# التغيرات التى تحدث فى مكونات محلول الكرش فى الأغنام خلال ٢٤ ساعة من التغذية على علائق مختلفة فى نسبة البروتين

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## الملخص

أجرى هذا البحث على ثلاثة مجموعات من كل من الأغنام الأوسيمى والصعيدى كل مجموعة مكونة من أربعة خراف أثنان من كل نوع واستخدمت ثلاثة علائق متساوية فى الطاقة ومختلفة فى نسبة البروتين وكانت نسبة البروتين فى هذه العلائق ٧٪ ، ١٤٪ ، ٢٤٪ على التوالى .

وقد أجرى على محلول الكرش الذى كان يتحصل عليه باستخدام اللى المعدى تقدير درجة تركيز الايدروجين والتركيز الكلي للاحماض الدهنية الطيارة ، النسب المختلفة للمركبات الازوتية أجريت هذه التقديرات قبل التغذية ثم على فترات ٤، ٨، ١٢، ٢٤، ساعة بعد تقديم العلائق وفيما يلى ملخص للنتائج التى أمكن الحصول عليها :

١ ــ لا يوجد فرق فى درجة تركيز الايدروجين بين العلائق المختلفة ولو أنه وجد فرق جوهرى بين درجات التركيز للعينات المأخوزة على فترات مختلفة من وقت تناول العليقة .

٢ ــ كمية البروتين المأكولة لها تأثير معنوى على التركيز الكلى للاحماض الدهنية الطيارة فى سائل الكرش .

٣ ــ توجد علاقة قوية بين محتوى العليقة المأكولة من الأزوت الكلى والامونيا فى محلول الكرش كما أن ميعاد أخذ العينة له تأثير معنوى على تركيز هذه المكونات .

٤ ــ وجد ارتباط قوى بين المحتوى الأزوتى لمحلول الكرش والتغيرات فى الوزن الحى للحيوانات .

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# DIURNAL CHANGES OF RUMINAL FLUID COMPONENTS AT THREE LEVELS OF PROTEIN IN RATIONS OF RAMS

(with five tables)

*By*

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## SUMMARY

Three diets contained different protein levels were fed to three groups, two animals in each, of Ossimi and Saidi rams. The three diets contained 7%, 14% and 24% dig. Protein. The PH value, concentration of total volatile fatty acids and the distribution of nitrogen in the ruminal liquor were measured before feeding as well as at 4, 8, 12 and 24 hours after feeding. The following results were obtained :

1. There were no significant differences in ruminal pH due to protein treatment. However, significant differences between sampling times were present.
2. Protein intake sigrificantly affected the concentration of volatile fatty acids.
3. Total nitrogen and ammonia nitrogen contents of the ruminal liquor were closely related to the nitrogen content of the diet Diurnal changes in the concentration of these components were significant
4. A close relationship (r = 0.849) was proved between liveweight changes and the nitrogen content of their ruminal liquor.

## INTRODUCTION

The importance of rumen in relation to metabolism of the whole body is well illustrated by a considertion of sources of energy to the ruminant (ANNISON and LEWIS, 1959). The influence of composition and physical form of the ration on rumen fermentation has been widely investigated (WEISS *et al.*, 1967). Also, the level of protein intake affects the digestibility of the diet and ruminal volatile fatty acids concentration (BATH and

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ROOK, 1963). Thus for assessment of the nutritive value of ruminant food, a knowledge about the production of VFA from the food is necessary. Carbohydrates in the rumen are mainly fermented to acetic, propionic and butyric acids which are readily absorbed.

Most dietary protein is converted in the rumen to form ammonia. Part of this ammonia is then used in the synthesis of microbial protein in which accounts for most of the nitrogen passing to the omasum (WELLER *et al.*, 1962). Loss of nitrogen from the stomach contents occurs when the amounts of nitrogen in the diet is high (RIDGES & SINGLETON, 1962) or when little digestible carbohydrate is available to encourage microbial protein synthesis (PHILLIPSON *et al.*, 1962).

Although many experiments have been carried out to clarify the effect of protein level on diurnal changes of the composition of the ruminal fluid, however, contradictious results were obtained. There-fore, the aim of the present work is to study the effect of various levels of protein in the ration of rams upon : firstly, the distribution of nitrogen in ruminal liquor. Secondly, the diurnal changes of ruminal PH as well as concentration of volatile fatty acids and nitrogen throughout the 24 hours following feeding of these various levels

Also, a relationship between the end products of ruminal liquor and body weight changes have been studied.

## MATERIALS AND METHODS

This study was carried out in the Experimental Farm of the Faculty of Agriculture, Assiut University. Twelve Ossimi and Saidi rams (18 month age, of 43-47 kg. live body weight) were used in this study. Animals of each breed were distributed at random into three groups, 2 animal in each. Experimental groups were designated as I, II, III, IV, V and VI. The first three groups were Ossimi and the others were Saidi. Groups I and IV; II and V and III and VI were fed rations contained 7% 14%, and 24% dig. protein respectively. The composition of the experimental diets is shown in Table (1). Animals were fed individually. The energy intake was 1.7 times of maintenance level. The average daily starch value (S.V.) and dig. protein (D.P.) intake is shown in Table (2). Animals were weighed monthly. This study was extended for 6 months.

The sampling time was proceeded with a 30-day preliminary feeding period. Ruminal samples were collected via stomach tube from all animals using a suction pump, and the samples were taken before feeding as well as at 4, 8, 12 and 24 hours after feeding.

Free ammonia in freshly strained ruminal liquor samples was determined by the CONWAY microdiffusion method (CONWAY, 1957). Ruminal fluid PH was measured immediately after collection by the use of glass electrodes

TABLE 1. **Composition of the experimental rations**

| | Low protein | Medium protein | High protein |
|---|---|---|---|
| | % | % | % |
| Undecorticated cotton seed cake . . . . | 20 | 40 | — |
| Decorticated cotton seed meal . . . . | — | 10 | 50 |
| Barley . . . . . | 42 | 27 | 7 |
| Wheat bran . . . . . | 35 | 20 | 25 |
| Horse beans . . . . . | — | — | 15 |
| Lime stone. . . . . | 2 | 2 | 2 |
| Common salt . . . . . | 1 | 1 | 1 |
| Dig. protein %. . . . . | 7.0 | 14.0 | 24.0 |

TABLE 2. **Average daily starch value (S.V.) and dig. protein (D.P). intake).**

| Breed | Ossimi | | Breed Groups | Saidi | |
|---|---|---|---|---|---|
| Groups | S.V. (kg) | D.P. (gm) | | S.V. (Kg) | D.P. (gm) |
| I | 0.78 | 91 | IV | 0.73 | 84 |
| II | 0.85 | 182 | V | 0.81 | 169 |
| III | 0.84 | 312 | VI | 0.73 | 288 |

PH meter (Model Multiscope). Total concentration of volatile fattty acids (TVFA) in ruminal liquor was dterminated using MARKHAM distillations apparatus after MARKHAM (1942). Total and nonprotein nitrogen were determined in the filtrate of the ruminal content using microkjeldahl method, while the protein nitrogen was calculated by the differences between the total and nonprotein initrogen (NPN).

The weighed mean of total volatile fatty acids, total nitrogen, protein nitrogen, nonprotein nitrogen and ammonia nitrogen were obtained in the following manner according to KLLIOT and TOPPS (1964). Simple means for eachof the four intervals between the five observations were first clacultaed and

each of these was then multiplied by the time interval it represented. The products were summed and the summation was divided by the total time of 24 hours.

Statistical analysis was carried out according to SNEDECOR (1962).

## RESULTS AND DISCUSSION

*PH Values* :

The mean PH values of the rumen contents were markedly similar for all groups, irrespective of the level of protein intake (Table 3). Differences between treatment, breeds and the interaction between breeds and treatment, were not significant (Table 4). Similar results were reported by ELLIOT *et al* (1964); and FONNESBECK *et al* (1970). The regulation of the PH in the rumen, mainly takes place by the addition of alkali to the ruminal ingesta through the flow of the saliva and through the elimination of fatty acids from the rumen by absorption and buffering action of rumen ingesta (ANNISON and LEWIS, 1959) Significant differecnes ($P<0.05$) between sampling times were found (Table 4). Diurnal variations in the PH in the ruminal liquor have been observed by FONNESBECK *et al* (1970) and SHEHATA (1972). These differences were directly related to the alteration in the rumen microbial pop- ulation (EL-KHOLY, 1972).

*Total concentration of VFA* :

The highest level of protein intake (24%) cuased significant increase ($P<0.01$) in VFA concentration in the rumen which continued over the entire 24 hr. (Table 3). Linear increase in the concentration due to the protein treatment were also detected ($r=0$ 596). The obtained results are in agreement with those reported by BATH and ROOKE (1963), that protein intake affected ruminal VFA concentration).

Significant differences ($p<0.01$) between breeds in TVFA were found. These differences may be attributed to differences in energy intake between breeds, since high levels of energy intake seemed to have a stimulating effect on the production of ruminal VFAS (ABOU-AKKADA *et al* 1971). Also available protein content of the ration may be reason for such differences between breeds (Table 2).

The highest concentration of VFA which was recorded 8 hours after feeding seems to be due to an optimum assimilation of ration by the ruminal microorganism. MORRIS et *al* (1965) on a range experiment, found the highest concentration at the ninth hour. By 12 *th* hours of feeding, the

TABLE 3. Diurnal changes in pH and total volatile fatty acids of rumen liquor.

| Breeds | Groups | pH values | | | | | | T.V.F.A. (m. eq/100 ml) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr. | 4 hr. | 8 hr. | 12 hr. | 24 hr. | Aver. | 0 hr. | 4 hr. | 8 hr. | 12 hr. | 24 hr. | Average |
| Ossimi | I | 6.50 | 6.42 | 6.53 | 5.98 | 6.35 | 6.35 | 55.25 | 76.81 | 125.12 | 86.58 | 78.51 | 86.58 |
| | II | 6.40 | 5.98 | 6.40 | 6.40 | 6.45 | 6.32 | 61.50 | 95.52 | 127.72 | 89.54 | 73.22 | 90.48 |
| | III | 6.30 | 6.12 | 6.53 | 6.25 | 6.58 | 6.36 | 80.40 | 62.64 | 143.50 | 103.29 | 128.40 | 112.17 |
| Saidi | IV | 6.45 | 6.20 | 6.65 | 6.52 | 6.48 | 6.46 | 57.43 | 82.07 | 86.37 | 69.28 | 57.56 | 70.34 |
| | V | 6.35 | 6.03 | 6.65 | 6.48 | 6.03 | 6.38 | 66.69 | 92.15 | 89.84 | 78.21 | 68.02 | 78.96 |
| | VI | 6.35 | 6.20 | 6.70 | 6.50 | 6.30 | 6.41 | 70.06 | 108.03 | 119.36 | 61.50 | 73.51 | 82.61 |

TABLE 4. Analysis of variance of the effect of breeds, treatments and periods on ruminal fluid components

| S.O.V. | d.f. | Means squares | | | | | |
|---|---|---|---|---|---|---|---|
| | | pH | Total VFA | Total N | NPN | PN | Ammonia N |
| Between periods "P" | 4 | 0.2852* | 4124.4329** | 87850.3** | 502.6428** | 93865.1849** | 576.6554** |
| Between treat. "T" | 2 | 0.0290 | 2203.7045** | 85616.4** | 415.9962** | 70939.8265** | 1620.9678** |
| Between breeds "B" | 1 | 0.0140 | 3766.4942** | 25814.3** | 892.6640** | 14814.4067** | 146.0785* |
| T × B . . . . . | 2 | 0.0325 | 364.3736 | 639.3 | 337.7836** | 558.8481 | 128.2048 |
| T X P . . . . . | 8 | 0.0477 | 196.6173 | 2013.9 | 265.7608** | 3915.5758* | 22.8407 |
| P X B . . . . . | 4 | 0.1167* | 891.483* | 7042.4* | 172.2410* | 4086.1529* | 170.2438** |
| T X P X B . . . | 8 | 0.1525* | 906.1876* | 6490.6* | 582.5819** | 6420.7769** | 194.9789** |
| Error . . . . . | 30 | 0.0215 | 232.861 | 2329.9 | 58.0082 | 1392.7694 | 28.6902 |

* Significant at 5 % level.
** Significant at 1% level.

concentration of fatty acids had declined markedly from the maximum values, but for all diets, they were still greater than the concentration before feeding. This diurnal pattern agrees with the findings of SHEHATA (1972) who rported that the concentration of VFAs' increased to a peak 1 to 6 hours after feeding depending on the nature of the diet and then decreased gradually till the next feeding.

Analysis of variance of ruminal volatile fatty acids concentration between sampling times was sinificant ($p<0.01$). The striking difference between sampling times in the concentration of VFA is an indication of the relative differences in rate of ruminal fermentation.

*Total nitrogen* :

Values obtained at the different sampling times are shown in Table (5) with weighed means conetration for the complete 24 hours. At all sampling times, the average level of ruminal nitrogen (N) was correlated with the protein content of the diet, since linear increases occureed in ruminal N content as dietary protein increased ($r= 0.832$). Maximal conentration of ruminal N was observed at eight hour after feeding. The rise in N conetnt after feeding became more fast as the protein level of the diet increased. This relatively sharp increase in ruminal N suggests that the N components of the diets with the highest proteint content were rapidly dissimilated in the rumen from the coarse particles of food to either particles of small size or soluble forms of N (ELLIOT and TOPPS, 1964).

*Ruminal ammonia* :

The means of conecntration of ammonia N in rumen liquor at the different sampling times are shown in Table (5). In general, the weighed mean of ammonia N was found to be positively and linearly related to the nitrogen content of the given food ($r = 0{\cdot}888$). Obtained results are in agrerent with those of FONNESBECK *et al.*(1970) that protein treatment couse significant increase in ammonia concentration.

The level of ammonia N in rumen liquor showed diurnal changes during the day on all diets. The results showed that the maximum levels of ammonia in ruminal liquor occurred from 4 to 8 hours after feeding, but usually these peaks occurred later. The level of ammonia reflects the difference between production from nitrogen sources and uptake by micro organism (ANNISON *et al.*, 1954). The observed levels of ruminal ammonia would depend upon the relative spead and time in these processes afterr feeding. In addition, the diffusin of ammonia through the rumen wall would be an important factor in determining its concentration in the rumen at any time (JOHINS, 1955).

The second peak in ammonia levels, which occurred 24 hours after feeding, may be due to recycling of ammonia through the saliva (HOSHINO *et al* 1966).

TABLE 5. The diurnal changes of different nitrogen fraction in rumen liquor.

| Groups | Total N (mg %) | P.N. (mg %) | P.N. (% of T.N) | NPN (mg %) | NPN (%of T.N.) | Ammonia N (mg %) | Ammonia % of T.N |
|---|---|---|---|---|---|---|---|
| | | | *First analysis (before feeding)* | | | | |
| I | 235.51 | 114.74 | 78.72 | 120.79 | 51.28 | 26.52 | 11.26 |
| II | 261.50 | 118.37 | 45.17 | 143.13 | 54.73 | 35.75 | 13.67 |
| III | 359.03 | 198.06 | 55.17 | 160.98 | 44.83 | 53.78 | 14.98 |
| | | | *Second analysis (4 hrs aftetr feeding* | | | | |
| I | 435.62 | 264.72 | 60.77 | 146.40 | 39.23 | 44.49 | 10.21 |
| II | 466.96 | 322.88 | 69.15 | 143.84 | 30.85 | 45.52 | 9.75 |
| III | 578.96 | 466.95 | 80.65 | 164.27 | 19.35 | 68.95 | 11.91 |
| | | | *Third analysis (8 hrs qfter feeding)* | | | | |
| I | 451.69 | 283.91 | 62.86 | 167.79 | 37.14 | 45.52 | 10.08 |
| II | 480.99 | 330.65 | 68.74 | 150.36 | 31.26 | 51.61 | 10.73 |
| III | 554.72 | 405.30 | 73.06 | 149.38 | 26.94 | 61.06 | 11.00 |
| | | | *Fourth analysis (12 hrs after feeding)* | | | | |
| I | 338.55 | 243.64 | 62.71 | 144.91 | 37.29 | 35.29 | 9.08 |
| II | 391.00 | 240.11 | 61.41 | 145.89 | 38.88 | 39.28 | 10.05 |
| III | 470.07 | 293.51 | 62.44 | 176.07 | 37.56 | 54.72 | 11.64 |
| | | | *Fifth analysis (24 hrs after feeding)* | | | | |
| I | 318.35 | 155.95 | 48.99 | 161.92 | 51.01 | 60.92 | 19.14 |
| II | 328.85 | 171.40 | 52.12 | 157.46 | 47.88 | 54.82 | 16.67 |
| III | 381.92 | 223.58 | 58.54 | 158.39 | 41.46 | 47.02 | 19.38 |
| | | | *The weighed mean* | | | | |
| I | 372.62 | 221.20 | 59.23 | 151.42 | 40.77 | 44.22 | 11.90 |
| II | 390.65 | 241.65 | 62.04 | 148.98 | 37.96 | 49.72 | 12.77 |
| III | 490.16 | 315.61 | 64.31 | 174.55 | 35.69 | 62.89 | 12.98 |

TABLE 5. (*Cont.*)

| Groups | Total N (mg %) | P.N. (mg %) | P.N. (% of T.N. | NPN (mg %) | NPN (% of TN.) | AmmoniaN (mg %) | Ammonia % of T.N. |
|---|---|---|---|---|---|---|---|
| | | | *First analysis (before feeding)* | | | | |
| IV | 278.00 | 152.41 | 54.82 | 125.59 | 45.18 | 34.13 | 12.34 |
| V | 290.91 | 156.79 | 53.89 | 139.62 | 46.11 | 50.12 | 17.22 |
| VI | 348.34 | 204.93 | 58.83 | 143.41 | 41.17 | 52.09 | 14.95 |
| | | | *Second analysis (4 hours after feeding)* | | | | |
| IV | 372.12 | 226.20 | 60.79 | 145.91 | 39.21 | 40.52 | 10.89 |
| V | 472.02 | 307.09 | 65.06 | 164.93 | 34.94 | 52.07 | 11.03 |
| VI | 589.86 | 440.98 | 74.76 | 148.87 | 26.24 | 52.51 | 8.90 |
| | | | *Third analysis (8 hours after feeding)* | | | | |
| IV | 375.33 | 229.44 | 61.13 | 145.89 | 38.87 | 50.81 | 13.54 |
| V | 431.64 | 292.79 | 67.83 | 138.85 | 32.17 | 45.72 | 10.59 |
| VI | 546.24 | 398.35 | 72.93 | 148.39 | 28.07 | 64.42 | 11.79 |
| | | | *Fourth analysis (12 hours after feeding)* | | | | |
| IV | 309.93 | 174.03 | 56.15 | 135.90 | 43.85 | 31.52 | 10.17 |
| V | 312.89 | 171.51 | 54.82 | 141.38 | 45.18 | 38.56 | 12.32 |
| VI | 386.35 | 247.48 | 64.06 | 138.86 | 35.94 | 43.08 | 11.15 |
| | | | *Fifth analysis (24 hours after feeding)* | | | | |
| IV | 217.43 | 65.51 | 30.13 | 151.92 | 69.87 | 38.77 | 17.83 |
| V | 269.02 | 111.62 | 41.50 | 157.41 | 58.50 | 50.83 | 18.89 |
| VI | 332.03 | 183.13 | 55.15 | 149.40 | 44.85 | 60.06 | 18.09 |
| | | | *The weighed mean* | | | | |
| IV | 305.41 | 163.03 | 53.28 | 241.38 | 46.72 | 38.28 | 12.47 |
| V | 346.82 | 196.87 | 56.59 | 149.95 | 43.41 | 46.00 | 13.33 |
| VI | 429.42 | 285.25 | 61.23 | 144.17 | 38.77 | 53.23 | 12·34 |

The protein N content of ruminal liquor presented in Table (5) showed that increasing level of prtcein intake caused a significant ($p<0.01$) increase in the level of protein N of rumen liquor. Linear relationship ($p=0.860$) between the N contents of rumen liquor and of the food eaten was found. These results agree with ELLIOT and TOPPS (1964).

Dirunal changes in level of PN were very pronounced. Values recorded before feeding and at 24 hours after feeding were lower than those observed at other times. The highest level was observed at 4 to 8 hours of feeding. Significant differences ($p<0.01$) were existed between the sampling dates. Similar results were repoted by ELLOT and TIOPPS (1964).

The protein N expressed as percentage of total N reveals that the lowest values of protein N correlated with the highest percentage of ammonia N. Similar results were reported by HUME *et al.* (1970, The diurnal changes in the relative values of prcetein N are in accordance with the dynamic changes in the relative values of ammonia N in the rumen liquor. These results are in agreement with those of ELLIOT and TOPPS (1964) and ABD El-HAFIZ (1973)

A close positive relationship ($r=0.849$) existed between live weight changes of the rams and the protein N content of their rumen liquor. ELLIOT and TOPPS (1964) foud that maintenance of body weight was associated with a N content of rumen liquor of 0.34 mg N/ml. This close association between N content of rumen liquor and body weight change was observed by ELLIOT *et al.* (1964)

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