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Effect of Whey-to-Casein Protein Ratio in Chocolate-Vanilla Milk Beverage on Satiation and Acute Energy Intake

Amelinda Angela, Lina Antono[1,*], Astri Kurniati, Kamalita Pertiwi and Mardi Wu

ABSTRACT

The satiating effect of dietary protein could be beneficial to fight obesity by improving weight loss. Whey and casein protein have different digestion rates and combining them may result in a prolonged satiating effect. This study investigated the effect of the whey/casein ratio on subjective appetite ratings and acute energy intake. Twelve healthy men with normal weight received a standardized breakfast followed by one of the three preloads (milk beverage containing 15 g protein with whey to casein protein ratios of 20:80, 50:50, or 80:20) in a randomized, single-blind, cross-over study. Subjective appetite ratings were measured using a visual analog scale while consecutive energy intake was measured through *ad libitum* lunch and dietary recalls of food and drinks consumed during the remainder of the experimental day. The results showed that there was no significant effect of the whey to casein protein ratio in milk beverages on the appetite ratings and subsequent energy intake. A high protein content, as opposed to the type of protein, may be of greater importance in determining the satiating properties of protein and should be taken into account when developing weight loss products. Further investigation is needed to study the effect of the ratio on metabolic satiety properties and the shelf life of the product.

Keywords: whey, casein, protein, satiety, energy intake

INTRODUCTION

Non-communicable diseases (NCDs), which mainly comprise cardiovascular diseases, diabetes, cancer and chronic respiratory diseases, represent a significant and unfortunately growing burden worldwide (Wagner and Brath, 2012). Popular belief presumes that NCDs are found in high income populations due to a sedentary lifestyle, while in fact, nearly 80% of NCD deaths occur in low-income and middle-income countries (Wagner and Brath, 2012).

Being overweight and being obese, both caused by an unhealthy diet and physical inactivity, are well-known risk factors for NCDs. Although the highest prevalences of being overweight and of being obese are observed in the World Health Organization region of the Americas, the prevalence of being overweight in middle-income countries is also high, with the fastest rise in being overweight found in the lower-to-middle-income countries (World Health Organization, 2010; Wagner and Brath, 2012). For example, Indonesia Health Profile showed that by 2010, the prevalence in adults of being overweight and being obese in Indonesia is relatively high, with rates of 10 and 11.7% respectively (Indonesia Ministry of Health, 2011).

Nutrifood Research Center, PT. Nutrifood Indonesia, Jakarta 13920, Indonesia.
* Corresponding author, e-mail: lina.antono@nutrifood.co.id

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Achieving a negative calorific balance by combining a low calorie diet and physical activity, thus leading to weight loss, is a common recommendation for people who are either overweight or obese; however, this concept seems to be outdated due to its failure to achieve long term success (Hafekost *et al.*, 2013). Therefore, a multi-factorial approach is needed to improve long-term weight loss.

A high protein diet has a positive effect on body weight and body composition. Dietary protein also has an important role in weight loss and weight maintenance because of its satiating effect (Westerterp-Plantenga *et al.*, 2009). However, the satiating effect of protein may vary between protein types. There is some evidence showing that different protein sources may differ in their satiating capacity, though the results are still inconclusive (Lang *et al.*, 1998; Lang *et al.*, 1999; Anderson *et al.*, 2004; Veldhorst *et al.*, 2009a; Abou-Samra *et al.*, 2011).

Some studies have shown that whey protein is more satiating than other proteins, including casein protein (Hall *et al.*, 2003; Luhovyy *et al.*, 2007; Veldhorst *et al.*, 2009a). Essentially, these two milk proteins have different physical properties and this difference may influence their satiating effect. Whey protein is rapidly digested, and thus could cause a rapid increase in plasma amino acids, while in contrast, casein is more slowly digested, and thus results in a slow and steady increase in plasma amino acids (Luhovyy *et al.*, 2007). Although it might be useful to prolong the satiating effect, the synergism of both proteins has received little attention. Therefore, the current study investigated the effect of the whey protein to casein ratio on subjective appetite ratings and the acute energy intake.

MATERIALS AND METHODS

Subjects

The subjects were twelve men with a healthy-weight (BMI 18.5–22.9 kg.m^{-2}). None were on a restricted diet, nor were any allergic to dairy products and none were cognitively restrained eater (Three Factor Eating Questionnaire Factor 1 score points ≤ 9; Stunkard and Messick, 1985). Subjects reported that they had been weight-stable for the last 6 mth.

Before the trial, subjects were asked to complete the International Physical Activity Questionnaire (IPAQ; Craig *et al.*, 2003). Their weight, body composition and basal metabolic rate (BMR) were also measured (InBody720, Biospace, Korea). These data were then used to calculate daily energy requirements (DER) by multiplying the measured BMR by an index of physical activity level which was estimated using the IPAQ. Table 1 displays subject characteristics.

Study design

A randomized, single-blind, cross-over trial was performed. All subjects were studied on three separate occasions with at least 6 d between each study. For each test day, subjects were advised to refrain from doing vigorous exercise for 24 hr before and after the test day.

One day before the test day, subjects were instructed to fast after 22.30 hours and were only allowed to drink plain water until the next morning. On the test day, all subjects arrived at the laboratory at 0800 hours. Upon arrival, subjects were seated in individual cubicles and their appetite profile was assessed using a visual analog scale (VAS) questionnaire. After the assessment, subjects were given a standardized breakfast which had to be consumed within 10 min. After

Table 1 Subject characteristics.

	Subject (number = 12)	
	Mean	SD
Age	24.17	1.47
BMI (kg.m^{-2})	21.47	1.54
BMR (kcal)	1473.25	104.96
DER (kcal)	2395.62	279.87

BMI = Body mass index; BMR = Basal metabolic rate; DER = Daily energy requirement, SD = standard deviations.

finishing the breakfast, subjective appetite ratings and breakfast palatability scores were immediately measured using the VAS questionnaire. Subjective appetite rating measurements were repeated at 30, 60, 90 and 120 min after breakfast.

At 1030 hours (120 min after breakfast), subjects were provided with one of the three preloads. Subjective appetite ratings and preload palatability scores were immediately measured afterwards using the VAS questionnaire; then 30, 60 and 90 min after, subjective appetite ratings were measured again.

At 1200 hours, subjects were provided with a standard pasta-based meal to be consumed *ad libitum*. They were instructed to eat as much of the meal as they wished until they felt comfortably full, and then they completed the subjective appetite rating and meal palatability rating questionnaires. Afterwards, they were free to leave the laboratory. After all subjects had left, the amount of the meal consumed by each subject was measured.

On the day after the test, subjects were asked to recall all food and drinks consumed after the *ad libitum* lunch on the previous day. Food recall was performed by interviewing all of the subjects the next morning.

Breakfast

The standardized breakfast was a tuna sandwich, which consisted of white bread (Sari Roti, Indonesia) and tuna sandwich spread (Ayam Brand, Indonesia). On one slice of bread, 16 g of tuna spread was added. The meal was prepared to fulfill 20% of the individual DER with 16% of the energy coming from protein, 22% from fat and 62% from carbohydrate.

Preloads

The preloads were three energy-fixed (140 kcal), chocolate-vanilla-flavored milk beverages. Each preload contained 15 g protein with whey and casein protein ratios of 20:80, 50:50 and 80:20, respectively. Table 2 presents the nutritional composition of the preloads.

Lunch

The food which was provided in the *ad libitum* lunch consisted of spaghetti (La Fonte, Indonesia), fresh mushroom Italian sauce (Prego, Indonesia), corn oil (Tropicana Slim, Indonesia), cheddar cheese (Kraft, Indonesia), sugar, salt and pepper. The food was prepared based on a standardized recipe and was mixed homogenously by the same person for each test day. The calorie content of the meal was calculated using the nutrition facts on the packaging of the ingredients: 100 g of spaghetti contained 150 kcal with 13, 22 and 65% of the total energy provided by protein, fat and carbohydrate, respectively.

Before serving, the food was warmed up in a microwave for 1 min. Subjects were initially provided with 300 g of spaghetti. Before the dish was completely empty, a new portion of food (150 g of spaghetti, warmed up in a microwave for 30 s) was added to the plate while the subject continued to eat. This was to ensure that the cue of an empty dish did not prompt meal termination (Astbury *et al.*, 2010). The process was repeated until the subject indicated that they wished to terminate the meal.

Appetite and taste ratings

A visual analog scale (VAS), 100 mm in length with words anchored at each end, expressing the most positive and the most negative rating, was used to assess the subjective appetite rating and palatability score. Subjective appetite ratings were measured using questions about hunger,

Table 2 Nutrient composition of preloads.

	Amount per preload
Energy	140 kcal
Protein	15 g
Energy from protein (%)	44.78%
Fat	2 g
Carbohydrate	14 g
Energy density	0.7 $kcal.mL^{-1}$

satiety, fullness, prospective food consumption, desire to eat, urge to eat and thought of food. The palatability of breakfast, preload and lunch was assessed using questions regarding appearance, smell, taste, aftertaste, overall liking and overall palatability. Subjects did not discuss or compare their ratings with each other (Flint *et al.*, 2000).

Food recall

The dietary recall interview was conducted on the day following each experiment. Subjects were instructed to describe all the food and drinks consumed from 1230 hours after the *ad libitum* lunch until 2359 hours on the day of the experiment. Colored photographs of different portions of food (Waspadji *et al.*, 2010) were provided to help the subjects estimate the quantity of food they had consumed.

Statistical analysis

Data were analyzed using the IBM SPSS statistics software (version 21; IBM SPSS Statistics; Somers, NY, USA). All results were presented as means with standard deviations unless otherwise stated. Differences were considered significant at $P < 0.05$.

Analysis of variance (ANOVA) for repeated measures on two factors (preload × time) was used to analyze the differences in the VAS appetite ratings between preloads. If a significant main effect of preload was obtained, *post hoc* analysis was conducted using a two-tailed paired *t*-test with Holm-Bonferoni step-wise correction for multiple comparisons to determine the location of the difference.

ANOVA for repeated measures with preload as within-subject was used to analyze the area under the curve (AUC) of the appetite ratings, energy intake during the *ad libitum* lunch test meal, palatability scores for the meals and preloads and subsequent energy intake within the experiment day. If a significant effect of preload was obtained, *post hoc* analysis was conducted using two-tailed, paired *t*-tests with Holm–Bonferroni step-wise correction for multiple comparisons to determine the location of the difference.

RESULTS

Appetite profile

One of the objectives of this research was to evaluate the effect of the whey to casein ratio on the subjective appetite ratings. Subjective appetite ratings at specific times were measured using a visual analog scale. As expected, ratings of hunger ($F(3.639,40.028) = 25.769$, $P < 0.001$), fullness ($F(3.687,40.559) = 29.640$, $P < 0.001$), prospective consumption ($F(3.555,39.101) = 17.653$, $P < 0.001$), desire to eat ($F(10,110) = 23.537$, $P < 0.001$), urge to eat ($F(3.539,38.928) = 19.218$, $P < 0.001$) and thought of food ($F(3.338,36.719) = 8.147$, $P < 0.001$) displayed a significant main effect of time.

After breakfast, the fullness ratings increased while other appetite ratings (hunger, prospective consumption, desire to eat, urge to eat and thought of food) decreased. Over time, all appetite ratings except thought of food ratings returned toward baseline values until the preload was served. After consuming preloads, fullness ratings increased again while other appetite ratings except thought of food ratings (hunger, prospective consumption, desire to eat and urge to eat) decreased. Afterwards, all appetite ratings except the thought of food ratings returned toward baseline values until the lunch meal was served (Figure 1). There was no significant main effect of preload and the preload × time interaction for all appetite ratings and the AUC of all appetite ratings.

Palatability test

The palatability of every meal served during the experiment was assessed immediately after consumption. There was no significant difference in the palatability scores of the breakfast and lunch meals served on each day of the experiment (Figure 2). Furthermore, the visual appeal, taste, smell, aftertaste and overall palatability scores of the three preloads did not differ.

Energy intake at test meal and food recall analysis

The ratio of whey and casein had no significant effect on the subsequent energy intake during lunch (Figure 3a) and on the self-reported energy intake during the remainder of each experiment day (Figure 3b). Consequently, the total acute energy intake after preload consumption (calculated by adding the energy intake during lunch and the self-reported energy intake during the rest of the experiment day) was not different between preloads (Figure 3c).

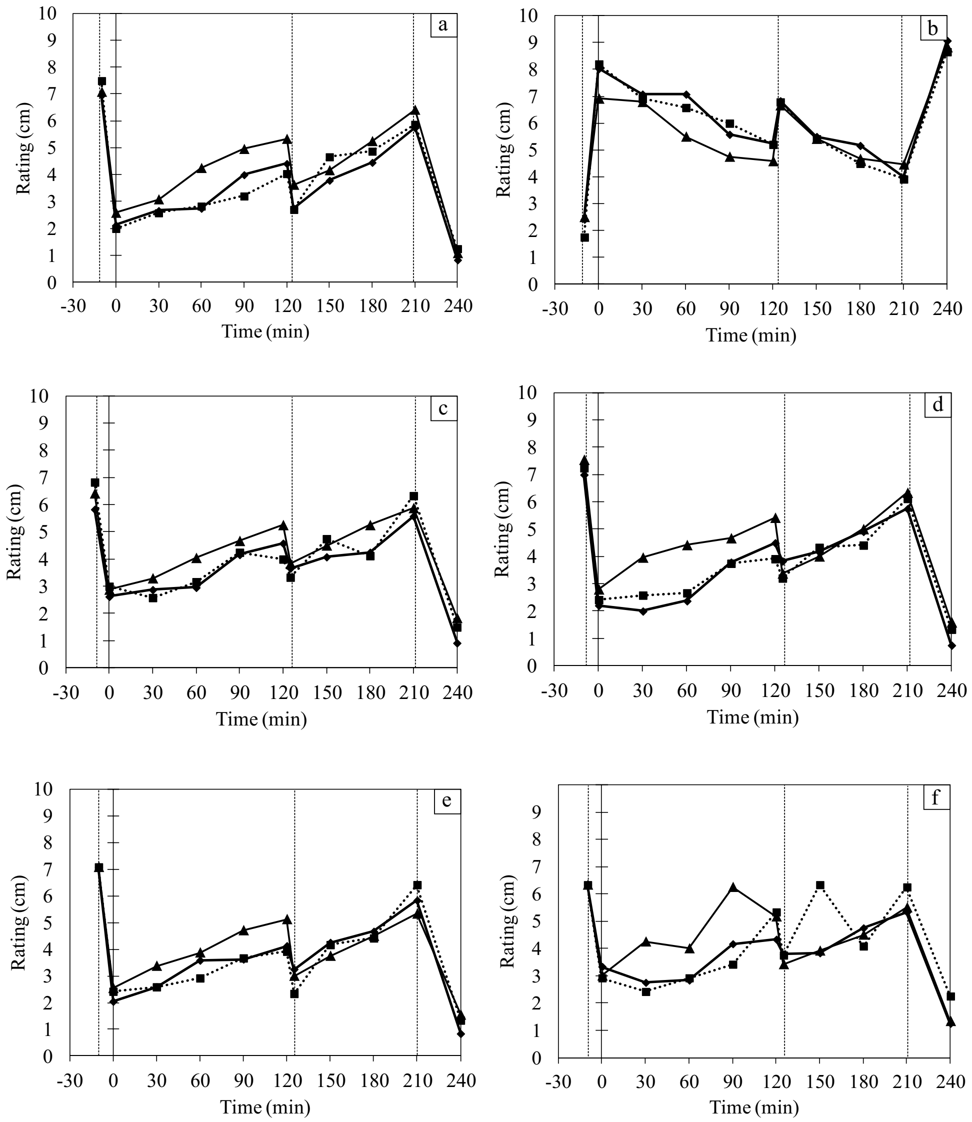

Figure 1 Changes in appetite ratings during the experiment for: (a) Hunger; (b) Fullness; (c) Prospective consumption; (d) Desire to eat; (e) Urge to eat; (f) Thought of food. Data are expressed as mean values. ◆ = Whey to casein ratio of 20:80; ■ = Whey to casein ratio of 50:50; ▲ = Whey to casein ratio of 80:20. The areas under the curve for all appetite ratings were not significantly different between all whey/casein ratios.

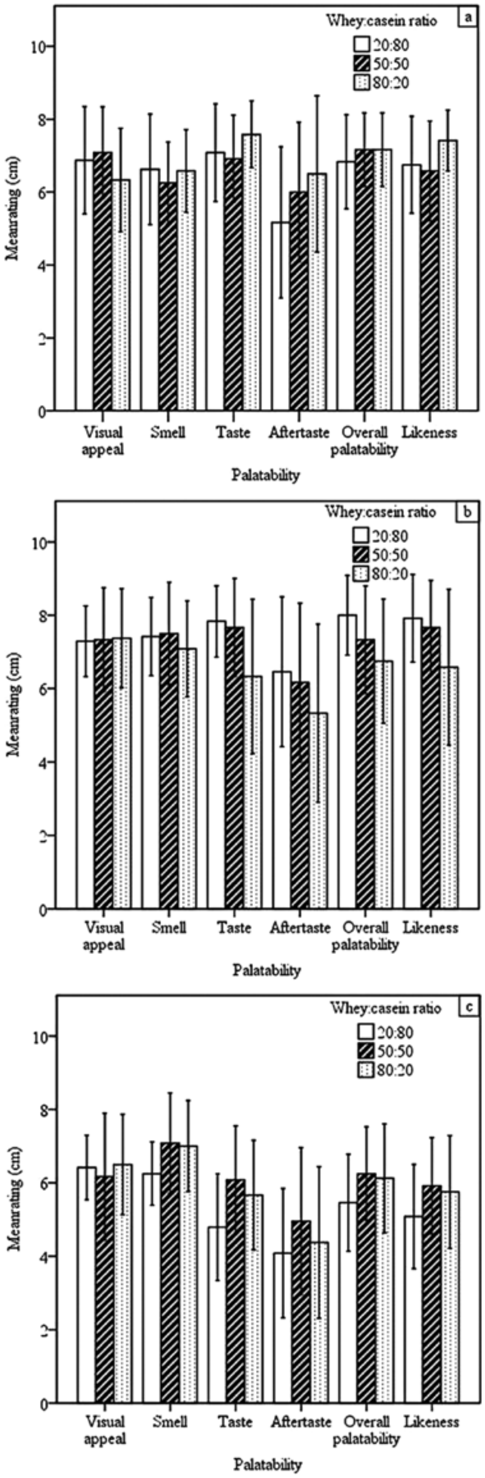

Figure 2 Mean palatability scores during each experiment of: (a) Breakfast; (b) Preloads; (c) Lunch. Error bars show the 95% confidence intervals.

DISCUSSION

Three fixed-energy milk beverages were given as preloads. All preloads had the same macronutrient composition but the ratio of whey protein and casein was manipulated (20:80, 50:50

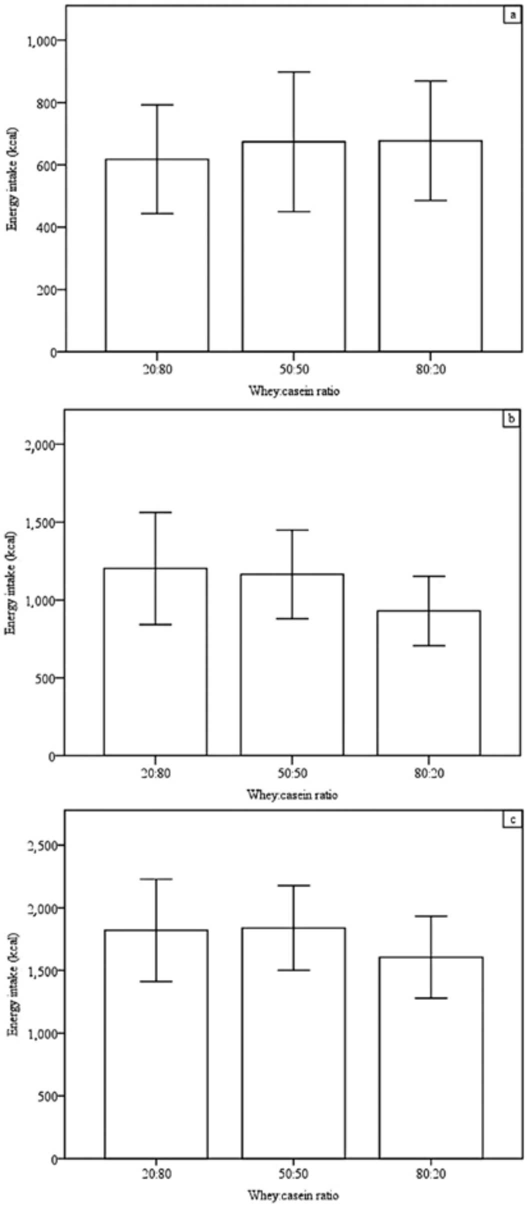

Figure 3 Mean subsequent energy intake during: (a) Lunch; (b) Remainder of the day; (c) Total energy intake. Error bars show 95% confidence interval.

and 80:20). The ratio of 20:80 was used to imitate the whey and casein ratio in milk (Luhovyy *et al.*, 2007). As far as the authors know, this is the first study to investigate the effect of the whey protein and casein ratio on subjective appetite ratings and the acute energy intake.

Based on the analysis of palatability scores, all meals served in this present study had the same taste, appearance and other organoleptic properties. The manipulated preloads also had the same flavor, color, absolute energy content, energy density, serving condition and volume. This avoided any bias as these factors have been shown to influence satiety (Livingstone *et al.*, 2000; Blundell *et al.*, 2010). The test meal in the present study was a single course meal which focused on the assessment of the food and energy intake rather than the nutrient intake. Therefore, it was suitable to be used to assess short-term energy compensation as a single course (Blundell *et al.*, 2010).

An effect size of 10% would be a reasonable and realistic difference to look for in studies of appetite (Flint *et al.*, 2000). To cover a test of an effect size of 10% with a study power of 0.9 of the appetite ratings in a paired design, 12 subjects would be sufficient (Flint *et al.*, 2000). With the 12 subjects involved in this study, it could be suggested that this study had sufficient power. As no statistically significant difference was observed, it appeared that the whey and casein ratio in milk beverages had no significant effect on the subjective appetite ratings and acute energy intake in this study.

The reason the whey and casein protein ratio failed to affect the subjective appetite ratings and acute energy intake might have been related to the high content of protein in the preloads served in this study. Other studies suggested that a relatively high amount of protein (greater than or equal to 50% energy) may have caused the lack of differences in satiety between different types of protein, for example when comparing the subsequent food intake after consuming whey, soy or gluten protein. Similar results were observed when comparing the satiating effect of protein and carbohydrate, such as comparing the appetite profile after consuming either casein, whey or carbohydrate preloads. These results suggested that it might not be possible to distinguish the satiating properties of different types of protein when the concentration of amino acids is above a threshold level (Veldhorst *et al.*, 2009b). The results of this study also implied that the amount of protein may be a greater importance in determining the satiating properties of a meal compared to the type of protein.

Nevertheless, there were some limitations in the current study. First, there was no control treatment. Blundell *et al.* (2010) stated that satiety studies are best conducted by ensuring a control condition, either through the use of a non-preload or a placebo treatment. In the current study, two additional preloads, consisting of 100% whey and 100% casein protein, might be needed. Second, a 90 min interval for the preload and test meal was used in the current study. However, justifying the time interval based upon the latest time points when there were significant differences in ghrelin concentrations between treatments might be a more precise approach (Veldhorst *et al.*, 2009a; Blundell *et al.*, 2010).

It must also be noted that the whey and casein protein ratio may have an effect on metabolic satiety. Metabolic satiety refers to all the neural and hormonal signals that are transported from the gastrointestinal tract to the brain. These signals refer to stomach fullness as sensed by stretch receptors, but also to hormones involved in hunger and satiety, such as ghrelin, cholecystokinin, GLP-1 and PYY18 (Ahima and Antwi, 2008). Therefore, it would be interesting to continue this study involving other measurements related to metabolic satiety properties. It would also be interesting to study the shelf life of the beverages before developing them further as a weight loss product.

CONCLUSION

There was no significant effect of the whey to casein protein ratio in the milk beverages on the appetite ratings and acute energy intake. A high protein content, as opposed to the type of protein, may be of greater importance in determining the satiating properties of protein and should be taken into account when developing weight loss products. Further investigation is needed to study the effect of the ratio on metabolic satiety properties and the shelf life of beverages before further developing them as a weight loss product.

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