male pigs at different slaughter ages. Italian Journal of Animal Science,(2010); 9(2), 33.
5- Saeed AA. Effect of level and degradability of dietary protein fed without baker's yeast (saccharomyces cerevisiae) on Turkish Awassi lamb’s performance. Ph.D. Dissertation, (2011); College of Agriculture, University of Baghdad.
6- Crocker C. Rapid determination of urea nitrogen in serum or plasma without deproteinization. The American Journal of Medical Technology,(1967); 33(5), 361-365.
7- Henry R, Cannon D, Winkelman J. Determination of calcium by atomic absorption spectrophotometry, Clinical Chemistry, Principles and Techniques, (1974); 657.
8- Kassem MM. Feed intake and milk production in dairy cow with special reference to diets containing grass and Lucerne silage with barley supplement. Ph.D. Dissertation,( 1986); Hanna Research Institute. Ayr. Scotland.
9- Snedecor GW, Cochran WG. Statistical methods. 6th edition. Iowa state University press (1973).
10- Al-Fartosi Kh G, Talib Y J, Ali Sh. Comparative study of some Serum Biochemical parameters of cattle and sheep of the marshes in the south of Iraq. AL-Qadisiya Journal of Vet. Med. Sci.(2010); 9(2), 78-84.
11- Ghanim S, Jasim F, Abood HK. Comparative study of serum protein status of local breed's sheep and goats in Basra province, AL-Qadisiyah Journal of Vet. Med. Sci., (2016);15(2),16-19.
12- Spears JW, Hatfield EE, Clark JH. Influence of formaldehyde treatment of soybean meal on performance of growing steers and protein availability in the chick. Journal of Animal Science, (1980); 50,750-755.
13- Kanjanapruthipong J, Vajrabukka C, Sindhuvanich S. Effects of formalin treated soy bean as a source of rumen undegradable protein on rumen functions of non-lactating dairy cows on concentrate based-diets. Asian-Aust. Journal of Animal Science, (2002); 15(10),1439-1444.
14- Archibeque SL, Burns JC, Huntington GB. Nitrogen metabolism of beef steers fed endophyte-free tall fescue hay: Effects of ruminally protected methionine supplementation. Journal of Animal Science, (2002); 80, 1344–1351.
15- Tewatia BS, Khatta VK, Virk AS, Gupta PC. Effect of formaldehyde-treated faba beans (Vicia faba L.) on performance of lactating goats. Small Ruminant Research, (1995); 16,107-111.
16- Santos FAP, Huber JT, Theurer CB, Swingle RS, Simas JM, Chen KH, Yu P. Milk yield and composition of lactating cows fed steam-flaked sorghum and graded concentrations of ruminally degradable protein. J. Dairy Sci., (1998); 81 (1), 215-220
17- Husain RAK, Offer NW. Effect of formaldehyde treatment on the degradation of acid-preserved fish silage protein in vitro. Anim. Feed Sci. Techn., (1987); 16,297-304.
18- Abdul-Ghani E, Hyde KF, Marshall R. Emic and etic interpretations of engagement with a consumer-to-consumer online auction site. Journal of Business Research,( 2011); 64, 1060-1066.
19- Laborde CJ, Chapa AM, Burleigh DW, Salgado DJ, Fernandez JM. Effects of processing and storage on the measurement of nitrogenous compounds in ovine blood. Small Ruminant Research, (1995); 17, 159-166.
20- El-Ayek My, Gabr AA, Mehriz AZ. Influences of substituting concentrate feed mixture by Nigella sativa meal on 2. Animal performance and carcass traits of growing lambs. Egypt. J. Nutr. and Feeds, (1999); 2(special Issue), 265-277.
21- Harris PM, Sinclair BR, Toreloar BP, Lee J. Short term changes in whole body and skin sulfur amino acid metabolism of sheep response supplementary cysteine. Aust. J. Agric . Research, (1997); 48(2), 137-146.
23- Majeed SH, Mahmodj MM. Iraqi Medical Plants, 1st ed.,In, Iraqi herbal Between Popular Medicine and Scientific Research, (1998); 15-16 and 77- 105 Dar Al-Thoura - Baghdad.
24-Mert N, Gunduz H, Akgunduz V, Akgunduz M. Correlation between biochemical parameters and production trait in Merino cross sheep III. Glucose, Alkaline phosphatase, ceruloplasmin. Turk. J. Vet. Anim. Sci., (2003); 27,583-588. (Cited by Ramzi, 2010).

25-Al-Saigh MNR. A study on physical traits of wool in Arabi sheep. Basrah J. Sci., (1990); 3, 9-20.

26-Al-Saigh MNR, Al-Kass JE. Sheep and Goat Production. Dar Al-Hikma, (1992); University of Basrah.

27-Al-Hellou MAH. Uses of some hematological and biological criteria as growth guide and a study of sexual maturity and wool physical traits in Arabi lambs. Ph.D. Dissertation, (2005); College of Agriculture - Basrah University.

28-Gulati SK, Kitessa SM, Ashes JR, Simos G, Wynn P, Fleck E, Scott TW. Designing milk fat for the new millennium by dietary strategies. Asian Aust. J. Anim. Sci., (2000); 13, 538-541.

29-Wood JD, Enser M, Fisher AV, Nute GR, Sheard PR, Richardson RI. Fat deposition, fatty acid composition and meat quality: A review. Meat Sci., (2008); 78, 343–358.

30-Nieto G, Ros G. Modification of fatty acid composition in meat through diet: effect on lipid peroxidation and relationship to nutritional quality- a review. In: Biochemistry, Genetics and Molecular Biology, (2012); ed. Catala, A. ISBN: 978-953-51-0716-3, InTech, DOI: 10.5772/51114. Pages 239-258.

31-Maia MRG, Correia CAS, Alves SP Fonseca AJM, Cabrita A RJ. Technical note: stearidonic acid metabolism by mixed ruminal microorganisms in vitro. J. Anim. Sci., (2012); 90: 900-904.

32-Scerra M, Caparra P, Foti F, Cilione C, Zappia G, Motta C, Scerra V. Intramuscular fatty acid composition of lambs fed diets containing alternative protein sources. Meat Sci., (2011); 87,229-233.