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Full Length Research Paper

Role of dietary supplementation in the protein content of bovine milk

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This study was conducted to determine the effects of different diet on protein contents of cow milk. A total of one hundred and eighty (180) individual cows of three different breeds (jersey, HF and cross bred) were divided into three dietary treatments groups along with a control diet consisting of simple green fodder (GF). The dietry groups included cotton oil cake (COC), mustard oil cake (MOC) and maize seed cake (MSC). Results of protein contents of fodder (FOD) in comparison with concentrates that is, F-COC, F-MSC, F-MOC improved the protein contents significantly (P < 0.01) When fed only FOD, the protein contents was 32.85 µg/ml (3.3%), which increased to 34.08 µg/ml (3.4 %), 34.03 µg/ml (3.4 %) and 34.15 µg/ml (3.4 %) with the addition of F-COC, F-MSC, and F-MOC, respectively. Feed back response of the caseins and whey proteins was observed in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) profile by resolving these proteins upon 15% SDS PAGE which showed remarkable variation in the banding pattern of all caseins i.e., α-caseins, β-caseins, κ-casein and whey proteins β-lactoglobulin, α-lactalbumin and showed prominent bands after the dietary treatment.

Key words: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), α-caseins, β-caseins, κ-casein, β-lactoglobulin, α-lactalbumin.

INTRODUCTION

Cotton oil cake (COC), mustard oil cake (MOC) and maize seed cake (MSC) are very rich in energy, fiber, and protein. Because of their high nutritional value, their incorporation into the diets of dairy cattle is deemed beneficial because protein is essential for maintenance, growth, and milk production (Armstrong and Prescott, 1971). The protein requirement of dairy cattle is really a requirement for amino acids by the animal tissues. Amino acids are supplied by the digestion of microbial protein, and by feed protein that escapes microbial breakdown in the rumen. Protein requirements for the cattle are called dietary protein (DP), dietary protein is provided through dietary plan. DP is required in cattle for the production of amino acids which are building blocks for the proteins (Lodhi el al., 2003). Micro-organisms in the rumen convert nitrogen from non protein nitrogen (NPN) sources into amino acids for their use. Digestion of microbial protein in the small intestine releases amino acids for absorption and utilization, the same as amino acids released from the digestion of true proteins (composed of amino acids) in feeds (Malik, 1994).

Approximately, 60% of the DP in the typical dairy cow diet is broken down by microbial digestion to ammonia. The rumen microbes must convert the ammonia to microbial protein if the dairy animal is to receive any

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Abbreviations: COC, Cotton oil cake; **MOC,** mustard oil cake; **MSC,** maize seed cake; **DP,** dietary protein; **NPN,** non protein nitrogen; **DIP,** degradable intake protein; **SIP,** soluble intake protein; **UIP,** undegradable intake protein; **GF,** green fodder.

benefit. Fermentable energy must be available for the micro-organisms to grow and synthesize the necessary amino acids (Rehman et al., 2007). If rumen ammonia levels are excessively high, the ammonia is absorbed into the blood and recycled or excreted in urine as urea. All feed protein sources are not degraded in the rumen to the same extent. Three protein terms describe the fate of dietary protein in the rumen. Degradable intake protein (DIP) is the portion of feed protein broken down to ammonia or amino acids by the rumen microbes. Soluble intake protein (SIP) is the portion of DIP that is rapidly degraded in the rumen. Generally, SIP is about half of the DIP. Undegradable intake protein (UIP) is the portion of feed protein that is not degraded by the rumen microbes and remains intact as it passes through the rumen (Rodriguez et al., 1997). Protein synthesis by rumen microbes will depend on feed intake, organic matter digestibility, feed type, protein level, and feeding system. Since 4.5 pounds of microbial protein synthesis per day is near the maximum, the remainder of the protein must be derived from UIP sources (Ireland-Perry and Stallings., 1993).

The present study was conducted with the prime objective to notice the progressive improvement of protein content of milk with addition of dietary supplementation.

Table 1. Analysis of variance at (α 0.01) for dietary plan.

Source	DF	Sum of square	Mean Square	F Ratio	Prob > F
Diet:1GF,2 MOC,3-MSC, 4-CSC	3	200. 4217	66. 8072	22. 4667	(P < 0.01)
Error	308	914. 5297	2. 9693		
C. Total	311	1114. 9514			

MATERIALS AND METHODS

Selection and arrangement of animals

Three breeds, cross bred, HF and jersey, were selected from the dairy farm in collaboration with the Department of Livestock Management, Khyber Pakhtunkhwa Agricultural University Peshawar, and were arranged in groups.

Sample collection

About 100 ml samples of milk were collected from each animal and each sample was divided into five aliquots. Each aliquot was subjected to serial dilution to adjust the protein content for analysis. After dilution the aliquot were subjected to analysis.

Quantification of Proteins

For sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis, the quantity of total protein in milk was measured spectrophotometrically using the Bradford (1976) method, with bovine serum albumin as the standard.

SDS-PAGE analysis of the total protein content

PAGE was performed in a vertical slab gel apparatus. The acrylamid gel (15%), the buffer solution and samples were prepared according to the procedure. The electrophoresis was run at 20 milliampere for 20 min and then 100 milliampere until the marker dye (bromphenol blue) was 0.5 cm from the anodic end of the slab (about 3 h). The slabs were stained for 1 h with Coomassie brilliant blue G 250 in 3.5% per- chloric acid and destained with 7.5% (v/v) acetic acid.

Statistical analysis

For the statistical analysis, general linear model (GLM) procedure of the Statistical Package for the Social Sciences (SPSS) 10 was used. To follow statistical procedure, the variables were divided into independent (diet, pH, heat, lactation stage and breed) and dependent variables (proteins). The GML model was used to find out mean comparison for various treatment groups. Significance was declared at P < 0.01, unless otherwise stated.

RESULTS AND DISCUSSION

All the cows selected for the study were grouped and were provided green fodder (GF) and GF + dietary supplements with concentrates that is, mustard oil cakes (MOC), maize seed cakes (MSC) and cotton oil cakes (COC) for three weeks. Protein contents of milk were quantified by spectrophotometer before and after the supplementation of concentrates. Results after the dietary supplementation were compared with the results obtained from animals fed green fodder alone to determine the effect of concentrates on milk protein content. The statistical analysis of data presented in (Table 1) revealed that addition of concentrates caused an increase in the protein content of milk significantly ((P < 0.01) as compare with GF alone.

Spectrophotometer data (Table 1) were further analyzed for their mean values comparison between dietary plan and milk protein contents. Figure 1 shows that the changes in protein contents of milk after the supplementation of concentrates were highly significant (P < 0.01). Increase in the protein contents after all the three types of concentrates that is, MOC, MSC, COC, were synonymous and significantly higher as compared with milk protein without concentrates (GF). The protein content in the cows fed GF alone ranged between 28.1 - 35.4 μg.ml-1, which increased to 31.0 - 36.75, 31.05 -

36.80 and 31.3 - 36.5 µg.ml-1 after supplementing GF with MOC, MSC and COC, respectively. The highest mean protein content of 34.1 ± 0.5 µg.ml-1 (Figure 1).

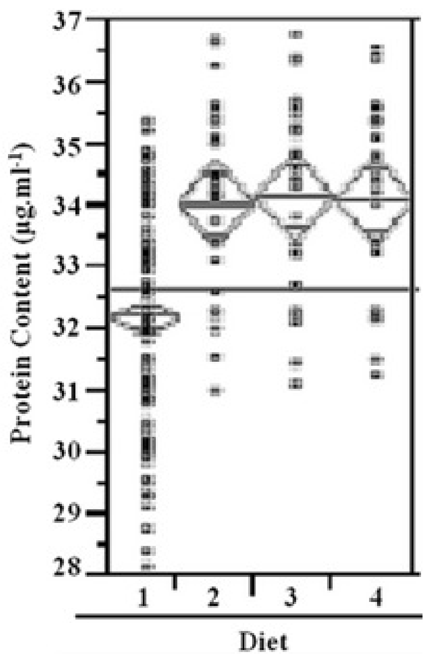

Figure 1. Graphical presentation of mean values of the milk protein content (µg.ml-1) after feeding the animals different types of diet. Diets were 1 – GF, 2 – MOC, 3 – MSC and 4 – COC. Diamonds represent the mean protein content ± SD of the animals in each group

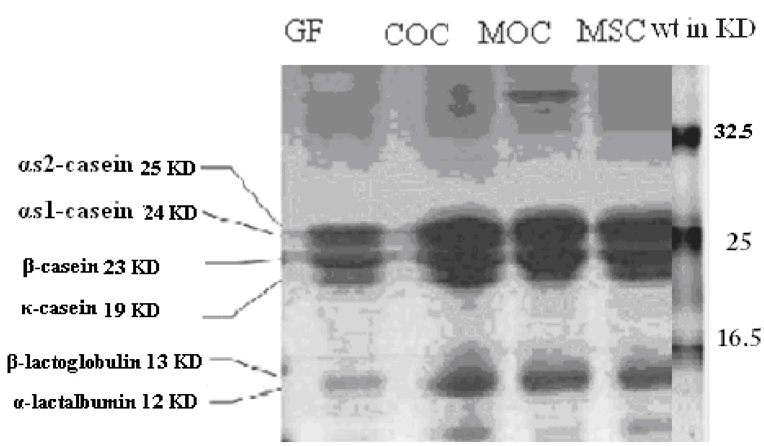

Figure 2. SDS-PAGE analysis for the composition of milk protein constituents after administration of different diets. GF, Green fodder; COC, cotton oil cake; MOC, mustard oil cake; MSC, maize seed cake.

Furthermore, previous studies have reported separation of different protein components through SDS-PAGE (Snezana et al., 2007). The relative composition of various milk protein components was analyzed by SDS-PAGE (Figure 2). Separation of milk proteins on SDS-PAGE profile revealed an indication of remarkable variation in the banding pattern of all caseins, that is, α-caseins, β-caseins, κ-casein and whey proteins β-lactoglobulin, α-lactalbumin and showed prominent bands after the dietary treatment (Figure 2).

Intake of energy, density of energy in the diet and source of energy in the diet potentially influence the concentration of protein in milk and the amount of milk protein produced (Canonica and Passalacqua, 2003). Since intake of energy usually is adjusted by substitution of concentrates for fodder, effects of energy

intake through different types of concentrates and concentration on milk proteins are usually compounded (Everett et al., 1990; Yousef et al., 1970). In the present study, the protein contents of milk were significantly higher (($P < 0.01$) after the supplementation of concentrates as compared to the protein contents in milk of animal given the fodder alone. The major reason for the fluctuation of protein contents in milk is the availability of amino acids to the mammary gland for protein synthesis as the concentrates are rich in nutrients including proteins which provide a bulk amount of raw amino acids and hence the protein contents increase in milk up to the significant level ($P < 0.01$) (Wu and Huber, 1994). When a thorough comparison was carried out, it was observed that all the concentrates contributed equally in the enhancement of protein contents of milk (Lodhi el al., 2003).

SDS-PAGE profile of milk protein from the animals provided diet supplemented with mustard oil cakes, maize seed cakes and cotton oil cakes also showed remarkably, prominent bands as compared with animals provided green fodder alone. However, the composition of different milk protein constituent remained unchanged irrespective of the dietary concentrates consumed and all the major protein components, αs-caseins, β-casein, κ-casein, α-lactabumin and β-latglobulin, were present in the milk sample of animals fed different dietary concentrates (Rehman et al., 2007). Dietary composition fulfills the requirements of all amino acid need to compose the proteins present in milk that is why all kinds of proteins increased equally. It is also hypothesized that decrease in protein concentration has been due to decreased glucose. Thus, the green fodder may be incapable of providing all the raw materials to enhance the proteins in milk (Pecquet et al., 2000). In a nutshell, this experiment demonstrated that there were significant ($P < 0.01$) differences in the protein content of milk due to supplementation of diet.

Conclusion and recommendations

Dietary supplements along with green fodder that is, COC, MSC and MOC showed a significant increase in the protein contents of milk. From the above results, it is suggested that the dietary supplements are very important for the enhancement of protein contents in milk. So it is interpreted that the dietary concentrates could be a major cause of enhancement of protein contents in the milk.

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