Effects of whey protein on intestinal integrity in heat-stressed growing pigs

July 2013

By:
Lance H. Baumgard, PhD.
Iowa State University, Department of Animal Science

Nicholas K. Gabler, PhD.
Donald C. Beitz, PhD.
Victoria Sanz-Fernandez, DVM

Partners:
Midwest Dairy Association

TABLE OF CONTENTS

Abstract

Heat stress (HS) reduces livestock productivity and this may in part be mediated by reduced intestinal integrity or "leaky gut". Dairy products improve intestinal integrity in a variety of human and small animal models. Consequently, we hypothesized that dietary bovine colostrum (CL) and whey protein (WP) would mitigate HS-induced leaky gut in pigs. Crossbred gilts (39 ± 3 kg BW) were ad libitum fed 1 of 4 dietary treatments (n = 8 pigs/treatment): 1) control (CT), 2) diet A, containing test product A (98% WP, 2% CL); 3) diet B, containing test product B (80% WP, 20% CL); and 4) diet *C,* containing test product C (100% WP). Diets were formulated to provide 100 g/d of protein from the test products. After 7d (Period 1, P1) on experimental diets, all pigs were exposed to constant HS conditions for 24 h (P2; 32°C, 26% RH). Production parameters and body temperature indices were recorded throughout the experiment. At the end of the study, pigs were euthanized and fresh ileum and colon sections were isolated and mounted into modified Ussing chambers. Intestinal permeability was assessed via measures of transepithelial electrical resistance (TER) and apparent permeability coefficients (APP) for the fluorescein isothiocyanate-labeled macromolecule dextran (FITC-Dextran). During P1, diet C-fed pigs had a slightly increased (*P* < 0.05; 0.1 oq rectal temperature (Tr), but respiration rates (RR) were not different between treatments. As expected, during P2, both Tr (*P* < 0.01; 40.29 vs. 39.43°C) and RR (*P* < 0.01; 116 vs. 40 bpm) increased, but no dietary treatment differences were detected. There were no differences in growth or feed efficiency during PI. Heat stress markedly reduced feed intake (*P* <0.01; 44%), and none of the dietary treatments ameliorated this decrease. After 24 h of HS, pigs in all treatments lost a similar amount of BW (-0.5 kg). Ileal TER was decreased (*P* = 0.02; 37%) and tended to be decreased (*P* = 0.10; 27%) in B and C diet-fed pigs, respectively; when compared to CT. No differences were detected in any of the remaining intestinal permeability variables. These data demonstrate that supplementing CL and WP in the proportions present in the test products does not ameliorate the effects of severe HS on intestinal integrity in pigs.

Materials and Methods

Animals

Thirty two crossbred gilts (39 ± 3 kg body weight) were assigned to 1 of 4 dietary treatments (n = 8 pigs/treatment) at the Iowa State University Swine Nutrition Farm. Pigs were housed individually in 1 of 2 environmental rooms for the length of the experiment. All procedures were reviewed and approved by the Iowa State University Institutional Animal Care and Use Committee.

Diets

All pigs were fed 1 of 4 iso-energetic and iso-nitrogenous diets formulated to meet or exceed the predicted requirements (NRC, 1998) for energy, essential amino acids, protein, minerals, and vitamins (Appendix A):

1) Control (CT) diet
2) Diet A: containing test product A (98% whey protein concentrate, 2% colostrum)
3) Diet B: containing test product B (80% whey protein concentrate, 20% colostrum)
4) Diet C: containing test product C (100% whey protein concentrate)

Diets were formulated to provide 100 g/d of protein from the test products; based upon an assumed total feed intake of 1.8 kg/d. Diets were fed ad libitum from 7 d prior to the environmental exposure and through the end of the experiment.

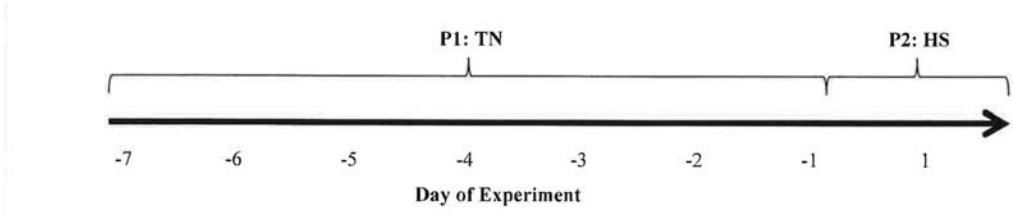
Experimental design

Pigs were selected and assigned to 1 of 4 dietary treatments based upon initial body weight (BW). The study began after 3 d of acclimation to the individual crates and was divided into two experimental periods (P): P1 and P2. During P1, pigs remained in constant thermo-neutral (TN) conditions (19°C;

46% humidity; temperature-humidity index::: 63; Figure 1) for 7 days. During P2, pigs were exposed to constant heat stress (HS) conditions (32°C; 26% humidity; temperature-humidity index::: 76; Figure 1) for 24 h. At the end of P2, pigs were sacrificed using the captive bolt technique followed by exsanguination. Intestinal tissues were immediately harvested at death.

Due to logistical constraints in sample collection and analysis, groups of 4 pigs (i.e. 1 pig/treatment) were sacrificed twice a day (8 pig/d) for 4 days. Each group of 4 pigs was considered a "set" for statistical purposes. The timing of each measured variable was similar between sets.

Experimental schematic



Thermal status measurements

Body temperature indices (respiration rate and rectal temperatures) were obtained four times daily *(0800, 1200,* 1600 and 2000 h) on d *-3,* -2, and -1 for baseline

determination. During P2, temperature indices were obtained at *0, 4, 8,* 12 and 24 h relative to the initiation of HS. Respiration rates (RR) were determined by counting flank movements and rectal temperatures (Tr) were measured using a standard digital thermometer (V901H Vicks®, Hudson, NY).

Environmental conditions measurements

Throughout the experiment, ambient temperature was controlled, but humidity was not governed. Each *room's* temperature and humidity were monitored and recorded every 30 minutes by a data logger (Lascar® model EL-USB-2-LCD, Erie, PA).

Production parameters*

Body weights were collected at the initiation of the study (i.e. beginning of P1), at the beginning of P2, and immediately prior to sacrifice. Feed intake (FI) was recorded for the last 5 days of P1to determine average daily Fl and feed efficiency.

*Production variables were measured only to provide a context for the more mechanistic measurements of the primary objective.

Tissue harvesting

Fresh sections from the proximal ileum (1.5 m proximal to the ileocecal valve) and distal colon (SO em proximal to the rectum) were harvested immediately following sacrifice. Tissues were flushed (with saline) of luminal content, placed immediately into Krebs-Henseleit buffer (KHB; containing 25 mM NaHC03, 120 mM NaCl, 1 mM MgS04, 6.3 mM KCl, 2 mM CaCl and 0.32 mM NaH 2 P04, pH 7.4) under constant aeration, and transported to the laboratory for mounting into modified Ussing chambers.

Ussing chambers

Ileal and colonic segments of each animal were mounted into modified Ussing chambers (Physiological Instruments and DVC 1000; World Precision Instruments, New Haven, CT) for intestinal integrity and permeability assessment. Tissue samples were pinned and placed vertically into the chambers with the mucosal side facing one half of the chamber and the serosal side facing the other half. Each side of the membrane was bathed in 4 mlof KHB and tissue was provided with a constant O 2-C02 mixture. Individual segments were initially voltage clamped (0 mV). Transepithelial electrical resistance (TER) was calculated by averaging the resistance during the first 10 min of tissue stabilization (Gabler et al., 2007).

Ileal and colonic segments were also assessed for the flux of the macro molecule fluorescein isothiocyanate labeled dextran (FITC-Dextran; 4,400 kDa; Sigma®, St. Louis, MO) across the intestinal barrier. After 20 min of stabilization, KHB was replaced with fresh buffer on both the mucosal and the serosal sides of the chamber. In addition, the mucosal side received 2.2 mg/mlof FITC- Dextran. Samples from the serosal side were obtained in

duplicate every 20 min for 120 min and read in a fluorescence spectrophotometer (495 nm excitation and 520 nm emission). FITC-Dextran permeability is reported as an apparent permeability coefficient (APP) calculated (as previously described by Tomita et al., 2004) using the following formula: APP =dO/ (dt x Ax C0).

where dO/dt is the rate of FITC-Dextran transport in IJ.g/sec, which is the slope of the regression line obtained by spectrophotometry; Co is the concentration in mg/ml; and A is the area of the membrane
in cm^2 .

Statistical analyses

Data are reported as LSmeans and considered significant if *P:::* 0.05 and a tendency if 0.05 > *P:::* 0.10. Variables with single measurements were statistically analyzed using the PROC GLM procedure of SAS version 9.2 (SAS lnst. Inc. Cary NC). The model included treatment and set as fixed effects. For a given variable, when an initial measurement was available (i.e. BW, ADG and Fl) it was used as a covariate. Variables with multiple measurements (i.e. Tr and RR during P2) were analyzed using the PROC MIXED procedure of SAS. Each animal's respective parameter was analyzed using repeated measures with an autoregressive covariance structure. The model included treatment and set as fixed effects, and covariate when available. The repeated effect was hour after initiation of HS. For both procedures, set and the covariate were only kept in the model if their *P* ::; 0.20. Post-hoc contrasts were utilized to estimate differences between each dietary treatment and the control diet.

Results

1. Body temperature indices

Overall, there were no treatment differences in daily average body temperature indices during P1(*P* >0.10; Table 1). When analyzing each time point individually, a treatment tendency *(P* ::; 0.10) and difference *(P:::* 0.05) were observed in Tr at 0800 and 1600 h, respectively; mainly due to an increased Tr (O.1aq for diet C-fed pigs.

As expected, during P2 Tr and RR increased an average of 0.85°C and 77 bpm, respectively relative to Pl. There was a time effect *(P* ::; 0.05; Table 2; Figure 2) for both indices; however, no treatment differences were detected. Rectal temperature progressively increased during the first 12 h of HS. At sacrifice, overall Tr was increased O.6 0C compared to P1 *(P* ::; 0.01; Table 2; Figure 2A). Respiratory rate increased *(P:::* 0.01) after 4 h of HS and remained elevated until sacrifice (Table 2; Figure 28).

2. Production parameters

2.a Body weight and average daily gain

Overall, the BW at the initiation of P1(i.e. at the beginning of the dietary treatments) was not different amongst treatments (39.48 kg; Table 3). However, when contrasting

individual groups, the pigs selected for the diet A and C treatments tended to be lighter (*P* = 0.06; 1.45 kg) and were lighter (*P* =0.05; 1.47 kg), respectively; compared to the pigs destined to be fed the CT diet (Table 3).

2.b Feed intake and feed efficiency

During P1, there were no treatment differences (*P* > 0.30) in FI (2.1 kg/d; Table 3} or G:F ratio (0.35; Table 3; Figure 5). During P2, all pigs reduced FI (*P* < 0.01; 44%L and the response was similar between treatments (*P* > 0.60; Table 4; Figure 6}.

3. Intestinal parameters

3.a Transepithelial electric resistance

Ileal TER was decreased (*P* :5 0.05) in diet B-fed pigs and tended to be decreased (*P* :5 0.10) in diet C-fed pigs compared to controls (37% and 27%, respectively; Table 5; Figure 7). Ileal TER for diet A-fed pigs did not differ (*P* > 0.50) from CT. There was no dietary effect on colonic TER (*P* > 0.30; Table 5; Figure 8).

3.b FITC-Dextran apparent permeability coefficient

There were no treatment differences (*P* > 0.40) in either ileal or colonic FITC-Dextran APP (Table 5; Figure 9 and 10}.

Discussion

Heat stress is one of the costliest issues for the US pork industry and it compromises America's global competitiveness. Despite advances in heat abatement systems, the warm summer months are still a financial burden for pig producers ($300 million; St Pierre et al., 2003; Pollman, 2010). Reasons for the HS-induced economic losses are reduced growth, poor sow performance, decreased carcass quality, and increased veterinary costs. Heat stress will be more of a concern if, as some have predicted, earth's temperatures keep rising as a consequence of global warming. In addition to climate change, genetic selection for leaner phenotypes decreases the pig's tolerance to elevated temperatures (selection for enhancing protein accretion results in increased basal heat production; Brown-Brandl et al., 2004).

In a recent study, we demonstrated that increasing dietary Zn improves small intestine permeability (60%) in severely heat-stressed growing pigs (Sanz-Fernandez et al., 2012}. This indicates that nutritional interventions are feasible tools to ameliorate the effects of HS in pigs, and undoubtedly represent a multibillion dollar market opportunity.

In the current study, during TN conditions (P1L diet C-fed pigs had a slight increase in Tr (0.1°C) compared to controls. This is difficult to understand, as this group of pigs did not have different FI or ADG: two key variables associated with increased basal heat production. Regardless, understanding why diet C-fed pigs had a slightly elevated Tr and whether or not this is of biological significance remains of interest. During P2, pigs were exposed to extensive HS conditions (constant 32°C; 26% humidity), and this environment is optimal for studying the physiological and metabolic consequences of severe thermal stress. Thus, HS pigs experienced a heat load well above their thermal comfort zone, which resulted in a marked increase in all the body thermal indices. The average differences in Tr and RR between P1and 2 were 0.85 °C and 77 bpm, respectively. However, no improvement was observed with any of the dietary treatments.

Feed intake during thermo-neutral conditions averaged 2.1 kg/d, which was 0.3 kg more than anticipated. Therefore, pigs received approximately 117 g/d of protein from the dietary treatment. As expected, HS conditions caused a sharp decrease in FI (44%) and the magnitude of the reduction did not differ between treatments. After 24 h of HS, pigs lost an average of 0.5 kg of BW and this was similar amongst dietary treatments.

The deleterious effects of HS are mediated, at least in part, by its effects on gastrointestinal health and function. During HS, blood flow is diverted from the viscera to the skin in an attempt to dissipate excess heat (Lambert, 2009). Reduced blood flow and hyperthermia lead to hypoxia and oxidative and nitrosative stress in the enterocyte (Lambert, 2004). As a result, cell membranes and tight junctions can be damaged, culminating in an increase in intestinal permeability: this is also known as "leaky gut" (Lambert et al., 2002). Due to the "loosening" of the junctions between the enterocytes, there is an increased passage of high molecular weight substances and pathogens, including LPS and other bacterial components from the lumen, into the splanchnic circulation.

Dietary bovine colostrum and whey protein have been demonstrated to improve the health of challenged intestines (Kelly, 2003; Krissansen, 2007). Both colostrum and whey protein are rich in antimicrobial proteins (i.e. glucomacropeptides, lactoferrin), immunoglobulins, specific amino acids (glutamine, cysteine, and threonine), and growth factors (Krissansen, 2007). However, the composition of these products is highly variable depending on the origin (i.e. breed, alimentation and health status), the time of collection (in the case of colostrum), and the post-collection processing and concentration; making difficult to identify the bioactive constituents responsible for their positive effects (Kelly, 2003).

In healthy human patients, colostrum supplementation ameliorated the effects of an indomethacin (a non-steroidal anti-inflammatory drug with adverse gastro-intestinal effects) challenge on intestinal permeability, assessed via a lactulose:mannitol test (Playford et al., 2001). A similar colostrum product was used as an enema for the treatment of ulcerative colitis in humans, and results indicated improved symptomatic and histological scores (Khan et al., 2002). Playford and colleagues (1999) compared the

effects of bovine colostrum and whey protein on indomethacin-induced gastric (gastric damage and ulcer score) and small intestine (reduction in villus height) injury of mice and rats, respectively; and reported that colostrum supplementation was more effective than whey. In vivo observations are supported by in vitro studies where bovine colostrum totally prevented and goat milk ameliorated the decrease inTER experienced by MOCK cells after an ethylen glycol tetraacetic acid insult (a disruptor of the tight junctions; Prosser et al., 2004).

In an attempt to ameliorate the effects of HS on intestinal permeability, rats were supplemented with bovine colostrum and goat milk for 7d prior to HS exposure and both dairy products improved intestinal permeability (determined via radiolabelled EDTA transport; Prosser et al., 2004). In addition, treating human colonic cells (cell line T48) with colostrum ameliorated the effects of HS on TER (Marchbank et al., 2011). In the same study, an increase in heat-shock protein 70 concentration was observed in colostrum treated NCM460 and HT29 human colonic cells after HS exposure, suggesting a possible role of these chaperone proteins on colostrum's mechanism of action.

In the present study, there was no improvement on intestinal permeability variables from pigs fed milk whey/colostrum. Reasons for this lack of response are not clear; however, we hypothesize that the severe HS conditions (constant 32oC without a recovery period during the night) may have blunted the potential beneficial effects of colostrum and whey protein on intestinal health. Whether or not mild and cyclical HS conditions (more typical of industry), would allow for improvement on intestinal permeability variables is of obvious interest. Furthermore, feeding diet B (the diet with the highest content in colostrum: 20%) significantly decreased ileal TER (37%, Figure 7). This was unexpected, but it is in agreement with a human report, where subjects supplemented with bovine colostrum had a greater increase in intestinal permeability after a standardized exercise program compared to controls and individuals receiving whey protein (Buckley et al., 2009).

Conclusions

Heat stress is one of the largest impediments to efficient animal production and likely the most expensive issue that global animal agriculture industries face. A hallmark of heat-stressed animals is a compromised intestinal integrity and the subsequent inflammation undoubtedly contributes to reduced animal productivity. We have now demonstrated that feeding a combination of bovine whey protein and colostrum did not ameliorate the effects of severe and constant heat stress on intestinal integrity. Whether milder and cyclical HS conditions (resembling those in industry settings) and different levels of inclusion of the test products would allow for improvement on intestinal health remains of interest.

Literature Cited

Brown-Brandl T, Nienaber J, Xin H, Gates R, ASAE. 2003. A literature review of swine heat and moisture production. Swine Housing li, Proceedings:31-40.

Buckley JD, Butler RN, Southcott E, Brinkworth GD. 2009. Bovine colostrum supplementation during running training increases intestinal permeability. Nutrients 1(2):224-34.

Gabler NK, Spencer JD, Webel DM, Spurlock ME. 2007. In utero and postnatal exposure to long chain (n-3) PUFA enhances intestinal glucose absorption and energy stores in weanling pigs. J Nutr 137(11):2351-8.

Kelly GS. 2003. Bovine colostrums: a review of clinical uses. Altern Med Rev 8(4):378-94.

Khan Z, Macdonald C, Wicks AC, Holt MP, Floyd D, Ghosh S, Wright NA, Playford RJ. 2002. Use of the nutriceutical', bovine colostrum, for the treatment of distal colitis: results from an initial study. Aliment Pharmacal Ther 16(11):1917-22.

Krissansen GW. 2007. Emerging health properties of whey proteins and their clinical implications. JAmColi Nutr 26(6):713S-23S.

Lambert GP. 2004. Role of gastrointestinal permeability in exertional heatstroke. Exerc Sport Sci Rev32(4):185-90.

Lambert GP. 2009. Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. J Anim Sci. United States. p. E101-8.

Lambert GP, Gisolfi CV, Berg DJ, Moseley PL, Oberley LW, Kregel KC. 2002. Selected contribution: Hyperthermia-induced intestinal permeability and the role of oxidative and nitrosative stress. J Appl Physiol92(4):1750-61; discussion 1749.

Marchbank T, Davison G, Oakes JR, Ghatei MA, Patterson M, Moyer MP, Playford RJ. 2011. The nutriceutical bovine colostrum truncates the increase in gut permeability caused by heavy exercise in athletes. Am J Physiol Gastrointest Liver Physiol 300(3):G477-84.

National Research Council. 1998. Nutrient Requirements of Swine, lOth ed. Nat. Acad. Press, Washington, DC.

Playford RJ, Floyd DN, Macdonald CE, Calnan DP, Adenekan RO, Johnson W, Goodlad RA, Marchbank T. 1999. Bovine colostrum is a health food supplement which prevents NSAID induced gut damage. Gut 44(5):653-8.

Playford RJ, MacDonald CE, Calnan DP, Floyd DN, Podas T, Johnson W, Wicks AC, Bashir O, Marchbank T. 2001. Co-administration of the health food supplement, bovine colostrum, reduces the acute non-steroidal anti-inflammatory drug-induced increase in intestinal permeability. Clin Sci (Land) 100(6):627-33.

Pollmann DS. 2010. Seasonal effects on sow herds : Industry experience and management strategies. J. Anim. Sci. 88 (Suppl. 3):9.

Prosser C, Stelwagen K, Cummins R, Guerin P, Gill N, Milne C. 2004. Reduction in heat-induced gastrointestinal hyperpermeability in rats by bovine colostrum and goat milk powders. J Appl Physiol 96(2):650-4.

SanzFernandez MV, Pearce SC, Mani V, Gabler NK, Patience JF, Wilson ME, Socha MT, Rhoads RP, Baumgard LH. 2012. Effects of supplemental zinc amino acid complex on intestinal integrity in heat-stressed pigs. Xllth International Symposium on Digestive Physiology of Pigs, 29 May - 1 June, Keystone, USA.

St-Pierre NR, Cobanov B, Schnitkey G. 2003. Economic Losses from Heat Stress by US Livestock Industries1.Journal of dairy science 86:E52-E77.

Table 1 Effects of whey protein on body temperature parameters in growing pigs during thermo-neutral conditions (P1)

	Treatment (Trt)					*P*	Contrast		
Parameter	CT	A	B	C	SEM	Trt	A vs CT	B vs CT	C vs CT
Rectal Temperature, °C									
0800 h	39.40	39.43	39.40	39.50	0.03	0.09	0.52	0.91	0.03
1200 h	39.35	39.42	39.41	39.48	0.06	0.49	0.41	0.48	0.13
1600 h	39.40[a]	39.47[ab]	39.39[a]	39.51[b]	0.03	0.02	0.10	0.83	0.01
2000 h	39.47	39.44	39.50	39.58	0.10	0.76	0.82	0.82	0.43
Daily average	39.40	39.43	39.40	39.50	0.06	0.65	0.81	0.95	0.29
Respiratory Rate, bpm									
0800 h	40	40	42	40	1	0.60	0.88	0.24	0.88
1200 h	38	38	38	39	1	0.90	0.76	0.64	0.85
1600 h	42	44	44	44	2	0.80	0.43	0.38	0.46
2000 h	37	39	40	37	2	0.50	0.51	0.30	0.71
Daily average	39	40	41	39	1	0.55	0.48	0.21	0.90

[a,b] Represent treatment differences ($P \leq 0.05$)

Table 2 Effects of whey protein on body temperature parameters in heat-stressed growing pigs (P2)

	Treatment (Trt)					*P*			Contrast		
Parameter	CT	A	B	C	SEM	Trt	Time	Trt*Time	A vs CT	B vs CT	C vs CT
Rectal Temperature, °C	40.33	40.34	40.27	40.23	0.07	0.61	<0.01	0.97	0.85	0.55	0.32
Respiratory Rate, bpm	118	117	116	113	5	0.92	0.04	0.77	0.88	0.77	0.50

Table 3 Effects of whey protein on production parameters in growing pigs during thermo-neutral conditions (P1)

	Treatment (Trt)					*P*	Contrast		
Parameter	CT	A	B	C	SEM	Trt	A vs CT	B vs CT	C vs CT
BW[1], kg									
Initial	40.50	39.05	39.35	39.03	0.51	0.17	0.06	0.13	0.05
Final	44.69	44.28	44.43	44.54	0.25	0.71	0.27	0.47	0.68
ADG[2], kg/d	0.76	0.71	0.73	0.74	0.04	0.73	0.29	0.48	0.69
FI[3], kg/d	2.15	2.11	2.06	2.10	0.07	0.80	0.71	0.33	0.61
G:F[4]	0.36	0.34	0.35	0.35	0.02	0.73	0.27	0.61	0.51

[1]Body weight
[2]Average daily gain
[3]Feed intake during P1
[4]Gain to feed ratio

Table 4 Effects of whey protein on production parameters in heat-stressed growing pigs (P2)

	Treatment (Trt)					*P*	Contrast		
Parameter	CT	A	B	C	SEM	Trt	A vs CT	B vs CT	C vs CT
ΔBW[1], kg	-0.64	-0.52	-0.43	-0.41	0.33	0.96	0.80	0.66	0.63
FI[2], kg	1.24	1.19	1.11	1.15	0.11	0.87	0.76	0.42	0.59
ΔFI[3], kg	-0.90	-0.93	-0.98	-0.98	0.11	0.95	0.83	0.63	0.62

[1]Change in body weight (P1 – P2)
[2]Feed intake during P2
[3]Change in feed intake (P1 – P2)

Table 5 Effects of whey protein on intestinal permeability parameters in heat-stressed growing pigs (P2)

	Treatment (Trt)					*P*	Contrast		
Parameter	CT	A	B	C	SEM	Trt	A vs CT	B vs CT	C vs CT
Ileum									
TER[1], Ω/cm^2	141.98^{bc}	156.9^{c}	89.81^{a}	103.53^{ab}	15.69	0.02	0.51	0.03	0.10
FITC-Dextran APP[2], µg/ml/min/cm^2	17.23	15.21	20.54	23.16	8.52	0.91	0.84	0.77	0.63
Colon									
TER, Ω/cm^2	90.15	89.30	88.52	100.28	7.39	0.66	0.94	0.87	0.34
FITC-Dextran APP, µg/ml/min/cm^2	10.13	10.95	9.84	5.99	4.24	0.76	0.90	0.97	0.40

[1]Transepithelial electrical resistance
[2]Apparent permeability coefficient

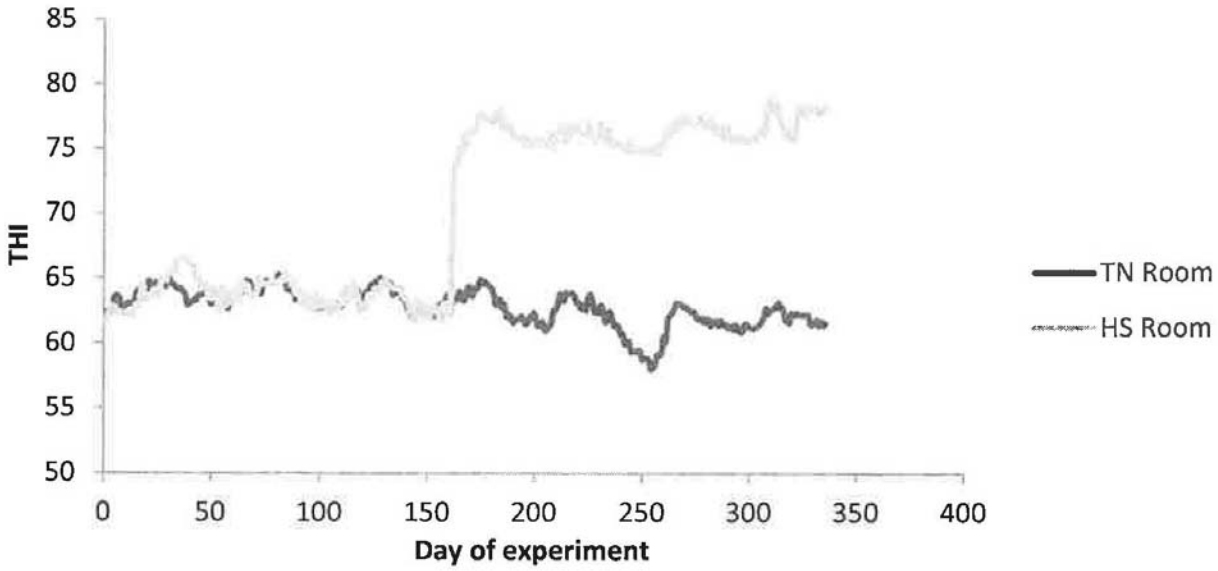


Figure 1 Average daily temperature-humidity index (THI) in thermo-neutral (TN) and heat stress (HS) rooms

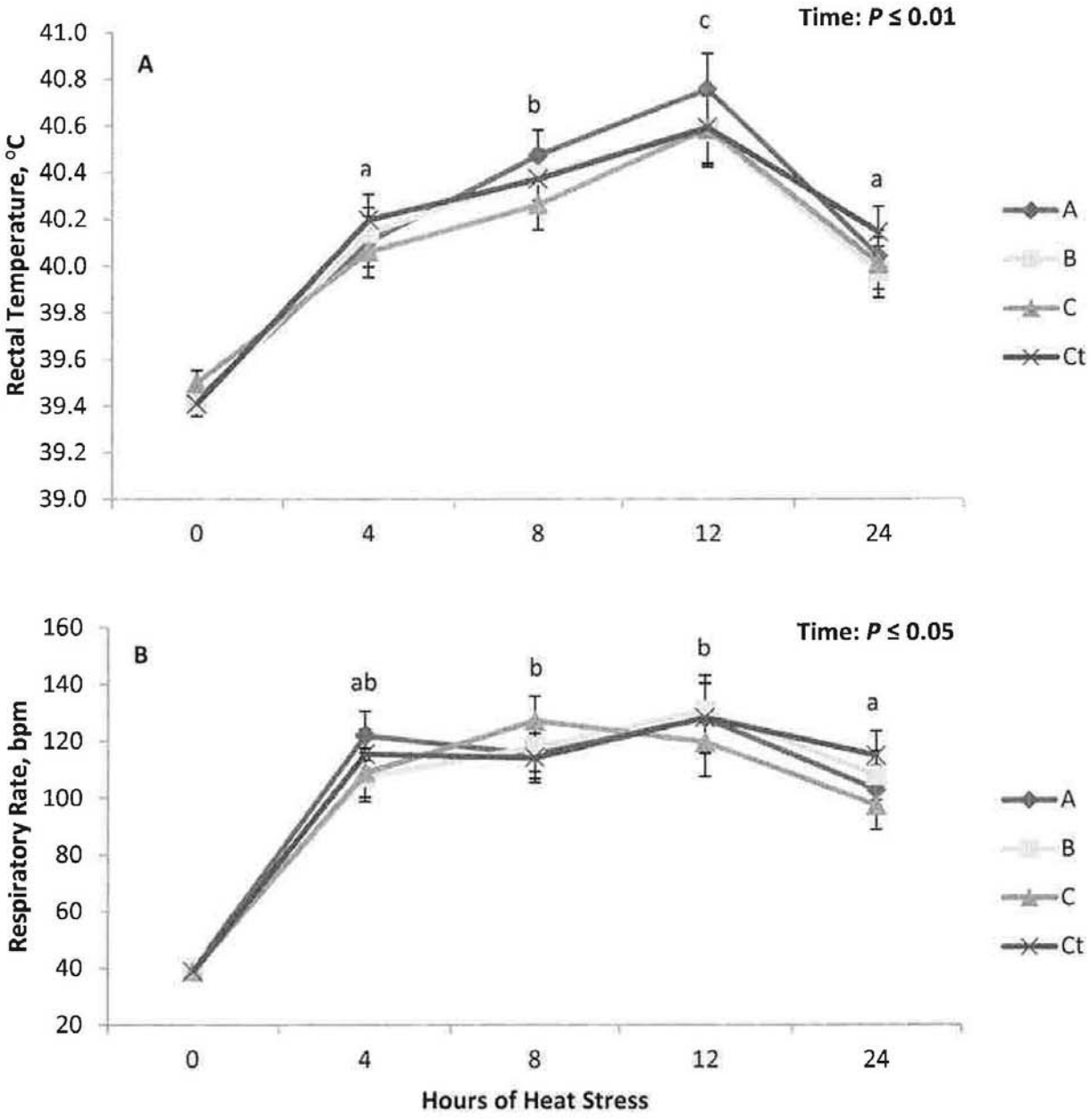


Figure 1 Effects of whey protein on (A) rectal temperature and (B) respiratory rate of growing pigs exposed to constant heat stress conditions (32°C) for 24 hours

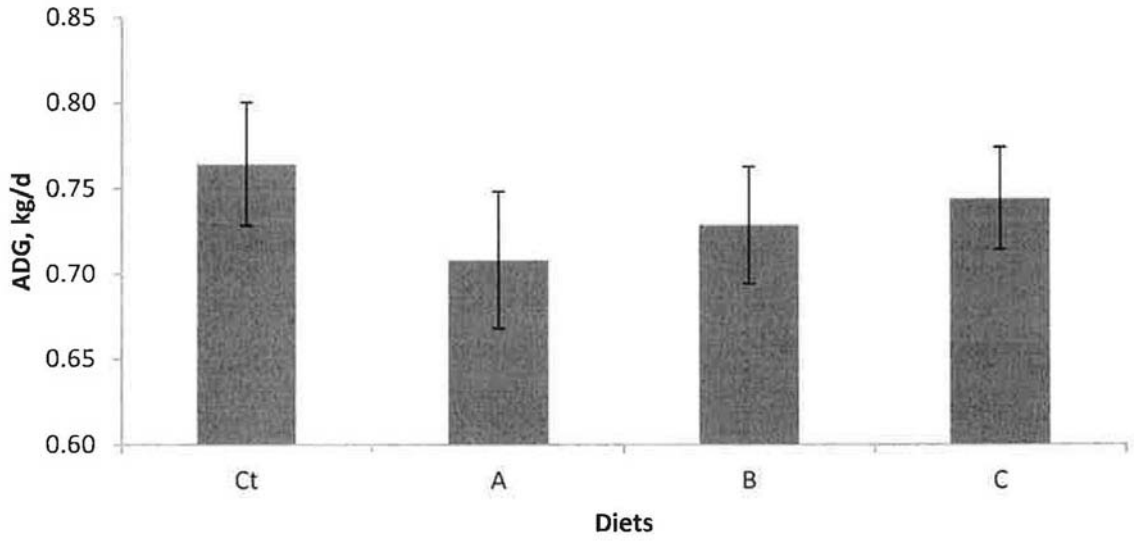


Figure 2 Effects of whey protein on average daily gain of growing pigs exposed to thermo-neutral conditions (19°C)

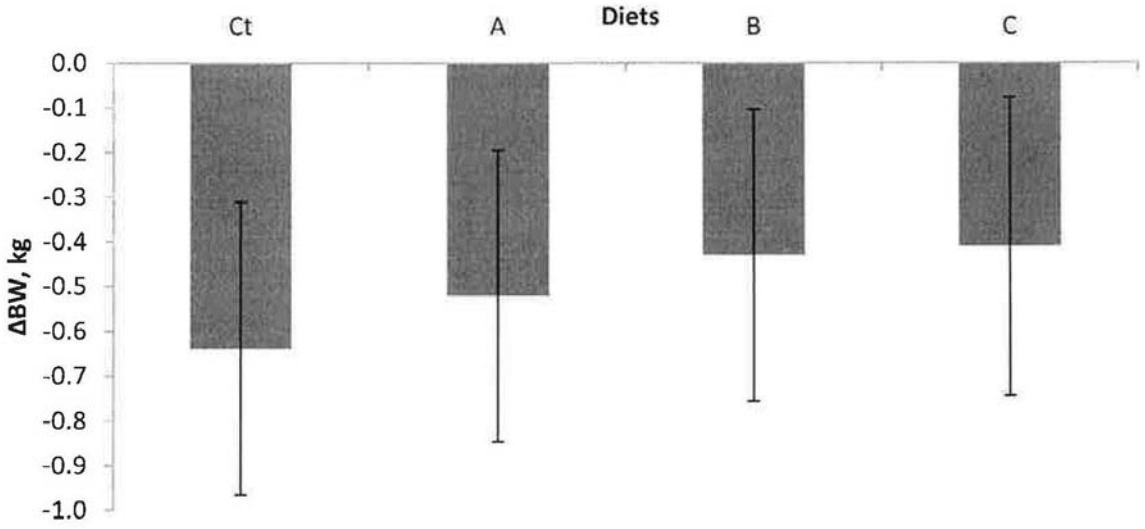


Figure 3 Effects of whey protein on body weight change of growing pigs exposed to constant heat stress conditions (32°C) for 24 hours

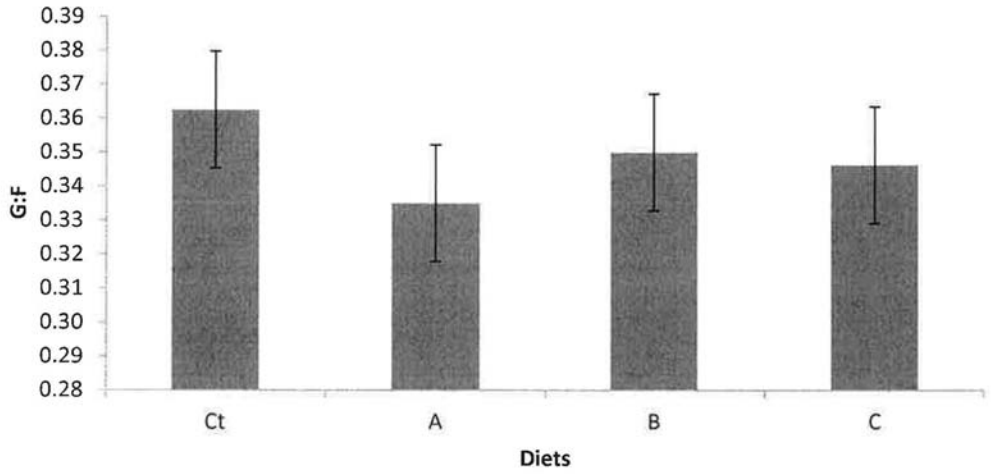


Figure 4 Effects of whey protein on gain to feed ratio of growing pigs exposed to thermo-neutral conditions (19°C)

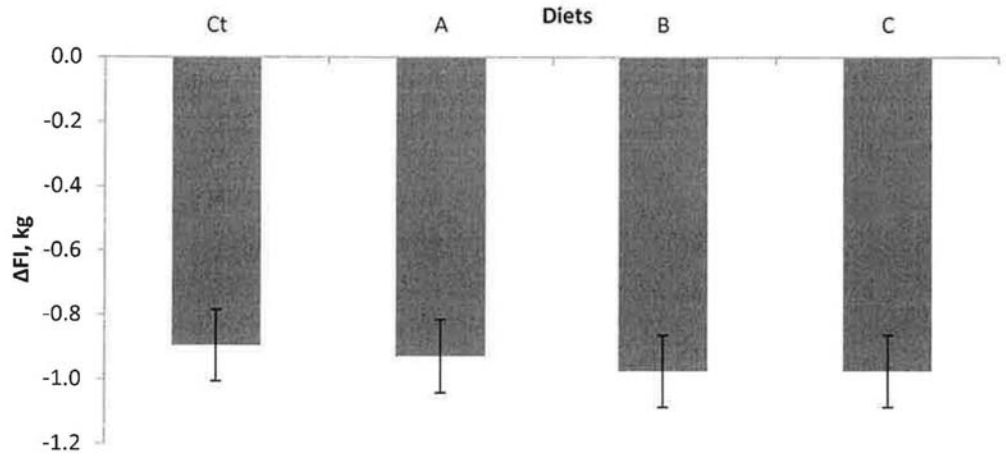


Figure 5 Effects of whey protein on feed intake change of growing pigs exposed to constant heat stress conditions (32°C) for 24 hours

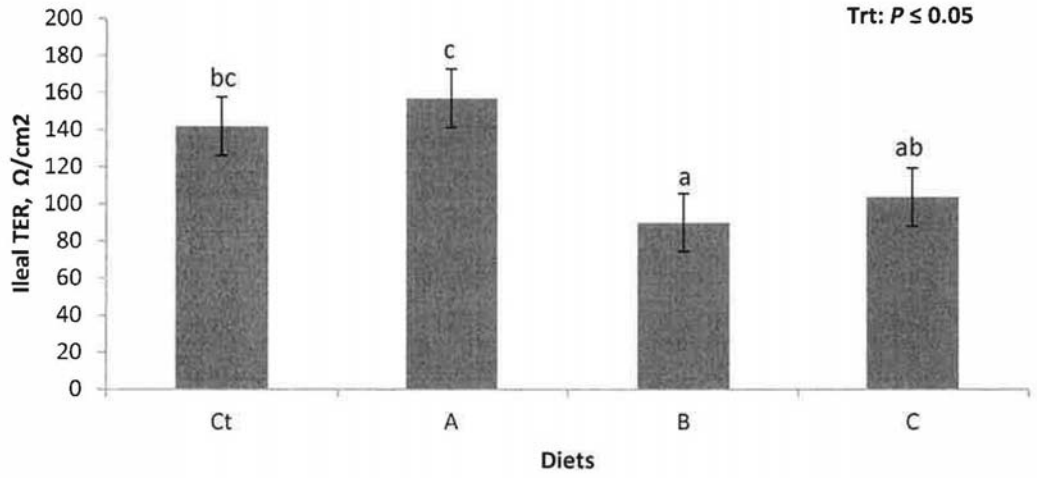


Figure 6 Effects of whey protein on ileal transepithelial electrical resistance coefficient of growing pigs exposed to constant heat stress conditions (32°C) for 24 hours

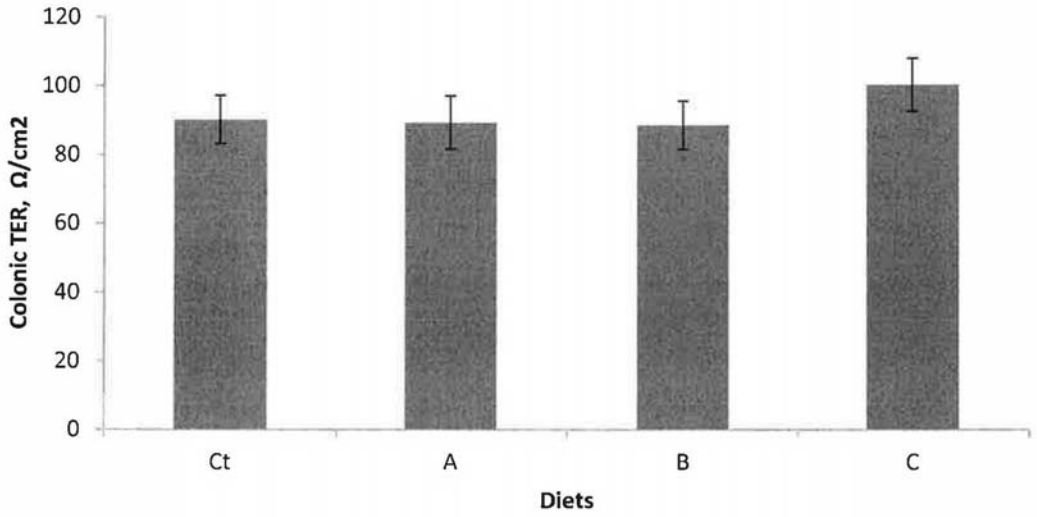


Figure 7 Effects of whey protein on colonic transepithelial electrical resistance coefficient of growing pigs exposed to constant heat stress conditions (32°C) for 24 hours

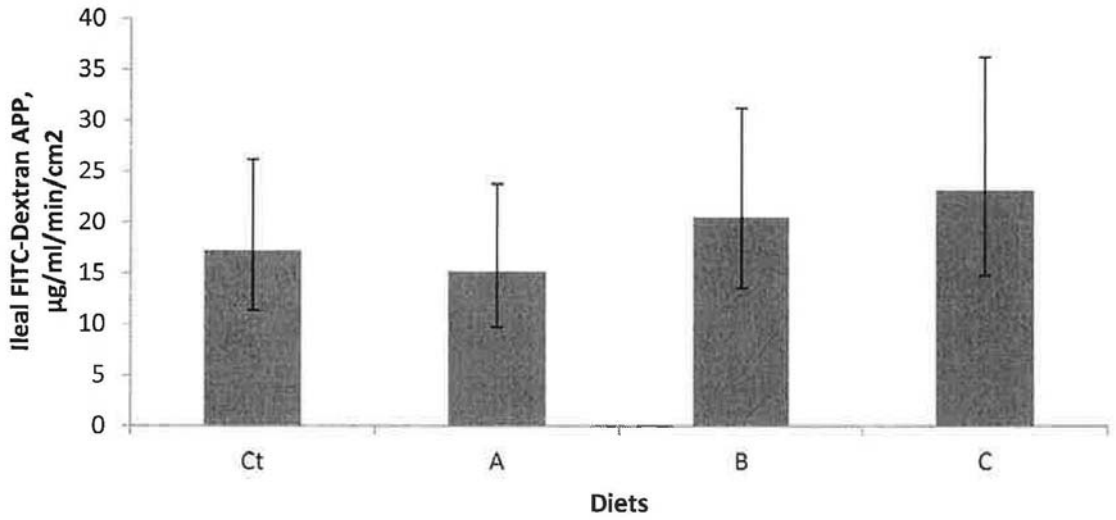


Figure 8 Effects of whey protein on ileal FITC-Dextran apparent permeability coefficient of growing pigs exposed to constant heat stress conditions (32°C) for 24 hours

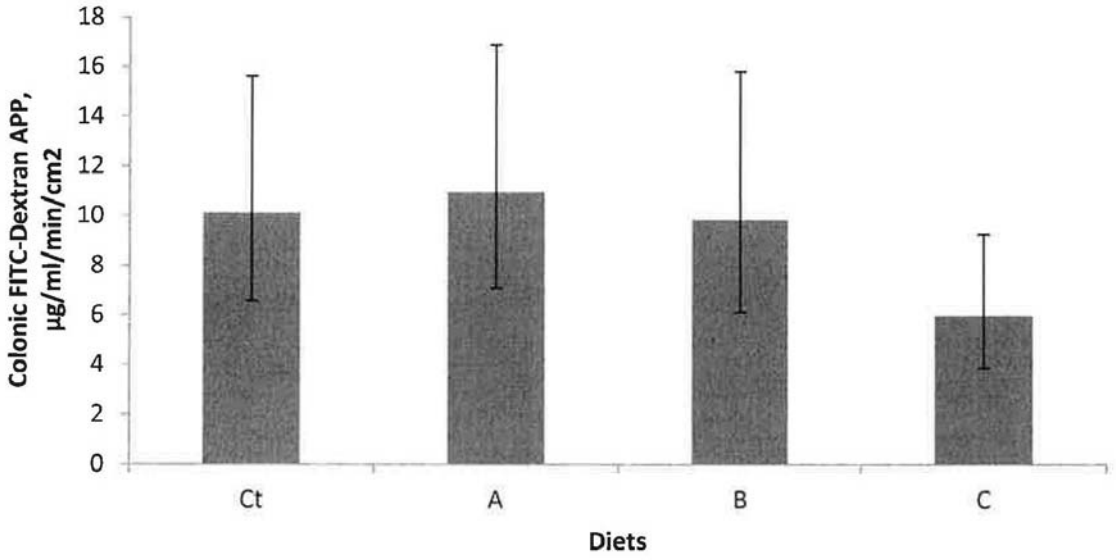


Figure 9 Effects of whey protein on colonic FITC-Dextran apparent permeability coefficient of growing pigs exposed to constant heat stress conditions (32°C) for 24 hours

APENDIX A:

Table A.1 Ingredients and formulated dietary nutrients

Parameter	Diets	
	CT	Test Diets
Ingredients (%)		
Corn	73.51	84.27
Soybean meal (46.5)	22.30	5.75
Soybean oil	1.04	-----
L-lysine HCl	0.30	0.20
DL-methionine	0.06	0.02
L-threonine	0.09	-----
Monocalcium phosphate	1.14	1.16
Limestone	0.94	0.98
Salt	0.35	0.35
Vitamin Premix[1]	0.15	0.15
Trace Mineral Premix[2]	0.12	0.12
Test Product A, B, or C	-----	7.00
Nutrients		
ME – kcal/kg	1530	1533
NE – kcal/kg	1126	1159
Crude Protein %	16.90	15.3
ADF %	3.50	2.70
NDF %	9.30	8.70
Crude Fiber %	2.70	2.20
Crude Fat %	4.60	4.40
SID Lys %	0.97	0.97
SID Thr %	0.61	0.61
SID Met %	0.31	0.29
SID Met+Cys%	0.56	0.56
SID Trp %	0.16	0.20
Calcium %	0.65	0.66
Phos. % - t total	0.60	0.55
Phos. % - a available	0.30	0.31
Phos. % - d digestible	0.30	0.30
Sodium %	0.16	0.16
Chlorine %	0.25	0.25

[1]Provided the following per kg of diet: vitamin A, 7,656 IU; vitamin D, 875 IU; vitamin E, 62.5 IU; vitamin K, 3.75 mg; riboflavin, 13.75 mg; niacin, 70 mg; pantothenic acid, 33.75 mg; vitamin B_{12}, 62.5 µg [2]Provided the following per kg of diet: Fe, 121 mg as ferrous sulfate; Zn, 121 mg as zinc sulfate; Mn, 28.6 mg as manganese sulfate; Cu, 12.1 mg as copper sulfate; I, 0.22 mg as calcium iodate; Se, 0.22 mg as sodium selenite

Table A.2 Composition of the test products

Parameter	Test Product		
	A	B	C
Protein %	79.70	78.44	79.85
Fat %	6.27	5.76	6.33
Lactose %	6.70	7.53	6.58
Ash %	2.88	2.93	2.88
Moisture %	4.36	4.32	4.36

Table A.3 Analyzed dietary nutrients

Parameter[1]	Test Product			
	CT	A	B	C
Crude Protein %[2]	17.82	16.27	16.80	16.46
Crude Fat %	4.61	3.48	3.09	3.25
Crude Fiber %	2.28	2.04	1.95	1.80
Ash %	4.87	4.24	4.37	4.43
Moisture %	12.46	12.67	12.84	12.76
Calcium %	0.73	0.78	0.77	0.78
Phosphorus	0.61	0.60	0.56	0.58
Zinc (ppm)	125	122	221	128
GE (Cal/100g)[3]	404	400	398	399

[1] Results (except moisture) are presented on a dry matter basis

[2] Percentage N x 6.25

[3] Calculated form proximate data