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Values of urinalysis dipstick in evaluating high-protein, low-carbohydrate, low-fat diets in male wistar rats

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Abstract. One popular diet pattern currently recommends a high-protein, low-carbohydrate diet as a weight-loss strategy in obese patients. Diets high in protein appear to reduce appetite, energy intake, body weight, and fat buildup. The purpose of this study was to determine the effect of long-term high-protein, low-carbohydrate and low-fat (TPRKRL) diets on body function. With a focus on urine analysis using the urinalysis dipstick and the Urine Analyzer Verify U120 tool from 22 samples of male Wistar rats that were given the standard diet and the TPRKRL diet for 8 weeks. The results showed that the TPRKRL diet on 8 variables had lower mean values than standard rats and there were significant differences in leukocyte counts, pH, specific gravity, ketones and bilirubin between the two groups. Whereas in the urobilinogen, protein and blood variables there were no significant differences. As well as other parameters namely nitrite and glucose abnormal abnormalities were not found.

1. Introduction

Of the various types of dietary patterns including low carbohydrate diets [1], low fat diet [2], high fat diets or ketogenic [3], etc. One of the popular dietary patterns that is promoted high protein diets can improve physical performance, body composition, and better health [4,5]. This diet is popular because it can increase satiety [6], reduce appetite, energy intake, body weight, and fat buildup [4]. But the benefits and disadvantages of this type of diet are still inconsistent [7,8].

A high protein diet is a protein intake that exceeds the RDA recommended protein standard of 0.8g / BW (kg) / day and is said to be high in protein if the intake exceeds 35% of total calories [9]. The protein requirement for physically active individuals is 1.4-2, 0 g / kg / day [10].

An increase in protein composition results in a decrease in the composition of other macronutrients namely carbohydrates and fats. It is said to be low in carbohydrates if it has a carbohydrate content <26% [1], which often uses <15% of total calories [11] and a low-fat diet if it has a fat content <30% of total calories [2].



There are studies that describe a high-protein diet that can cause nephrotoxicity [12], increase intraglomerular pressure and glomerular hyperfiltration which will cause rapid decline in kidney function [13].

Urine is a biological fluid that can describe the physiological state of the body both metabolic processes to pathological conditions, especially the state of the kidneys and the body in general [14]. Tests using urinalysis dipsticks are cost-effective, easy, fast, sensitive and effective tests that can be done anywhere [15,16]. The reagent dipstick test can produce valuable semiquantitative chemical results [16].

This study focuses on looking at the effects of high-protein, low-carbohydrate and low-fat diets on the body through observing urine using semiquantitative urinalysis dipstick.

2. Materials and Methods

2.1. Food preparation

The diet used for the control group was standard animal pellets (AD-II Japfa®, Indonesia) consisting of yellow corn, soybean meal (SBM), bone meal and meat (MBM), corn gluten (CGM) and palm oil. The feed used for the TPRKRL feed group is processed by itself using a nutrisurvey application guide with a composition consisting of mackerel, white and egg yolk, and cornstarch. Table 1 shows the results of the test feed used in the study.

Table 1. Macronutrient content comparison between the diets

Macronutrients	Standard Diet (AD-II)	TPRKRL Diet
Protein	24.52 %	73.65 %
Carbohydrate	61.01 %	7.14 %
Fat	7.17 %	12.21%

2.2. Animal preparation

The experimental animals were 100-day-old male Wistar Rattus norvegicus rats with body weight of 190-290 g (n = 22). The rats were adapted for 7 days given AD-II standard diet and water in an ad libitum manner. Rats were placed individually in cages, then rats were divided into two groups. The first group (n = 9) was given AD-II as a standard diet and the second group (n = 13) was given the TPRKRL diet, each rat was fed 10-15% of BB rat ~ 30g / head / day without calorie restriction. Both groups were given an ad libitum drink, monitored their health, measure both body weight and the remaining food which were left over the next day, every day. After 60 days, all the rats were put into a metabolic cage modification to maintain the quality of their urine and urine collection was carried out and a semiquantitative dipstick urinalysis was examined with 10 reagent parameters. All experimental animal procedures have been approved by the Animal Ethics Commission of the Faculty of Medicine, Hasanuddin University.

2.3. Urinalysis

Urine was collected in the morning before being fed, the urine is then put into a glass tube so that the urinalysis dipstick cushion can be immersed properly. The urinalysis dipstick is then removed and dried for 2 minutes, then the dipstick is put into the Verify U120® Urine Analyzer tool to be interpreted quantitatively. The urinalysis dipstick contains 10 reagents, leukocytes, nitrites, urobilinogen, protein, pH, blood, specific gravity, ketones, bilirubin, glucose.

2.4. Data Analysis

All data was analyzed using the IBM Statistics SPSS 22 program. Data distribution was tested for normality and homogeneity by Shapiro-Wilk and Levene's test. Normally distributed data was tested using unpaired T-test. Data were not normally distributed was analyzed with the Mann Whitney test where it was considered significant if the p value < 0.05 . Data is displayed in mean \pm SD.

3. Results

On urine examination using a semi-quantitative urinalysis dipstick with 10 parameters as shown in Table 2, the average value of the TPRKRL group (carbohydrate: protein, 7.14%: 73.65%) is lower than the control group (carbohydrate: protein, 61%:24.5%). Based on the data processing, there was a significant difference ($p < 0.05$) in the variables of leukocytes, pH, specific gravity, ketones and bilirubin between the two groups. Whereas in the urobilinogen, protein and blood variables there were no significant differences ($p > 0.05$), and nitrite and glucose were not found in the urine in the two groups.

Table 2. Semi-quantitative measurements of urinalysis dipsticks

Variables ^a	Mean \pm SD		p
	Standard diet (n=9)	TPRKRL diet (n=13)	
Leucocytes (Leu/ μ l)	108.33 \pm 151.86	22.69 \pm 39.98	0.032*
Urobilinogen (mg/dl)	21.50 \pm 11.02	18.38 \pm 4.99	0.411
Protein (mg/dl)	143.33 \pm 1.21	81.92 \pm 0.75	0.24
pH	6.89 \pm 0.78	6.08 \pm 0.19	0.005*
Blood (Ery/ μ l)	31.11 \pm 68.64	20.38 \pm 28.24	0.241
Specific gravity	1.02 \pm 0.01	1.03 \pm 0.00	0.003*
Ketone (mg/dl)	28.33 \pm 2.42	6.15 \pm 1.16	0.003*
Bilirubin (mg/dl)	2.67 \pm 23.31	1.31 \pm 16.70	0.01*

(a)=Mann-Whitney test, SD=standard deviation, n=sample number, p=value, (*)=significant ($p < 0.05$)

4. Discussion

A high-protein diet is popular with its benefits among people to lose weight, especially in obese individuals [6], but this has also been linked to its impact on long-term use [8].

In this study we see that the use of a high-protein diet where the composition of carbohydrates and fats is restrictive compared to mice given a standard diet where the carbohydrate content is higher shows that the average value of all the variables in the 8 parameters of urinalysis dipstick testing is lower than that of standard mice, where Two other parameters namely nitrite and glucose were not found abnormal abnormalities.

The presence of leucocyturia in the TPRKRL group was lower and there was a statistically significant difference ($p = 0.03$) compared to the control group, which could allow the process of inflammation or infection that occurs in the urinary tract. Common causes of leukocytes in the urine (pyuria) are pyelonephritis, cystitis, interstitial nephritis, glomerulonephritis, stones, corticosteroid drugs and cyclophosphamide [16]. However, this study requires further investigation.

Urobilinogen is a product of hemoglobin metabolism formed by normal intestinal flora. Normally not found in urine, but can be increased if there is an increase in bilirubin products such as hemolytic anemia or blockage of the portal vein due to cirrhosis of the liver and hepatitis [16]. But in this study both groups experienced an increase in urobilinogen with a lower TPRKRL value than the standard group.

Normally protein or blood cells cannot pass through the permeable selective glomerular capillary membrane [17]. The presence of proteinuria can indicate intrinsic damage to the kidneys and urinary tract [16]. In this study, abnormal values were obtained, namely the presence of proteinuria in both groups but the TPRKRL group had lower values than the group standard diet.

A pH check can work to see kidney, respiratory, and metabolic disorders. The normal urine pH in susceptible acids (~ 5.0) [18]. In this study, there was a significant pH difference between the two groups, where the pH of the TPRKRL diet group was more acidic than the standard diet. Urine pH will be more acidic in individuals who are given a diet high in protein.

The presence of hematuria is an important indicator for diseases of the urinary system [16]. Microscopic hematuria accompanied by proteinuria can indicate glomerular disorders or disease [19]. In this study there were no significant differences in the presence of hematuria in the two groups, but the TPRKRL diet group had a mean value lower than the standard group.

Specific Gravity in the two experimental groups found a significant difference, but both values are still vulnerable to normal values of specific gravity

In a state of low carbohydrate supply, the body will carry out the process of gluconeogenesis in which the second energy source other than carbohydrates, namely fatty acids through the β -oxidation pathway to the citric acid cycle. Where the end result of this process produces acetoacetic, β -hydroxybutyric acid and acetone known as ketone bodies [3,20]. In this study there are significant differences, especially the TPRKRL diet group having a lower ketone value than the control group. As it is known that carbohydrate intake in TPRKRL feed is very low (7%), of course the ketogenic process needs to occur, but this is proven through the results of dipstick urinalysis with a relatively lower number than imagined. This is suspected because of the lack of supply of fat sources where its use is only around 12% in the feed.

Bilirubin is a product of the degradation of red blood cells in the liver, spleen and bone marrow. Normally conjugated bilirubin is not found in urine, but conjugated bilirubin in very small amounts (~ 0.02 mg / dl). Can also be found if there is a blockage of the bile duct, carcinoma, drug induction and periportal inflammation. In this study, bilirubinuria levels were found to have significant differences between the two groups, but the levels were lower than the standard group.

5. Conclusions

In this study we see that the use of a high-protein diet where the composition of carbohydrates and fats is restrictive compared to rats given a standard diet where the carbohydrate content is higher by using a semiquantitative urinalysis dipstick test shows that there are significant differences in the variables leukocytes, pH, specific gravity, ketones and bilirubin between the two groups. Whereas in the urobilinogen, protein and blood variables there were no significant differences. We also obtained data that the mean values of all the variables in the 8 parameters of urinalysis dipstick testing were lower than standard rats, where the other 2 parameters namely nitrite and glucose were not found abnormalities. However, the above results need to be confirmed by plasma tests in subsequent studies.

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