Ciencia e Investigación AGRARIA

Cien. Inv. Agr. 38(1):31-39. 2011
www.rcia.uc.cl

ANIMAL PRODUCTION

RESEARCH PAPER

Nutrient absorption, feed intake and liveweight change as a consequence of Roux-en-Y gastric bypass with increasing alimentary limb lengths in the pig model

Mónica Gandarillas[1], Camilo Boza[2], Luis Barrales[1], Suzanne Marie Hodgkinson[3], and Fernando Bas[1]

[1]Departamento de Ciencias Animales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile. Casilla 306-22, Santiago, Chile.
[2]Departamento de Cirugía Digestiva. Facultad de Medicina. Pontificia Universidad Católica de Chile. Casilla 114-D, Santiago, Chile.
[3]Instituto de Producción Animal. Facultad de Ciencias Agrarias. Universidad Austral de Chile. Campus Isla Teja, Casilla 567, Valdivia, Chile.

Abstract

M. Gandarillas, C. Boza, S.M. Hodgkinson, L. Barrales, and F. Bas. 2011. Nutrient absorption, feed intake and liveweight change as a consequence of Roux-en-Y gastric bypass with increasing alimentary limb lengths in the pig model. Cien. Inv. Agr. 38(1): 31-39. Roux-en Y gastric bypass (RYGBP) combines a restrictive and malabsortive component of the digestive system. Metabolic outcomes of increasing the latter, is a matter of research interest. The aim of this study was to evaluate the nutritional consequences of RYGBP with different alimentary limb lengths using a pig model. Twenty-six pigs were randomly assigned to four laparoscopic procedures: RYGBP with 300 cm of alimentary limb (T300); RYGBP with 600 cm (T600); RYGBP with 900 cm (T900); sham operation (TS). RYGBP consisted of creating a proximal gastric pouch with a 300, 600 and 900 cm alimentary limb. Sham operation was performed by bowel transections and re-anastomosis in the ileum and proximal jejunum together with gastrotomy and closure. Animals were evaluated weekly for weight increase and monthly for food intake. Eighteen weeks after surgery, fecal *in vivo* apparent digestibility of protein, fat, fiber, calcium and phosphorous were determined. We performed ANOVA and Tukey tests to detect differences in weight, feed intake and digestibility. We observed significant differences in liveweight after 10 weeks among T300, T600 and T900 as compared to TS. No differences were found among T300, T600 and T900. We also observed significant differences in feed intake among T300, T600 and T900 versus TS. No differences among treatments for digestibility of fat; fiber; protein and energy were found. Calcium and phosphorous apparent digestibility differed among treatments. RYGBP reduced weight increase in the porcine model and calcium and phosphorous digestibility was reduced with longer limbs.

Key words: Gastric bypass, Roux-en-Y, pig nutrition, obesity.

Introduction

Bariatric surgery has been developed as a successful treatment for weight reduction (40-100% of excess weight) among morbidly obese humans subjects. This is due to a lower incidence of weight regain compared to drug treatments (Buchwald *et al.,* 2004). Weight reduction is followed by a reduction of related comorbidities (such as type II diabetes mellitus, hypertension, sleep apnea, dyslipidemia) in short and long term follow-ups (Bloomberg *et al.,* 2005).

Roux-en Y gastric bypass (RYGBP) is among the most frequently practiced bariatric surgery procedures used to treat morbid obesity. It com-

Received January 21, 2010. Accepted January 28, 2011.
Corresponding author: mgandari@uc.cl

bines a restrictive component, by constructing a 50 ml gastric pouch, with a malabsorptive mechanism through the creation of an alimentary limb of 150 cm (Herron, 2004).

The precise length of alimentary limbs in order to obtain the best weight loss results in the morbid and super-obese subjects has been the matter of much research. Studies comparing different limb lengths by RYGBP in morbid obese and super obese (BMI $\geq$ 50 kg/m^2) patients have shown that only super obese benefit from longer alimentary limbs. Longer alimentary limbs in patients with BMI < 50 kg/m^2 has not resulted in greater weight reduction although a lower incidence of weight regains has been observed (Brolin *et al.,* 1992; MacLean *et al.,* 2001; Choban and Flancbaum, 2002; Dresel *et al.,* 2004; Inabnet *et al.,* 2005). The basis of success is thought to be a combination of both restrictive and malabsortive nutrient mechanisms (NIH, 1991).

The metabolic impact of the malabsorptive mechanism is not yet clear. Nutritional deficiencies (e.g., calcium, iron, vitamin D, etc.) have been observed in adolescent patients; where bariatric surgery is being performed more frequently (Deckelbaum and Williams, 2001; Surgeman *et al.,* 2003). Nutrient absorption studies have not been carried out in human subjects who have undergone RYGPB.

The pig (*Sus scrofa domestica*) is considered a suitable alternative to evaluate the metabolic consequences of these procedures, based on similarities between humans and pigs in terms of anatomic, physiologic and metabolic characteristics (USDA, 2000). While the digestive system of pigs has some anatomical differences compared to humans, the physiology of digestion remains the same. Swine are used as a general surgical model for most organs and systems, for cardiovascular research including atherosclerosis, for digestive system models, and in recent years in transplantation and xerographic research (USDA, 2000).

In general, experimental animal models have been used to assess the feasibility of surgical techniques rather than to evaluate the postoperative metabolic consequences (Cagigas *et al.,* 1999; Scott *et al.,* 2001). However, animal models such as the pig can also be used in studies that accurately establish the relationship between the input and the output of nutrients (Schneider and Flatt, 1975; McLean and Tobin, 1987; Whittemore, 1990).

The aim of this study was to evaluate the nutritional consequences of RYGBP with increasing alimentary limb lengths, utilizing an experimental pig model (*Sus scrofa domestica*).

Material and methods

Animals, experimental designed and treatments

Twenty-six male pigs (Large White x Landrace) of approximately 25 kg body weight were used in the study. Seventeen pigs underwent laparoscopic RYGBP with three different alimentary limb lengths as described below and were randomly allocated to three treatments. A fourth treatment (n=9) constituted the sham treatment (TS) against which the measured variables were compared. The first treatment, named T300 (n=9) had a 300cm alimentary limb; the second treatment named T600 (n=4), had a 600cm limb; and the third treatment named T900 (n=4) had a 900cm alimentary limb. The fourth experimental treatment (TS) was named the sham group.

Animal care and welfare protocol

All pre- and postoperative care of the animals was in accordance with a previously approved animal welfare protocol. Before this experiment was conducted, it was approved by the Comité de Bioseguridad de la Facultad de Agronomía e Ingeniería Forestal de la Pontificia Universidad Católica de Chile. The experiment was conducted according to internationally accepted principles and guidelines for the care and use of laboratory animals (for details see Wolfensohn and Lloyd, 2003).

Surgical Technique

The surgery was performed by digestive surgeons trained in advanced laparoscopy and bariatric surgery. Animals underwent general anesthesia, induced with 2% Xylazine hydrochloride (IM), ketamine (IV) and thiopental (IV), and later maintained with halothane through orotraqueal intubation.

Laparoscopic RYGBP was performed following a five trocar technique (20mm 512 SD, Johnson and Johnson®). A 90cm biliopancreatic limb was created using a 45mm laparoscopic linear stapler (TR45W, Johnson and Johnson®). The alimentary limb was 300cm, 600cm or 900cm depending on the experimental treatment. The jejuno-jejuno anastomosis was conducted using a linear stapler, closing the anastomotic gap with a 3-0 Vycril running suture. The gastric pouch was constructed firing 6 linear staplers (TR45G, Johnson and Johnson®), from the greater curvature towards the lesser curvature of the stomach, following a 34 F bougie. The gastric pouch staple line was later reinforced using a 1-0 Prolene running suture. The alimentary limb was mobilized following an antecholic route, creating later a 25mm hand sewn ante gastric gastro-jejunum anastomosis (Figure 1). In the sham treatment (TS), pigs were anesthetized and a laparoscopy operation was performed, three bowel transections and re anastomosis were performed in the ileum and in the proximal jejunum, followed by a gastrectomy and closure with staple.

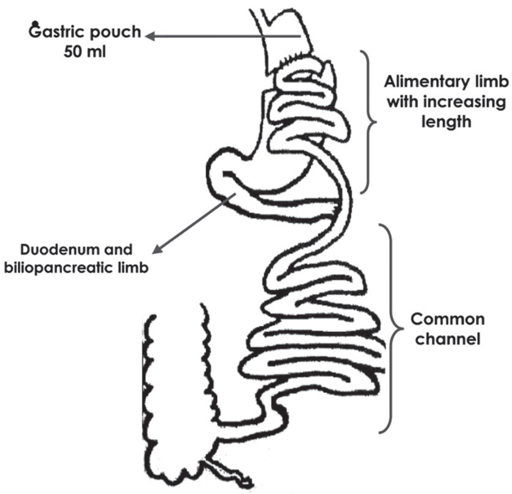

Figure 1. Roux-en-Y Gastric Bypass. In T300, T600 and T900 a gastric pouch was created, and a gastrojejunum anastomoses constituted the Alimentary limb, which length 300 cm in T300; 600 cm in T600 and 900 cm in T900. Simultaneous as the alimentary limb extents, the common channel (the place were digestion and absorption begins) decreases in length.

Animal feeding

Prior to the surgery, a liquid diet was provided to the animals for 24 hours to prevent vomiting or severe gastrointestinal distention caused by feed residues. Twelve hours before surgery, animals were maintained strictly free of water or any other liquid (Swindle, 1998). Following the surgical procedure, animals were maintained without food and water administrated orally for the first 12 hours. Then gradually they began to drink water, and 1-2 liters of a liquid diet consisting of semi-skimmed milk (prepared at the concentration recommended on the packaging). To increase the energy content of the milk, maltodextrine (110 g/L) was added. The liquid diet was administered to the animals so long as no side effects (vomiting, pain signs, and lack of appetite) appeared from digestion, until seven to eight weeks post-surgery. If such signs appeared, a veterinarian assistant checked the incidence of fever, feces aspect, texture, color and odor. This is critical because abdominal pain in most cases is due to a gastric or intestinal obstruction. In this case antibiotic, analgesic and anti-inflammatory therapy and/or endoscope exploration must be performed.

Animals that progressed properly had their diets changed gradually over a one week period to a solid, commercial diet for pigs (Concentrados Cisternas®).

Liveweight follow up

All animals were weighed weekly using an electronic scale (FX-1, Iconix ®) for eighteen weeks.

Handling Conditions

Following the surgery, animals were maintained in individual pens with a slat-type floor, nipple-type water disposers, and wide feeders. The environmental temperature was kept between 18 and 24 °C.

Post-operative follow up

Following the surgery, all animals were kept in individual or collective pens and received clear liquids for the first 48 hours. Animals were monitored for 18 weeks measuring liveweight increase and nutritional evaluation through *in vivo* digestibility trials as described below.

Feed intake measurements

Feed intake was measured in all animals over a 10-day period, at week number 24, when the pigs reached six months after surgery. During these periods, animals were housed in metabolic crates, and allowed *ad libitum* access to the diet.

Feed digestibility in vivo trials for measuring nutrient absorption

Thirty-four weeks after surgery, *in vivo* apparent digestibility trials were conducted following the method described by Schneider and Flatt (1975). The pigs were housed in individual metabolism crates during the 15-day evaluation period. First, an adaptation period of 4 days allowed the animals to get accustomed to the crates. During days 5 to 11, pigs received the diet *ad libitum* to establish the feed intake of each one. On days 12 to 15, pigs received 85% (This 85% allows the digest food to have a proper passage throughout the gastrointestinal tract) of the *ad libitum* intake recorded. A complete fecal collection was carried out on days 13 to 15. Feces were collected hourly and weighed. Of the total feces collected each day, a sample of 10% was frozen at -20 °C. Following this period the three samples per animal were thawed, mixed thoroughly and a sub sample of 500 g from each pig was taken.

Chemical analyses of feed and feces

The feed and fecal samples were analyzed as follows. Dry matter was determined according to the method described by AOAC (1990). Ash content was quantified by burning samples at 550°C for 12 h in a muffle furnace. Total fat was measured by the method described by Sukhija y Palmquist (1988); crude protein (N x 6.25) was measured by Kjeldahl method using a Kjell-Foss 16200 autoanalyzer; acid detergent fiber was determined by the Van Soest method (Goering and Van Soest, 1970). Gross energy was determined with an IKA-C 400 bomb calorimeter (IKA Werke, Janke & Knunkel, Staufen, Germany). Calcium was analyzed by flame atomic spectrophotometry (SpectrAA 220 FS Varian Springvale, Australia) and phosphorous was assayed using the Vanadate colorimetric method described by Biomérieux (Biomérieux 61571, Charbonnieres-les-bains, France).

Calculation made from the samples to total amounts of each nutrient (dry matter, ash, crude protein, total fat, fiber detergent acid, calcium, phosphorous) were determined by calculating the percentage of nutrient that appears on feces as:

$$\text{Fecal digestibility of nutrient (\%)} = [(I - F) / I] * 100$$

Where I = nutrient quantity in oral intake; and F = fecal excretion of nutrient

Statistical analysis

Data for liveweight change over time were analyzed using ANOVA for a repeated time measurements, to compare the between subject effect (the treatment effect), the within subject effect (time effect or changes within the measurement taken on each animal) and the interaction between time*treatment. Tukey test means were used when the variability sources were statistically different. For feed intake (measured as a percentage of metabolic weight) and the digestibility nutrient data (calculated as percentage), a "one way ANOVA" and a Tukey test was used. All data was processed using the statistical analyses software (SAS/STAT Version 6.10; SAS Institute, Cary, NC).

Results

Liveweight

All treatments remained without a significant liveweight increases for 6 weeks post-surgery. However, animals from TS treatment increased liveweight from week 10 until the end of the experiment (Figure 2) ($P \leq 0.05$). Moreover, from week 10th to18th after surgery no significant differences were observed on liveweight among T300 (31.1 +/- 7.4 kg), T600 (42.0+/- 12.8) and T900 (37.0 +/- 2.4 kg). In contrast, these three treatments had a lower liveweight gain when compared with TS (73.0 +/- 10.7 kg) ($P \leq 0.05$) between weeks 10th and 18th (Figure 2).

Feed intake

No significant differences were found among means of RYGBP treatments ($P>0.05$), but they differed with respect to the mean of TS animals (Table 1).

Nutrient digestibility

There were no significant differences among mean treatments ($P > 0.05$) for the digestibility of dry matter; total fat; ADF; crude protein and digestible energy (Table 2). However, the digestibility of ash, calcium and phosphorous significantly differed among treatments, with the TS animals having a greater digestibility than the T300, T600 and T900 animals ($P \leq 0.05$).

Discussion

Pigs have been used extensively in human nutrition research (Miller and Ullrey, 1987). The digestive system of swine has anatomical similarities and differences compared to humans. Pigs and humans have comparable gastrointestinal tract (GIT) anatomy, morphology and physiology. The GIT of a 30- to 40-kg pig is similar in total length to that of a human adult and the relative sizes of the sections of human and pig GIT are alike with a similar digestive physiology (USDA, 2000). Swine are omnivores, as are humans. Furthermore, there are many similarities between humans and pigs in their dietary requirements for nutrients, although the quantitative requirements for each nutrient differ between both species, for example the total requirement of crude protein in terms of dry matter are higher for piglets due to a higher growth rate of this specie (National Research Council, 1998). Surgically prepared pigs provide a powerful tool for investigating digestion and absorption of nutrients (Yen, 2000). Several types of surgeries have been carried out

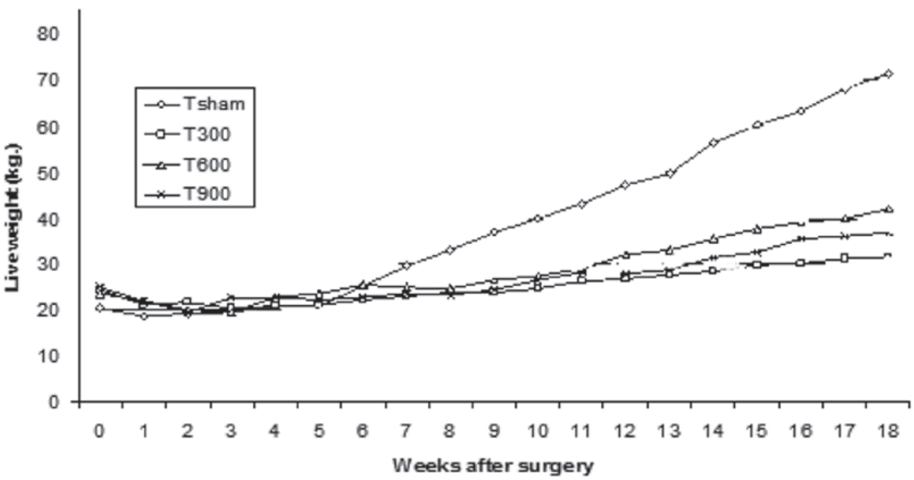

Figure 2. Liveweight evolution during the next 18 weeks following the surgery. Statistically significant differences began at week 10 postoperative between TS and all other treatments; $P < 0.05$ and were maintained from the onward. No significant differences ($P > 0.05$) were present between treatment T300, T600 and T900.

Table 1. Average feed intake as a percentage of the liveweight per treatment[1].

	TS	T300	T600	T900
Feed intake (g/day)	3147.6 a	1392.1 b	1664.1 b	1579.9 b
Liveweight (kg)	98.6	48.8	55.7	49.7

[1]Numbers in a row with different superscript significantly differed ($P \leq 0.05$).

Table 2. Fecal apparent digestibility *in vivo* (mean $\pm$ sem) in pigs with Roux-en-Y gastric bypass with and alimentary limbs of a 300 cm (T300), 600 cm (T600), and 900 cm (T900) and pigs with a sham operation (TS)[1].

	TS	T300	T600	T900
Dry Matter (%)	73.0 $\pm$ 1.1	70.6 $\pm$ 1.6	72.2 $\pm$ 1.9	69.4 $\pm$ 3.2
Ash (%)	42.9 $\pm$ 2.7 a	23.01 $\pm$ 3.8 b	23.3 $\pm$ 6.2 b	21.5 $\pm$ 2.7 b
Acid detergent fiber (%)	20.6 $\pm$ 2.7	16.1 $\pm$ 3.7	20.6 $\pm$ 8.7	12.8 + 3.0
Total Fat (%)	63.8 $\pm$ 2.7	56.7 $\pm$ 5.1	64.9 $\pm$ 9.4	75.7 $\pm$ 4.4
Crude Protein (%)	76.6 $\pm$ 1.3	73.2 $\pm$ 1.5	73.2 $\pm$ 3.7	74.8 $\pm$ 1.5
Calcium (%)	41.1 $\pm$ 1.6 a	13.8 $\pm$ 5.9 b	7.3 $\pm$ 3.8 b	2.7 $\pm$ 1.7 c
Phosphorous (%)	44.7 $\pm$ 1.8 a	27.8 $\pm$ 6.0 b	22.3 $\pm$ 11.9 b	3.4 $\pm$ 3.3 c
Energy (%)	72.8 $\pm$ 1.2	70.75 $\pm$ 1.6	73.4 $\pm$ 2.9	74.3 $\pm$ 1.3

[1]Numbers in a row with different superscript significantly differed ($P \leq 0.05$).

with the porcine model, to test their applicability to human surgery, to try new equipment or to develop the necessary skills for surgeons. More recently, endoscopic and laparoscopic surgical models have been developed and used extensively on pigs (Tumbleson, 1986; Tumbleson and Schook, 1996).

Bariatric surgery has been shown to be an effective treatment for patients with severe clinical obesity (Buchwald *et al.,* 2004). In addition to significant weight loss, it has also been associated with improvements in comorbidities (Alvarez-Leite, 2004). Roux-en-Y gastric bypass (Figure 1) is designed to limit food intake by creating a small gastric pouch and to reduce nutrient absorption by bypassing the long limb of the intestine (Brolin, 2005). Unfortunately, bariatric surgery can also potentially cause a variety of nutritional and metabolic complications, mostly due to the extensive surgically induced anatomical changes incurred by the patient's gastrointestinal tract, particularly with roux-en-Y gastric bypass. The most common micronutrient deficiencies that have been observed are for vitamin B12, iron, calcium, and vitamin D (Malinowski, 2006). These results should be useful when compared with young adolescents which are candidates for bariatric surgery, especially those that are in a growing phase of their lives.

Results on liveweight observed during 18 weeks following the RYGBP with different length limb indicated that there were no significant differences in weight progression among experimental groups with 300, 600 and 900 cm alimentary limbs. Similar results have been observed in different clinical studies practiced with humans with different limb lengths such as Maclean and co-workers (Chapin *et al.,* 1996), who studied morbid patients (BMI $\geq$ 40) who underwent RYGBP, with 40 cm alimentary limb, compared to super obese patients (BMI $\geq$ 50) with 100cm alimentary limb. Only super obese patients proved significant excess weight reduction. In the same line, Choban and Flancbaum (2002) compared morbid obese patients who underwent RYGBP with alimentary limb lengths of 75 cm and 150 cm versus super obese patients with alimentary limbs of 150 cm and 250 cm. After a two years follow up, only super obese patients proved significant differences in excess weight reduction with longer limbs. Despite these results in human patients with a significant excess weight, the porcine model did not show differences among different limb lengths, probably due to that not using obese or morbidly obese pigs threw out the experiment.

Significant differences were observed between TS animals when compared to T300, T600 and T900 animals probably due to differences in feed intake (Table 1). This suggests that the reduced growth rate was due to a reduction in gastric volume which was the same for T300, T600 and T900 animals. It appears that increasing the malabsorptive effect by increasing the length of the bypass limb done in RYGBP in growing non-obese pigs, did not affect their growth rate.

In the *in vivo* apparent digestibility study there were no significant differences between treatments in terms of dry matter, crude protein, acid detergent fiber, total fat and energy digestibility among treatments. This further suggests that no malabsortive effect existed with increased alimentary limb length, and there was no malabsortive effect between short or long alimentary limbs versus sham pigs. There was no weight increase of the animals that were subjected to RYGBP with alimentary limbs of 300, 600 and 900 cm. This appears to be due to the restrictive effect on stomach size and its consequent effect on feed intake, which was the same for the three surgical groups.

Overall mineral absorption (measured as ash digestibility), and specific calcium and phosphorous absorption (measured as calcium and phosphorous digestibility), differed among treatments (Table 2). A lower level of absorption of a portion of the diet, representing the mineral fraction, was demonstrated in animals with TS treatment in comparison with those that received different alimentary limb length (T300, T600, and T900 animals). Also, significantly different calcium and phosphorous absorptions were demonstrated between the sham group versus T300, T600, and T900. Underwood and Suttle (1999), reported that apparent absorption of calcium from the diet in pigs of the same age varies between 20 to 50%, depending on the physiological status of the animals. In human studies, Chapin *et al.,* (1996) found that the most common complaint was fractured bones or a reduction in bone density; observations showing "severe bone loss". Probably due to fat malabsorption, severe vitamin D deficiency can develop along with the reduced ability to absorb calcium. In our experiment, pigs received 6-7% of total fat as percentage of dry matter intakes, and no differences in absorption were found at these fat concentrations. However, this might not hold true as fat content in the diet increases to reach concentrations more frequent on western diets. As fat was absorbed in the present study, vitamin D (a fat-soluble vitamin) should also have been well absorbed as they both utilize the same digestion and absorption route (Henry and Norman, 1984).

As a conclusion and in line with the pig model, the decreased liveweight, when using a growing non-obese model, appeared to be due to a decreased feed intake. The surgical procedures did not have a malabsortive effect for protein, energy, fat and fiber. However there was a significant decrease for mineral, calcium and phosphorous absorption, which could have important consequences in operated subjects.

Acknowledgments

To Dirección de Investigación y Postgrado of the Pontificia Universidad Católica de Chile for providing funding (Proyecto de inicio 2531-073).

Resumen

M. Gandarillas, L. Barrales, S.M. Hodgkinson y F. Bas. 2011. Absorción de nutrientes, consumo de alimento y cambio en el peso vivo en cerdos operados con bypass gástrico en Y de Roux con creciente longitud de asa intestinal. Cien. Inv. Agr. 38(1): 31-39. El objetivo de este estudio fue evaluar las consecuencias nutricionales del BPGYR con distintas longitudes de asa alimentaria en el modelo porcino. Veintiséis cerdos fueron aleatoriamente asignados a cuatro tratamientos. T300 consistió en BPGYR con asa alimentaria de 300cm, T600 fue BPGYR con 600cm, T900 fue BPGYR de 900cm; TS fue la operación sham. En el BPGYR se creó una bolsa gástrica de 50 cc, anastomosada al yeyuno, constituyendo asa alimentarias de 300, 600 y 900cm. La operación sham (TS) fue realizada hacienda tres incisiones en el intestino, posteriormente re-anastomosadas. Los animales fueron pesados semanalmente durante 18 semanas. El consumo de alimento y la digestibilidad aparente fecal de macro y micronutrientes se realizó a la decimoctava semana. Un ANDEVA se usó para comparar los promedios de peso por tratamiento y el test de Tukey se utilizó cuando hubo diferencias en peso vivo, consumo de alimento y digestibilidad. Desde la décima semana postoperatoria, hubo diferencias significativas de peso entre TS vs. T300, T600 y T900. No hubo diferencias entre T300, T600 y T900. En el consumo de alimento, hubo diferencias significativas de peso entre TS vs. T300, T600 y T900. No hubo diferencias entre T300, T600 y T900 en la digestibilidad de la grasa, fibra, proteína y energía ($p > 0,05$), en cambio la digestibilidad del calcio y fósforo difirió significativamente entre TS vs. T300 y T600 vs. T900. Como conclusión, el BPGYR redujo el incremento de peso en el modelo porcino, pero mientras se alarga el bypass gástrico no se afecta la digestibilidad de macro nutrientes y no así de calcio y fósforo.

Palabras clave: Bypass gástrico, Y de Roux, nutrición porcina, obesidad.

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