**Original Research Article**

# Effect of Nutrient Formulations on Permeation of Proteins and Lipids through Porcine Intestine *In vitro*

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## Abstract

*Purpose: To investigate the effect of nutrient formulations on the permeation of proteins and lipids through porcine intestine in vitro.*
*Method: In vitro permeation studies of proteins and lipids of two peptide-based formulations, composed of various compounds and sources of hydrolyzed protein was carried out, and compared with a conservative polymeric formulation as control, The test was undertaken using Franz diffusion cell apparatus incorporating porcine intestine.*
*Results: The peptide-based formulation demonstrated higher protein absorption than the conservative polymeric one. However, there were some differences in protein absorption rates between the peptide-based formulations obtained from various sources. Formulation A with 1.0 and 1.5 kcal/mL exhibited significantly (p < 0.05) higher cumulative protein permeation (11.97 ± 0.23 and 12.54 ± 0.94 µg/cm$^2$) than formulations B (9.41 ± 0.36 and 9.67 ± 0.35 µg/cm$^2$) and C (8.34 ± 0.56 and 8.61 ± 0.71 µg/cm$^2$), respectively. Lipid permeation from formulations A and B (13.91 ± 0.26 and 12.94 ± 0.59 µg/cm$^2$ respectively for 1.0 kcal/mL formulation, and 13.31 ± 0.21 and 12.86 ± 0.16 for 1.5 kcal/mL formulation) which consist mainly of medium chain triglycerides (MCTs), were significantly (p < 0.05) higher than those from formulation C (11.49 ± 0.43 and 12.62 ± 0.38 µg/cm$^2$ for 1.0 and 1.5 kcal/mL formulation, respectively) which mostly contained long chain triglycerides (LCTs).*
*Conclusion: The results reveal that oligomeric formulations have higher absorption rate than polymeric formulations. However, the outcomes when administered to clinically ill patients need to be investigated.*

*Keywords: Nutrient formulations, Permeation, Proteins, Lipids, Porcine intestine, Medium chain triglycerides, Long chain triglycerides*

## INTRODUCTION

Malnutrition is a well-known risk factor influencing the occurrence of clinical complications in clinically ill patients [1-3]. Nutritional support is an essential component in the clinical management of critically ill patients, particularly when the illness is associated with gastrointestinal (GI) complication [4]. Enteral route has many advantages over parenteral route in case GI tract can function [5,6]. The cellular proliferation of brush border enzyme can be maintained by enteral feeding. Thus, enteral nutrition is the preferred route [7-9].

Enteral nutrition (EN) formulations can be classified as elemental, semi-elemental and polymeric. Many studies have confirmed the higher absorption of semi-elemental over elemental and polymeric [10]. Peptide-based or

protein hydrolysates formulae contain proteins that have been hydrolyzed and are also referred to as semi-elemental diets [11]. Compared with free amino acids (FAA) or intact-protein formulations, peptide-based feedings have been displayed to improve nitrogen balance; improve protein synthesis; improve absorption; reduce diarrhea; maintain gut integrity; and improve outcomes [12].

Due to the specific uptake system of the GI tract, small peptides consisting of 4 – 12 amino acids are absorbed more easily and consistently than corresponding mixtures of FAAs [10]. Peptide-based formulations can differ in protein quantity and the size of the peptides.

Some formulaions contain very large peptides; conversely, some contain very small peptides. Thus, two protein hydrolysates made by different methods, their absorption kinetics are likely quite different. Consequently, all peptide-based formulations are certainly not created equal [13-15].

This study investigated the absorption ability of protein and lipid of two peptide-based formulations composed of different composition and different source of protein hydrolyses in comparison to the conservation polymeric formulation using *in vitro* permeation studies through the porcine intestine.

# EXPERIMENTAL

## Nutrient formulations

The 6 formulations of nutrient consist of 2 formulations of A, peptide (1 and 1.5 kcal/ml), 2 formulations of B, oligimeric (1 and 1.5 kcal/ml) and 2 formulation of C, polymeric (1 and 1.5 kcal/ml) were prepared. The composition of the nutrient formulations are listed in Table 1.

## Preparation of nutrient sample

### Formulation A (oligomeric)
Formulation A with 1 kcal/ml and 1.5 kcal/ml were prepared by adding 21.65 g and 32.48 g, respectively, of formulation A in 60 ml of water. The mixtures were mixed and the final volume adjusted to 100 ml.

### Formulation B (oligomeric)
Formulation B with 1 kcal/ml and 1.5 kcal/ml were prepared by adding 22 g and 33 g, respectively, of formulation B in 84 ml of water. The mixtures were mixed and the final volume adjusted to 100 ml.

### Formulation C (polymeric)
Formulation C with 1 kcal/ml and 1.5 kcal/ml were prepared by adding 24 g and 36 g, respectively, of formulation C in 70 ml of water. The mixtures were mixed and the final volume adjusted to 100 ml.

**Table 1:** Composition of the formulations

| Ingredient | Formulation A | Formulation B | Formulation C |
|---|---|---|---|
| | *Oligomeric* | *Oligomeric* | *Polymeric* |
| Protein | Whey protein hydrolysate (8.4%) Caseine hydrolysate (7.6%) Caseine (1.4%) Glutamine (7.9%) Leucine (1.1%) | Whey protein hydrolysate (20.9%) | Whey protein isolate (11.5%) Soy protein isolate (11.5%) |
| Fat | MCT oil (9.0%) Canola oil (4.5%) Fish oil (4.1%) | MCT oil (12.6%) Soybean oil (4.6%) | Canola oil (9.2%) High oleic Safflower oil (4.6%) Ricebran oil (4.2%) Fish oil (2.3%) |
| Carbohydrate | Maltodextrin (30.0%) Isomaltulose (20.4%) | Maltodextrin (31.7%) Potato starch (8.0%) Sucrose (14.5%) | Maltodextrin (23.8%) Isomaltulose (9.2%) Maltitol (9.2%) Fibersol (4.6%) FOS (2.3%) |
| Vitamin & mineral And other | (5.3%) | (7.7%) | (7.6%) |

### *In vitro* permeation studies

### *Preparation of isolated jejunum intestine epithelium*

The porcine jejunum intestine was obtained immediately after slaughter in the general slaughterhouse (Nakhon Pathom, Thailand). The segment of 10 cm length of jejunum intestine was collected, rinsed and placed in ice-cold sterile saline (0.9 % w/v NaCl solution). Then, the intestinal segment was cut along the mesenteric border. Epithelium layer was separated from the muscular and serosal layer using a slide strip technique. The mucosal or epithelium layer was cut into small pieces around $3.5 \times 3.5$ cm$^2$.

### Permeation study

*In vitro* permeation studies of proteins and lipids through the porcine intestine were determined using Franz diffusion cell apparatus. The diffusion cell has an average diffusion area of 2.022 cm$^2$ and the receptor compartment has a volume of 6 ml approximately. Before starting the experiments, the receptor compartment is filled with phosphate buffer saline (pH 7.4) and maintained at 37 $^o$C using a water bath. The solution in the receptor compartment is continuously stirred at 400 rpm using a magnetic stirrer in order to maintain the sink condition during the experiments.

The isolated Jejunum intestine epithelium was mounted between donor compartment and receiver compartment of the Franz diffusion cell by giving the mucosal side contact to the donor compartment. The donor compartment is filled with 2 ml of mixed solution of samples with a bile acid solution and pancreatic enzyme solution (50 mL: 6.06 mL: 12.5 mL) [16]. The donor and receptor compartment are then fixed by clamper. The top of the donor compartment filled with nutrient sample solution is covered with parafilm in order to prevent isolated Jejunum intestine epithelium from water loss. To investigate the cumulative permeation profiles, 500 µl of the solution in the receptor compartment was sampled at 0.15, 0.30, 1, 2, 4, 6 and 8 h. After sampling, an equal volume of the fresh PBS was replaced. The amount of digestible protein and lipid in the solution are determined using Lowry assay and the triglyceride quantification kit (BioVision, USA), respectively. All experiments are performed 6 times.

### Statistical analysis

All experimental measurements were made in replicate (n = 6) and expressed as mean ± standard deviation (SD). Statistical significance of differences was examined using one-way analysis of variance (ANOVA), followed by least significant difference (LSD) post-hoc test (SPSS 16.0 software). Differences were considered significant at $p < 0.05$.

## RESULTS

### *In vitro* permeation of proteins

*In vitro* permeation studies of proteins through the porcine intestine were investigated using Franz diffusion cell apparatus. The permeability of protein from three formulations with a difference in composition was compared. In addition, the permeability of the same product with different caloric value was also evaluated. In general, nutrient concentrations of standard formulae vary from 1.0 – 2.0 kcal/mL. These formulations may be used with volume sensitive patients or patients needing fluid restriction. However, this intervention may not always be clinically significant. Calorically dense formulations are most practical for use in patients requiring tube feeding [17]. Thus, the difference in protein absorption from the nutrient formulations with different caloric values should be investigated. Figure 1 describes the cumulative amounts per area of protein permeated against time from three different formulations (A, B and C) with different caloric values. The results revealed that the permeation of proteins from the same nutrient formulations with caloric values of 1.0 kcal/mL and 1.5 kcal/mL were not significant difference in all of the tested formulations. Thus, the nutrient formulation with different caloric value (1.0 kcal/mL and 1.5 kcal/mL) can be used depending on clinical condition as previously mentioned without any effect on absorption ability.

The protein permeations of each formulation in comparison to the others are presented in Figure 2. Formulation A exhibited significant higher protein permeation for both caloric values (1.0 and 1.5 kcal/mL) in comparison to formulation B and formulation C at all time points. In addition, formulation B exhibited significant higher cumulative protein permeation than formulation C (polymeric) at 240 and 360 min for 1 kcal/mL and at 360 min for 1.5 kcal/mL.

### *In vitro* permeation studies of lipid

*In vitro* permeation studies of lipids through the porcine intestine were also elucidated using Franz diffusion cell apparatus and were illustrated in Figure 3.
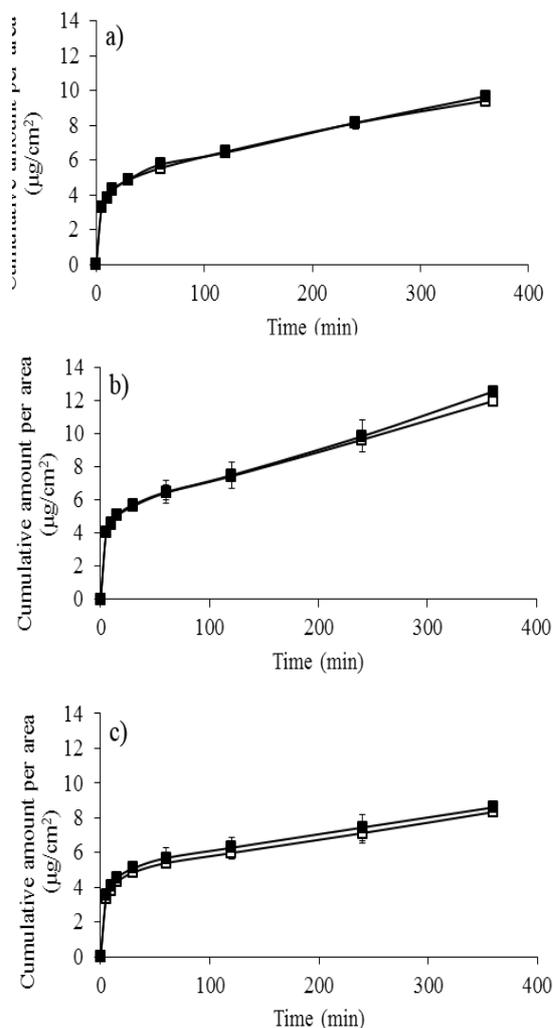
**Figure 1:** Cumulative amounts per area of protein permeated against time from a) formulation A, b) formulation B and c) formulation C with different caloric value: (□) 1.0 kcal/mL and (■) 1.5 kcal/mL

The permeation of lipid from formulation A and formulation B with 1.0 kcal/mL and were not significantly differ from 1.5 kcal/mL. However, the cumulative lipid permeation of formulation C with 1.0 kcal/mL was significantly differs from 1.5 kcal/mL since 30 min after permeation test.

The cumulative amounts per area of lipid permeated against time of formulation A, B and C with different caloric value are presented in Figure 4 above. At a caloric value of 1.0 kcal/mL, the cumulative amount of lipid permeated of formulation A were significantly differ from formulation B since 5 min after the permeation test excepted at 60, 120 and 240 min. However the cumulative amount of lipid permeated of formulation A were significantly differ from formulation C at all time points. The cumulative amount of lipid permeated of formulation B also

significant differs from formulation C since 30 min after beginning the permeation test. At a caloric value of 1.5 kcal/mL, the cumulative amount of lipid permeated of formulation A and formulation B were not significantly different. However, the cumulative amount of lipid permeated of formulation A and B were significantly higher than formulation C after 120 min of permeation time.
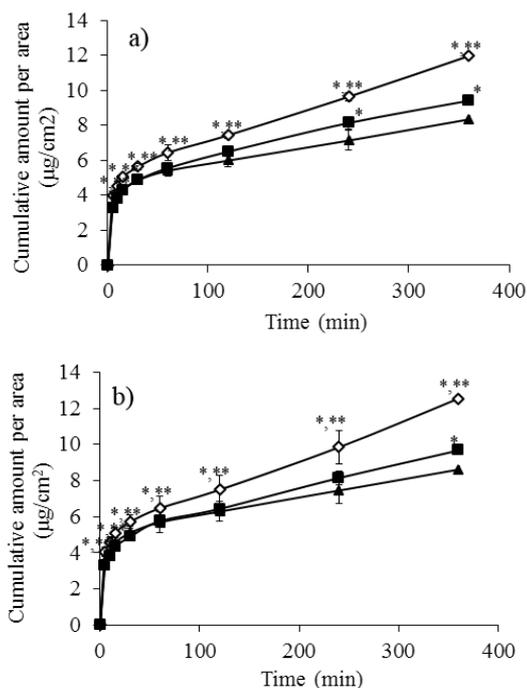


**Figure 2:** Cumulative amounts per area of protein permeated against time from different formulations: (◇) formulation A (■) formulation B (▲) formulation C at caloric value of a) 1.0 kcal/mL b) 1.5 kcal/mL *Statistically significant difference ($P < 0.05$) from control (formulation C), **Statistically significant difference between formulation A and formulation B

## DISCUSSION

The differences in amount of protein permeation among three formulations may be due to the difference in protein composition of each formulation. Formulation A and formulation B contained protein hydrolysate, which has smaller size of protein than the polymeric one (formulation C). Previous studies have reported that protein hydrolysates containing mostly di- and tripeptides are absorbed more rapidly than free form amino acids and much more rapidly than intact proteins [11,13]. The substantially greater absorption rate of amino acids from the dipeptide than from the amino acid mixture seems to be the effect of the transport capacity, which has a greater transport capacity than

amino acid carrier system [11]. The difference in protein absorption from formulation A and formulation B which are protein hydrolysate may be due to the difference in size of protein hydrolysate which obtained from different sources. Previous studies have reported that peptide-based formulations can differ in protein provided quantity and the size of the peptides.
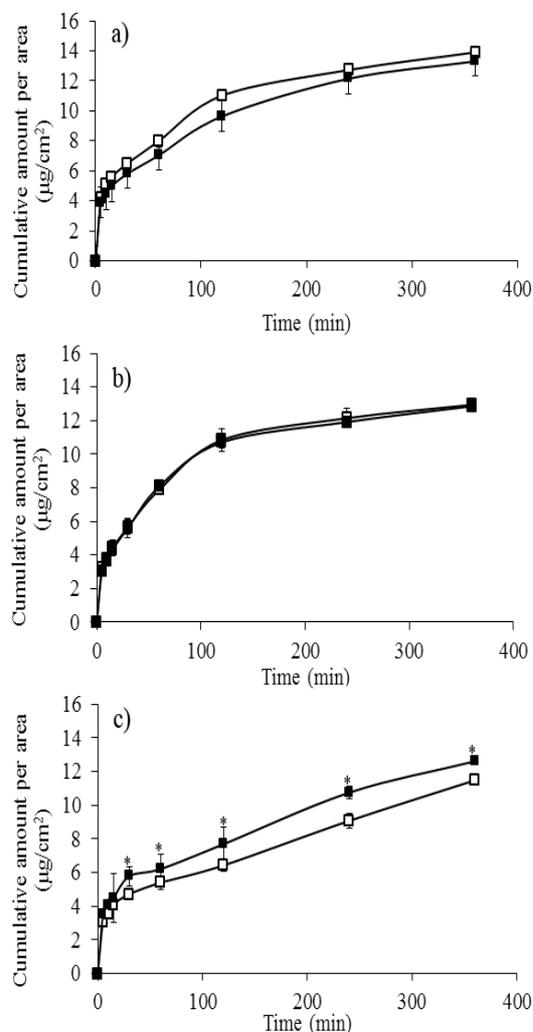
Both formulations A and B contained medium chain triglycerides (MCT) as a main component of fat which easily and fully absorbed. Conversely, formulation C contained long chain triglyceride which needs to digest or break down before absorption. Thus, formulation A and B exhibit higher rate and amount of lipid permeated as compared to formulation C.
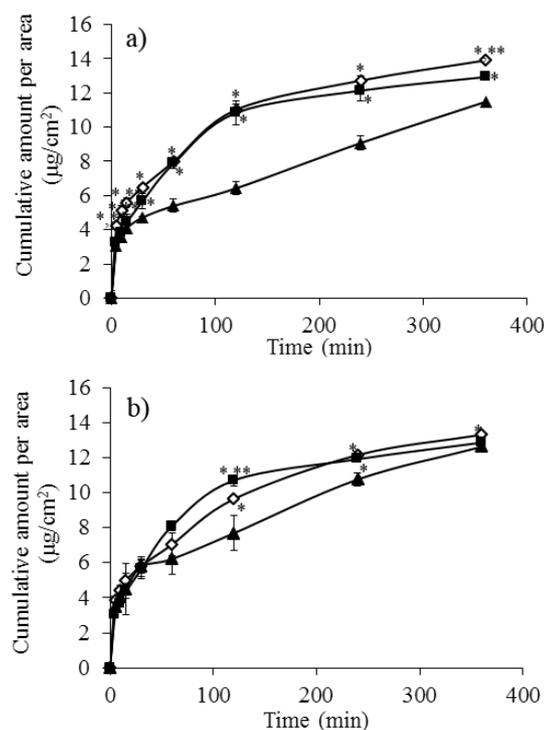


**Figure 3:** Cumulative amounts per area of lipid permeated against time from a) formulation A, b) formulation B and c) formulation C with different caloric values: (□) 1.0 kcal/mL and (■) 1.5 kcal/mL. * Statistically significant difference ($P < 0.05$)



**Figure 4:** The cumulative amounts per area of lipid permeated against time from different formulations: (◇) formulation A (■) formulation B (▲) formulation C at caloric value of a) 1.0 kcal/mL b) 1.5 kcal/mL. * Statistically significant difference ($P < 0.05$) from control (formulation C), ** Statistically significant difference between formulation A and formulation B

Some formulations contain peptides that are very large and similar to whole proteins. Conversely, some contain peptides that are very small and similar to free amino acids. Thus, their absorption kinetics are likely quite different [13-15].

The difference in cumulative amounts of lipid permeated through the porcine intestine from different formulations may be the result of the difference in kind of lipid used in formulations.

## CONCLUSION

The findings of this study indicate that oligomeric formulations exhibit higher protein and lipid absorption capacity than the polymeric formulation. In addition, either 1.0 kcal/mL or 1.5 kcal/mL can be used as nutritional support without any effect on absorption. However, actual clinical outcomes in patients needs to be investigated.

## ACKNOWLEDGEMENT

# REFERENCES

1. Villet S, Chiolero RL, Bollmann MD, Revelly JP, Cayeux RNMC, Delarue J, Berger MM. Negative impact of hypocaloric feeding and energy balance on clinical outcome in ICU patients.  Clin Nutr 2005; 24: 502-509.
2. Rubinson L, Diette GB, Song X, Brower RG, Krishnan JA. Low caloric intake is associated with nosocomial bloodstream infections in patients in the medical intensive care unit.  Crit Care Med 2004; 32: 350-357.
3. Petros S, Engelmann L. Enteral nutrition delivery and energy expenditure in medical intensive care patients. Clin Nutr 2006; 25: 51-59.
4. Kannan M. Nutrition in Critically Ill Patient. Indian J Anaesth 2008; 52: 642-651.
5. Marik PE, Zaloga GP. Meta-analysis of parenteral nutrition versus enteral nutrition in patients with acute pancreatitis. BMJ. 2004; 328: 1407.
6. Abou-Assi S, Craig K, O'Keefe SJ. Hypocaloric jejunal feeding is better than total parenteral nutrition in acute pancreatitis: results of a randomized comparative study.  Am J Gastroenterol 2002; 97: 2255-2262.
7. Gramlich L, Kichian K, Pinilla J, Rodych NJ, Dhaliwal R, Heyland DK. Does enteral nutrition compared to parenteral nutrition result in better outcomes in critically ill adult patients? Nutrition 2004; 20: 843-848.
8. Heyland DK, Drover JW, Dhaliwal R, Greenwood J. Optimizing the benefits and minimizing the risks of enteral nutrition in the critically ill: role of small bowel feeding. JPEN J Parenter Enteral Nutr 2002; 26: 51-55.
9. Moore FA, Feliciano DV, Andrassy RJ, McArdle AH, Booth FV, Morgenstein-Wagner TB, Kellum JM Jr, Welling RE, Moore EE. Early enteral feeding, compared with parenteral, reduces postoperative septic complications. Ann Surg 1992; 216: 172-183.
10. Silk DB, Grimble GK, Rees RG. Protein digestion and amino acid and peptide absorption. Proc Nutr Soc 1985; 44: 63–72.
11. Manninen AH. Protein hydrolysate in sport and excercise: a brief review. J Sports Sci Med 2004; 3: 60-63.
12. Zaloga, GP. Studies comparing intact protein, peptide, and amino acid formulas. In: Bounous, G., editors. Elemental Diets in Clinical Situations. Florida: CRC Press; 1993; pp 201–217.
13. Manninen AH. Protein hydrolysates in sports nutrition. Nutr Metab (Lond) 2009; 6: 38
14. Grimble GK, Rees RG, Keohane PP, Cartwright T, Desreumaux M, Silk DB. Effect of peptide chain length on absorption of egg protein hydrolysates in the normal human jejunum. Gastroenterology 1987; 92: 136-142.
15. Grimble GK, Guilera Sarda M, Sesay HF. The influence of whey hydrolysate peptide chain length on nitrogen and carbohydrate absorption in the perfused human jejunum.  Clin Nutr 1994; 13: 46.
16. Ehrlein H, Stockmann A. Intestinal absorption of nutrients is not influenced by soy fiber and does not differ between oligomeric and polymeric enteral diets.  Dig Dis Sci 1998; 43: 2099–2110.
17. Malone A. Enteral Formula Selection: A Review of Selected Product Categories. Pract Gastroenterol 2005; 44-74.