The Effect of Different Levels of Protein Supplementation in Rations on Feed Consumption, Milk Yield and Milk Composition in Anatolian Buffaloes of Different Ages [Bubalus Bubalis (Linnaeus, 1758)].

Taşkın DEĞIRMENCİOĞLU (taskin@uludag.edu.tr)
1Uludag University, Karacabey Vocational School

Research

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Abstract

Background

In this study, it was aimed to determine the optimal crude protein level in the ration for milk production and milk composition in milk buffaloes of different ages.

Materials, Methods & Results

A total of 20 Anatolian buffaloes were used as animal material. Buffalo cows were tested in the early lactation period (lactation period; 65-80 days). Water buffaloes are divided into four groups as young and adult; First, second groups (those who gave birth to the first), third and fourth groups (those who gave birth to the second and more). 5 compartments are allocated for each group. In the trial, buffalo cows with two different trial rations R1; (17.82% CP) and R2; (19.18% CP) were fed. Especially during the experiment, the total DM consumption of adult buffaloes in group 4 and group 3 increased when compared with young buffaloes (p <0.05). As a result of the research, the milk yield values determined in milk buffaloes fed with the R2 ration were found to be higher than the values found in milk buffaloes fed with the R1 ration (6.34-5.64kg / day) (p <0.05). However, the effect of R1 and R2 rations on milk production was similar in young buffaloes (4.22-4.36 kg / day) (p> 0.05).

Introduction

The protein requirement of water buffalo varies greatly according to body weight, body weight gain, pregnancy, lactation period and the protein content of the milk. Milk producers feed their cows with a ration with high crude protein content in order to increase the milk yield and protein content of their cows and encourage the necessary microbial protein in the rumen environment (Olmos Colmenoro & Broderick, 2006). In a study where they investigated the effects of different protein-containing rations on the productivity criteria of cows, increasing the crude protein content (CP) of the ration from 15–16% increased milk production by 0.75 kg / day, and the CP level of the ration was increased from 19–20%. determined that it positively affected milk yield (0.35 kg / day). The highest milk yield was obtained with a ration containing 23% CP Anonymous (2001). On the other hand, rations containing high levels of protein and low levels of energy cause fatty, infertility, laminitis and ketone formation in the mammary glands. In case of protein deficiency, animals reduce feed consumption (Değirmencioğlu, 2020). Similarly, Morrow (1976) states that cows with a high condition score in the early lactation period and fed a ration containing 15% Crude protein have a high incidence of metabolic disorders such as ketosis and anorexia, where feed consumption and milk yields are lower than expected. When the studies on the effects of different protein levels in the ration on milk yield were examined, it was seen that different results were obtained (Anonymous, 1978). As a matter of fact, while the increase in crude protein in the ration increases milk yield in some studies (Gardner and Park., (1973), Polan et al., (1976), Roffler et al., 1978), in some studies the protein level in the ration (16.1 %- It is stated that the increase from 16.7 CP; to 18.4–
18.9% CP) does not affect milk yield (Van Horn et al., 1976, Kwan et al., 1977, Grieve et al., 1980, Cunningham et al., 1996, Broderick, 2003 and Leonardi et al., 2003). When the crude protein level of the ration rises, it is broken down in the rumen and used in microbial protein synthesis, the remaining ammonia passes into the blood, then turns into urea in the liver. Some of the urea is excreted in urine. Urea in urine is rapidly hydrolyzed to ammonia and causes environmental pollution (Muck, 1982). In addition, excessive use of protein-based feed raw materials in the ration reduces the profit margin due to the high cost of production. In feeding buffalo and dairy cows, Turkey prefers sunflower meal obtained by extrusion as a protein feed. The number of buffaloes in our country has decreased rapidly due to reasons such as draining wetlands, opening pastures to collective housing, migration from village to city, and preference of high-yielding breeds. As a matter of fact, the number of Anatolian buffaloes is stated as 135,000 heads. Anonymous, (2018). Due to the small scale of water buffaloes and insufficient water buffalo breeding methods and limited capital in Turkey buffaloes are unidirectional and feeding, nutritional disorders occur in the first months of lactation and milk yield cannot reach the desired level (Degirmencioglu, 2016). The aim of this study is to determine the optimal crude protein level in the ration for milk production and milk composition in milk buffaloes of different ages.

**Materials And Methods**

**2.1. Trial material buffaloes**

The experiment was conducted in a semi-open type shelter between April and May 2018 in the Karacaoğlan buffalo union (Mustafakemalpaşa, Bursa). A total of 20 Anatolian buffaloes were used as animal material, young (3 old – 10 heads) and adult (4 years old 6 heads and 5 years old 2 heads and 6 years old 2 heads). Buffalo cows were tested in the early lactation period (lactation period; 65–80 days). Daily milk yield and live weight were taken as a basis for the distribution of water buffaloes to groups (Table 1).

**2.2. Trial material ratios**

Experimental animals were fed for a total of 30 days, 15 days of exercise and 15 days of main period. Water buffaloes are divided into four groups as young and adult; First, second groups (those who gave birth to the first), third and fourth groups (those who gave birth to the second and more). 5 compartments are allocated for each group. The mixture, which forms the basis of the ration consumed by the buffaloes in the research, was prepared in a special feed factory in Mustafakemalpaşa. In the research, 2 different rations were prepared, one for control and one for trial.

In the study, sunflower seed meal was added to the intensive feed mixtures of the groups at a level of 29% (R1) and 35% (R2), respectively. Care was taken to prepare the rations equally in terms of energy content. The structure of the dense feed mixtures used in the research, the nutrient contents of alfalfa hay and corn silage are given in Table 2. It was foreseen that the animals were given 5 kg of alfalfa hay shown in Table 2 and corn silage at ad-libitum level from corn silage to meet the necessary nutrients for survival and 4 liters of milk yield. At the end of the research, individual silage, alfalfa and intensive feed
consumption of buffalo cows in the main period was determined daily, taking into account the reports of Maynet (1984). Pasture consumption was not determined because of free pasture. The animals were milked twice a day at 6:30 a.m. and 7:30 p.m. Measuring tape (Galuna Balance, Purina brand) was used to determine the live weight of animals at the beginning of the experiment. In addition, while the milk yield of animals in the main period of 15 days was determined during milking, the milk obtained was sampled and the milk was kept in the refrigerator at 2–4 °C. The Weende Analysis method was used to determine the nutritional content of the feed raw materials used in the study. Anonymous (1990). Cell wall components of corn silage and alfalfa hay were determined according to the method reported by Robertson and Vansoest (1981). In the calculation of ME values of feeds, the equations stated below by Anonymous (1901) were used. The solids-non-fat content (SNF) and fat and protein components of the milk were analyzed using a Milcosan FT-120 device. SCC was determined with a Somacount 150 (Bentley Instruments, Chaska, USA).

2.3. Statistical analysis

In the study, an equality of variance between groups was not achieved. For this reason, in determining the differences between the mean values of the groups, the Cruska wals analysis and the Tamhanes T2 multiple comparison test in the post hoc were used to control the importance level of the differences between the groups (Turan 1995). All statistical analyzes were made in general linear model with the help of SPSS 15.0 package program.

Results And Discussion

As seen in Table 3, it was determined that the daily average dry matter consumption of buffaloes during lactation varied between 8.46 ± 0.13–11.65 ± 0.47 kg. The highest feed consumption was found in group 4, followed by groups 3, 2, and 1, respectively. Especially during the experiment, the total DM consumption of adult buffaloes in group 4 and group 3 increased when compared with young buffaloes (p < 0.05). It can be said that this situation arises as a result of higher body weight and milk yield of adult buffaloes compared to young buffaloes. Depending on the increase in the CP level in the diets, a slight increase was observed in the total dry matter consumption. As a matter of fact, when DM consumption is examined in Table 3, it is seen that the adult buffalo group fed with R2 ration in the main period was 0.35 kg / day higher than the adult buffalo group fed with R1 ration. Similarly, there was an increase of 0.18 kg / day in young buffaloes fed with R1 and R2 rations. However, the differences between the dry matter consumption of buffaloes between the groups fed with R1 and R2 rations were found to be insignificant. The results of this study are consistent with the research findings, which reported that there is no difference between the DM consumption of cows as the CP level of the ration increases (Polan et al., 1976; Foldager & Huber, 1979; Cressman, et al., 1980; Olmos Colmenoro and Broderick, 2006). However, some researchers reported a difference between the DM consumption of cows fed rations containing different CP content (Roffler et al., 1978; Van Horn et al., 1979). In a study conducted, Broderick (2003) reported that the increase in CP at the level of 15.1%, 16.7 and 18.3% in the ration linearly increased DM consumption in dairy cows, whereas the ration containing 18.3% CP was insignificant in increasing milk
yield. Cressman, et al., 1980) stated that DM consumption increased by 1.9 kg / day in the cows fed with 18% CP in the first period of lactation compared to the cow group fed 12% CP whereas it decreased in the group fed with 15% CP. He reported that this decrease was due to excessive conditioning stress. Similarly, Ipharraquerre and Clark (2005) reported on dry matter consumption (14-29.4 kg / day) and milk yield (15.1–45.5 kg / day) of feeding diets with different CP content (12.1–23.3%) in dairy cows. It was determined that it had a significant effect. The main differences in the literature are thought to be due to differences in animal factor, temperature inside the shelter, daylight, humidity, stress, particle size of the ration, energy level of the ration and structural polysaccharide content of the ration.

As seen in Table 3, the milk yield of buffaloes according to the groups are; It was determined that it varied between 4.22 ± 0.35–6.34 ± 0.06 kg / day.

As a result of the research, daily milk yield of buffaloes in the 4th group fed with R2 ration increased compared to the other groups (P < 0.05; Table 3). On the other hand, while the increase in crude protein level in the ration increased the milk yield of adult buffaloes, it had an insignificant effect on young buffaloes. As seen in Table 3; At the end of the main period, the average daily milk yield of adult buffalo cows in the 3rd and 4th groups fed with rations containing CP at two different levels (R1; R2;) varied between 5.64 ± 0.14 and 6.34 ± 0.06 kg / day, respectively. It was observed that the average daily milk yield of buffaloes increased due to the increase in the pulp level added to the ration. It was determined that 35% pulp layer (R2) in the ration significantly increased the average daily milk yield in buffaloes (11.04%; p < 0.05). These increases in milk yield levels are the result of higher DM consumption in this group. In young buffaloes, average daily milk yield ranged between 4.22 ± 0.35, 4.36 ± 0.05 kg / day, and their milk yield was higher in the group fed with R2 and low in the group fed R1. However, as a result of the analysis, it was determined that the difference between the groups was statistically insignificant and the crude protein changes in the ration did not have a significant effect on the average daily milk yield of heifers (p > 0.05). In a study on the subject, it was stated that cows in the 2nd and 3rd lactation fed with mixed feed with 15–16% crude protein (CP) produced more milk than cows fed with mixed feed with 12% CP content. However, it was reported that no significant difference was found in young cows consuming similar compound feed Roffler et al. (1978). Investigating the effects of rations containing CP at different levels (12%, 15% and 18%) on milk yield in cows and heifers, Cressman et al. (1980) It was determined that the milk yield of cows in the experimental group fed with a ration containing 18% CP was 18.85% higher than the milk yield of cows in the group fed with a 12% CP ration (p < 0.05), whereas the protein increase in the ration did not affect the milk yield. However, Leonardi et al., 2003; stated that when the CP level of the ration is increased to (16.1–18.8%), the increase in milk yield is lower. Similarly, it was reported that there was no significant difference in milk yield in dairy cows fed with a ration containing 16.5% 17.9 and 19.4 CP (Olmos Colmenoro and Broderick, 2006). However, a linear increase was observed in milk yield depending on the increased CP ratio (7.82 and 14.94%). The results obtained were found to be lower than the findings of Olmos Colmenoro and Broderick (2006). It can be said that this difference is due to species difference. As a result of the research, all the findings obtained from the research regarding the protein differences and milk yield of the rations are in great agreement with the results of similar studies on this subject and are supported by the results of these studies (Gardner and Park.,
At the end of the main period, the average daily milk yields of the experimental animals in groups 1, 2, 3 and 4, adjusted for 4% fat, were determined as 6.94 ± 0.17, 7.01 ± 0.21, 7.42 ± 0.50 and 7.97 ± 0.08, respectively. The differences between the groups in terms of milk yield corrected for 4% fat were statistically insignificant.

When the effects of using pulp at different levels in dense feed on the components of milk were examined, it was determined that the average milk protein levels of the experimental animals fed rations containing R1 and R2 dense feed varied between 4.04 ± 0.14–4.49 ± 0.25, respectively (Table 3). Differences between rations in terms of milk protein levels in the main period were statistically insignificant. It can be said that the reason for this is the starch-based feed raw materials in the protein formation in milk compared to the protein-based feed raw materials included in the ration (Degirmencioglu and Karabulut, 2010).

As it is known, with the increase in the amount of starch given to the animal, glucose provides more amino acids to the mammary glands by preventing the deamination of amino acids in the small intestine tissues and liver, resulting in an increase in milk protein level (Chamberlain and Wilkinson 1998). The conclusion obtained from the study that diets containing different levels of CP do not affect the milk protein content (Van Horn et al., 1976, Kwan et al., 1977, Grieve et al., 1980, Cunningham et al., 1996, Broderick, 2003 and Leonardi et al., 2003)'s reports. As seen in Table 3, the average milk fat levels of buffaloes ranged from 5.72 ± 0.06 to 8.32 ± 0.33. When young buffaloes in the same lactation period are compared with the adult buffalo group, the increase is striking (P < 0.05). It can be said that low milk yield in these young buffaloes may cause an increase in milk fat.

However, it was observed that using different levels of CP in the dense feed mixture did not affect the fat level in milk.

As a matter of fact, in a similar study conducted by Olmos Colmenoro and Broderick, 2006) on the subject, researchers determined that increasing levels of CP in dense feed (17.9–19.4%) did not affect milk fat (3.47–3.44%).

**Conclusion**

Based on the findings obtained in Table 3 as a result of the research, it is seen that rations containing sunflower seed meal have high feeding value for buffaloes. It was concluded that the intensive feed mixture R2 with 19.18% CP should be preferred at first due to the increase in milk yield (0.70 ml, 11.04%) (p < 0.05) observed in the adult buffalo group. However, the effect of R1 and R2 rations on milk production was similar in young buffaloes (4.22–4.36 kg / day) (p > 0.05). In general, R1 and R2 did not cause any difference in the milk composition of the buffaloes examined. This study is a pioneering study for future studies in different protein-containing diets and different age groups.

**Declarations**

**Ethics approval and consent to participate**
There is the consent of the owner of the business where I work on the buffaloes. The photo of the business owner is attached. The owner helped me to work personally. Ethical approval has not been obtained since it is not taken into the blood and tissues of animals.

**Consent for publication**

I hereby declare that the author of this manuscript, familiar with its content, have given their consent to publish the manuscript in the presented form in the Journal of Animal Science and Biotechnology.

**Availability of data and material**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The author declare that they have no competing interests" in this section.

**Funding**

Materials for analysis were provided from a previous project and from the researcher’s budget.

**Author contribution**

T. D. designed and conducted most of experiments, statistical analysing the result and writing. T.D was also involved to choosing sample of animals and collecting data.

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Tables

Table 1: Trial animals used in the research, body weights and milk yields and standard error values (mean±SE)

| Groups           | Number of animals | Body weight (kg) | Milk yield (kg / day) |
|------------------|-------------------|------------------|-----------------------|
| Group 1 Young buffalo | 5                | 386.20±2.75      | 3.38±0.24             |
| Group 2 Young buffalo | 5                | 384.20±2.78      | 3.52±0.22             |
| Group 3 Adult buffalo | 5                | 492.00±4.63      | 5.39±0.16             |
| Group 4 Adult buffalo | 5                | 512.00±15.09     | 5.30±0.16             |

Table 2: Ingredient composition and chemical analysis (%) (DM) of concentrate feed mixes, alfalfa hay and corn silage nutrient. (Naturally)
| Feed                                      | Groups                              | Roughages       |
|-------------------------------------------|-------------------------------------|-----------------|
|                                           | Group (1)/Young Buffalo             | Group (2)/Adult buffalo |
|                                           | Ration 1                            | Ration 2        |
|                                           | 17.82 % CP                          | 19.18 % CP      |
|                                           | Ration 1                            | Ration 2        |
|                                           | 17.82 % CP                          | 19.18 % CP      |
| Barley                                   | 34                                  | 34              |
| Wheat                                    | 35                                  | 35              |
| Sunflower Seed                           | 29                                  | 35              |
| Marble powder                            | 1                                   | 1               |
| Salt                                     | 0.75                                | 0.75            |
| Vitamin + - Mineral                      | 0.25                                | 0.25            |
| Total                                    | 100                                 | 100             |

**Nutrient content**

|                   | Group (1)/Young Buffalo | Group (2)/Adult buffalo |
|-------------------|-------------------------|-------------------------|
| Dry matter/(Natural) $^1$ | 90.17                   | 90.37                   |
| Organic matter    | 86.71                   | 86.59                   |
| Crude protein      | 17.82                   | 19.18                   |
| Crude fiber        | 9.00                    | 9.05                    |
| Crude oil          | 2.20                    | 2.21                    |
| Crude ash          | 3.46                    | 3.78                    |
| Nitrogen free ext  | 45.03                   | 36.41                   |
| Starch             | 28.88                   | 41.63                   |
| NDF (Neutral Detergent Fibre) | 21.66                  | 28.79                   |
| ADF (Acid Detergent Fibre)       | 11.71                   | 13.67                   |
| Energy ME (kcal kg) | 2649.09                | 2649.34                 |
| NR                 | 148.65                  | 138.13                  |

DM, Dry Matter; OM, Organic matter; CP, Crude protein; CO, Crude oil; NDF, Neutral detergent fibre; ADF, Acid detergent fibre; NFE, Nitrogen free ext.; CA, Crude ash; ME, Metabolizable energy. NR Nutrient ratio $^*$, Nutrient content of feed raw materials according to Weende analysis method Akyildiz, (1984)) and Metabolizable energy calculated according to the equation of TSE (1901)

**Table 3: Performance of Groups (mean±SE)**
| Features | Ration crude protein levels (%) | Significant |
|----------|--------------------------------|-------------|
|          | Group 1 Ration (R1) (17.82% CP) | Group 2 Ration (R2) (19.18%CP) | Group 3 Ration (R1) (17.82%CP) | Group 4 Ration (R2) (19.18%CP) |
| Total Dry matter | 8.46±0.13 b | 8.64±0.05 b | 11.30±0.16 a | 11.65±0.47 a |
| Milk yield (kg/day) | 4.22±0.35 c | 4.36±0.05 c | 5.64±0.14 b | 6.34±0.06 a |
| 4FCM % | 6.94±0.17 | 7.01±0.21 | 7.42±0.50 | 7.97±0.08 |
| Milk protein (%) | 4.21±0.18 | 4.49±0.25 | 4.04±0.14 | 4.14±0.14 |
| Milk fat (%) | 8.32±0.33 a | 8.04±0.19 a | 6.09±0.46 b | 5.72±0.06 b |
| SNF | 10.02±0.18 | 10.12±0.22 | 10.09±0.26 | 9.94±0.14 |
| SCC(x log10 mL⁻¹) | 140.400±27.16 | 149.00±33.45 | 292.400±67.77 | 185.200±23.87 |

%4FCM; %4 Fat Corrected milk, SNF Fat free  SCC; Somatic Cell Count
§ § a-b, c-d: (p<0.05) Different letters in a same line are significantly different.
NS: Non significant