Effect of crude protein content in milk replacer on heat production due to feed intake in veal calves

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Abstract

The use of milk replacers containing casein is known to favour clotting of protein and fat in the abomasum of veal calves but it would not affect the flow of carbohydrates. The clot formation, dependent on the dietary balance between protein and fat would alter digestion and heat production (HP) kinetics. These effects on HP have been assessed during 2 experiments. In the first experiment, veal calves received isocaloric diets with 4 levels of crude protein (CP) at 3 stages of fattening (mean body weight: 72, 136 and 212 kg). They were placed in an open-circuit respiration chamber for measuring their HP, feed intake and physical activity during 6 days. Their protein fractional degradation rate (FDR) was estimated by measuring urinary 3-methylhistidine excretion. Their HP kinetic was partitioned between components due to physical activity, intake, digestion and metabolic utilization of dietary nutrients (thermic effect of feeding (TEF), short term component) and resting metabolism. During an additional fasting day, their fasting HP was estimated and the difference with HP due to resting metabolism was assumed as a long term component of TEF. During the first stage, but not later, short term TEF increased when dietary CP decreased, resulting in a negative long term TEF for the lowest CP levels at the first stage. These differences can be explained by differences in digestive kinetic and/or in metabolic use of nutrients since FDR increased when dietary CP increased. Both hypotheses have been tested in a second experiment where 2 batches of 4 calves received the 4 dietary CP levels during successive periods of 2 days. This design may not affect digestive kinetic of nutrients but prevent metabolic adaption to CP level. As short term TEF was no more affected by dietary CP level, the differences observed in the first experiment may be due to metabolic adaptation to dietary CP level.

Introduction

French veal calf production is mainly characterized by raising an animal from 50 to more than 250 kg body weight (**BW**) over a period of about 6 months with an average BW gain higher than 1.2 kg/d. During this period, about 90% of dry matter intake (**DMI**) consist in liquid milk replacer. Due to this particular feeding technique, the calf remains at a preruminant stage and milk replacer enters straight the abomasum by the way of oesophageal groove without entering the rumen (Guilhermet et al., 1975). Several sources of proteins (milk proteins (casein), whey proteins, vegetable proteins…) are commonly used in the composition of milk replacer but they differ in their digestive and metabolic utilizations. Indeed, the use of milk replacers containing casein is known to favour clotting of protein and fat in the abomasum of veal calves resulting in additional retention time of those nutrients in the abomasum whereas carbohydrates leave the abomasum faster (Mathieu, 1968; Guilloteau et al., 1975; Miyazaki et al., 2009). Therefore, these differences in flows of nutrients at the end of the abomasum result in differences in postprandial kinetics of blood nutrients (Grizard et al., 1982) and may thus alter heat production (**HP**) kinetic after a meal. Furthermore, coagulation in the abomasum of the calf is similar

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with clot formation in cheese factory. Indeed, the content in protein or fat and their balance in milk entering the process may affect coagulation properties of the milk during cheese production (Malacarne et al., 2006; De Marchi et al., 2007). Consequently, variations in protein and fat contents of milk replacer would alter coagulum formation and therefore digestive and metabolic kinetics, resulting in differences in HP kinetic. The objectives of the experiments were to determine and to explain the effect of crude protein (**CP**) content in isocaloric milk replacers on HP kinetic following a meal.

Materials and Methods

Experimental design

Two experiments were conducted jointly at the Institut de l'Elevage facilities (Le Rheu, France) and at the INRA facilities (Saint-Gilles, France), using male Prim'Holstein veal calves. During both experiments, the calves were offered reconstituted (liquid) milk replacer at a controlled temperature ranging from 45 to 50°C in 2 equal meals per day at 0845 and 1800 h and they did not receive any solid feed. For measurements, they were housed in individual metabolism cages with wood-slatted floor and equipped (experiment 1) with a harness and an attached plastic bag to allow total feces collection. They were placed in a 12-m^3 respiration chamber, whose temperature and relative humidity were maintained constant at 18°C and 70%, respectively. A 12-h lighting time span (from 0730 to 1930 h) was used.

The first experiment (Labussière et al., 2008a) was designed to determine the long-term effect of 4 levels of dietary CP on HP in veal calves during 3 stages: stage 1 (from 5 to 8 week in rearing), stage 2 (from 13 to 16 week) and stage 3 (from 21 to 24 week). Week 1 corresponds to the first week after purchasing and arrival at the facilities at 7 to 15 days of actual age. At each stage, measurements were conducted on 4 calves per dietary CP level during 4 successive weeks (one calf per week). After 2 weeks of adaptation to one of the 4 CP level, DMI, nitrogen and energy balances (including HP measurements) of each calf was measured during 6 days in respiration chamber. These measurements were followed by an estimation of fasting HP (**FHP**) during an additional day in the respiration chamber when the calf did not receive its evening meal. The 4 dietary CP levels were obtained by using 4 isocaloric grower diets differing in their CP content (used during stage 1) and 4 isocaloric finisher diets differing in their CP content (used during stages 2 and 3). Diets included skim milk powder, 50%-fat enriched skim milk powder and lactose (**Table 1**). They were formulated to achieve 4 levels of CP calculated relative to a reference CP level, which was 21% DM for grower diets and 20% DM for finisher diets. For each type of diet, the 4 CP levels contained 76, 88, 100 and 112% of the reference CP level and referred to as **G76**, **G88**, **G100** and **G112** for grower diets, and **F76**, **F88**, **F100** and **F112** for finisher diets.

The second experiment was designed to determine the short-term effect of dietary CP level on HP. Two batches (one per type of diet) of 4 calves received the same diets as in the first experiment but during short periods. Each calf received successively the 4 diets (grower or finisher type) according to a latin-square design with successive periods of 2 days. Its DMI and HP were measured in respiration chamber during the 2 days without any adaptation period before measurements. However, a 14-day adaptation period to metabolic cages preceded the actual measurements periods.

Measurements and calculations

For both experiments, calves were weighed at the beginning and at the end of the measurements in respiration chamber, before the morning meal. DMI was calculated as the difference between amounts of DM offered and refused by the calf. Both were calculated by measuring amounts and DM contents of fresh feed offered to or refused by the calf at each meal. Additionally, time of eating was recorded. During the first experiment, feces and urine were also collected and digestibility and metabolizability coefficients of energy were calculated according to standard procedures (Labussière et al., 2008a). From results of the first experiment, the ME content of each diet was calculated to calculate the amount of ME ingested by each calf in the second experiment from DMI. Urine was also analyzed in the first experiment for creatinine and 3-methylhistidine contents to calculate fractional degradation rate (**FDR**, Nishizawa et al., 1979; Schroeder et al., 1990).

When in the respiration chamber, the calf was housed in a cage mounted on force sensors which produced an electrical signal indicative for its physical activity. Gas concentrations (CO_2, O_2 and CH_4) of outgoing air and ventilation rate were recorded continuously according to methods described by van Milgen et al. (1997). The O_2 concentration was measured with a paramagnetic differential analyser (Oxymat 6, Siemens AG, Munich, Germany), whereas the CO_2 and CH_4 concentrations were measured with infrared analysers (Ultramat 6, Siemens AG, Munich, Germany, or Unor 600, Maihak AG, Hamburg, Germany for CO_2, and Unor 6N, Maihak AG, Hamburg, Germany for CH_4). Gas extraction rate was measured with a mass gas meter (Teledyne Brown Engineering, Hampton, Virginia, USA). Gas concentrations, the signal of the force sensors, gas flow rate, temperature and relative humidity in the respiration chamber were measured 60 times per second, averaged over 10-second intervals and average values were recorded for subsequent calculations.

The kinetic of HP (**Figure 1**) was studied according to methods developed by van Milgen et al. (1997), considering that total HP was the sum of components due to physical activity (**AHP**), feed intake (short-term component of thermic effect of feeding (**TEF**), **TEF_s**) and resting metabolism (**RHP**). The modelling approach developed by van Milgen et al. (1997) considered that the variations in O_2 and CO_2 concentrations in the respiration chamber were related to events occurring in the chamber (physical activity and feed intake). These events resulted in gas exchanges due to physical activity and feed intake, in addition to the gas exchanges corresponding to resting metabolism. Each component was estimated from respective O_2 consumption and CO_2 production using the formula of Brouwer (1965), excluding CH_4 production and urinary nitrogen losses. The model considers TEF_s as a gamma distribution function of time after the meal, which was defined using 2 parameters: the amount of feed intake during the meal and the duration after the meal (**TTEF**) which is necessary so that HP due to feed intake has declined to half of its maximal value. During the fasting day, only the second part of the day was considered in the calculation involving only 2 components for calculating total HP: one due to physical activity, and the other one corresponding to basal metabolic rate (**FHP**). The latter was estimated as the horizontal asymptotic value of HP kinetic from the time when the calf did not receive any feed, assuming a first-order decline between fed and fasted states. The difference between RHP and FHP corresponded to a long term component of HP due to feed intake (**TEF_l**). All HP data were expressed relative to metabolic BW calculated as $BW^{0.85}$ (Labussière et al., 2008b).

Statistical analyses

For the first experiment, BW, DMI, ME intake and FDR were analyzed separately for each stage and the model included the effects of dietary CP level and week of measurement within a stage (PROC GLM, SAS, 2004). The model also included the effect of ME intake as a covariance effect for HP data and TTEF. For the second experiment, BW and DMI were analyzed separately for each type of diets (grower vs finisher) and the model included the effect of CP level, the repeated effect of period of the latin square and the random effect of calf (PROC MIXED, SAS, 2004). The model also included the covariance effect of ME intake for HP data and TTEF.

Results

Results for the first experiment are presented in **Table 2**. At each stage, BW, DMI and ME intake were not affected by dietary CP content. They averaged 71.9 kg, 1.31 kg and 672 kJ/kg $BW^{0.85}$/d during stage 1, 136.0 kg, 2.12 kg and 611 kJ/kg $BW^{0.85}$/d during stage 2, and 212.3 kg, 2.77 kg and 529 kJ/kg $BW^{0.85}$/d during stage 3. The short term component of TEF significantly decreased when dietary CP content increased during stage 1 (P<0.01) but not later. It decreased from 62 kJ/kg $BW^{0.85}$/d (or 9.2% of ME intake) with diet G76 to 30 kJ/kg $BW^{0.85}$/d (or 4.7% of ME intake) with diet G112 whereas it averaged 45 (7.3% of ME intake) and 42 kJ/kg $BW^{0.85}$/d (8.2% of ME intake) during stages 2 and 3, respectively. The long term component of TEF increased from -7 kJ/kg $BW^{0.85}$/d with diet G76 to 22 kJ/kg $BW^{0.85}$/d with diet G112 during stage 1 whereas it remained constant during stages 2 and 3 and averaged respectively 0 and -1 kJ/kg $BW^{0.85}$/d. Due to opposite variations in short and long term components of TEF, their sum remained constant at each stage and averaged 53, 45 and 43 kJ/kg $BW^{0.85}$/d during stage 1, 2 and 3,

respectively. Therefore, 7.9, 7.4 and 8.1% of ME intake were lost as TEF during stage 1, 2 and 3, respectively. During stage 1, TTEF significantly decreased from 7.0 to 5.5 h when CP content increased from 76 to 112 % of the reference level (P=0.06). During stages 2 and 3, variations in dietary CP content did not affect TTEF which averaged 6.4 and 6.7 h, respectively. Protein turnover was estimated using FDR, which was significantly affected by dietary CP level. It increased from 1.3 to 1.6% during stage 1, from 1.4 to 1.6% during stage 2 and from 1.5 to 2.2% during stage 3 when CP content of the milk replacer increased from 76 to 112% of the reference level.

Results for the second experiment are listed in **Table 3**. There was no effect of dietary CP content on BW and DMI for each type of diet: BW averaged 127.9 and 161.2 kg and DMI averaged 2.29 and 2.45 kg/d during measurements for grower and finisher diets, respectively. The short term component of TEF was not affected by dietary CP content and it averaged 57 kJ/kg $BW^{0.85}$/d for grower diets and 42 kJ/kg $BW^{0.85}$/d for finisher diets. Additionally, TTEF remained constant whatever the dietary CP content: it averaged 6.0 h for grower diets and 6.4 h for finisher diets.

Discussion

In the model developed by van Milgen et al. (1997), HP relative to intake and utilization of nutrients is divided into 2 components. The short term component is considered to be associated with the ingestion and digestion of the meal whereas the long term component is associated with metabolism of the digested nutrients (van Milgen and Noblet, 2000). The long term component is estimated as the difference between RHP and FHP values which corresponds to the difference between the horizontal asymptotic values of HP kinetic of fed and fasted days (at a zero activity level). Therefore, both asymptotic values were not directly measured but they were estimated from the decline of HP consecutive to the last meal for the fed days, or from the decline of HP when the animal progressively became in a fasted state during the fasting day. In the first experiment, results for the first stage indicate that TEF_l with the lower dietary CP contents were slightly below 0. Due to the experimental error, these values were not significantly different from 0 but they were significantly lower than values measured for higher dietary CP contents. As indicated by van Milgen and Noblet (2000), the separation between short and long term components of TEF did not reflect biological mechanisms relative to the ingestion, digestion and metabolic utilization of nutrients since involved processes occur simultaneously and cannot be distinguished. Our results therefore indicate that the modelling approach, first developed for analysing HP kinetic of swine, may not be fully adequate for analysing HP kinetics of veal calves, despite their similar digestive tract and processes (at least when the calf is maintained in a preruminant stage).

As explained earlier, TEF_s is modelled as a gamma distribution function of time after the meal (**Figure 2**). When considering results for experiment 1 during stage 1, it appears that HP consecutive to a meal increased more with diet G76 than with diet G112. This could be explained by the lower CP content of the diet, which was associated with higher fat and carbohydrate contents due to the isocaloric property of the diets. Therefore, clot formation in the abomasum might be altered with diet G76, resulting in a faster passage of nutrients in the small intestine. With diet G112, the higher CP content (including casein) might result in a slower passage of nutrients (specifically protein and fat) in the small intestine. Therefore, the absorption of amino acids and fat is more extended after the meal and small absorption of protein and fat could thus occur during the whole day. As a non dynamic process, the latter may be included in the long term component of TEF, resulting in a lower TEF_s with diet G112 than with diet G76. During subsequent stages, there were no more differences in short and long term components of TEF, despite variations in dietary CP content. As the calf gets older, the increase in amount of liquid milk replacer offered can result in passage of liquid milk from the abomasum back to the rumen, causing alteration of digestive kinetic (Hostettler-Allen et al., 1994) and therefore of kinetic of nutrient absorption and utilization.

In agreement with Gerrits et al. (1998), protein turnover increased when dietary CP content increased, irrespective of the stage, as suggested by values of FDR. This may suggest that the calf is able to adapt its metabolism to dietary characteristics and specifically to dietary CP content. This adaptation may result in variations in HP kinetic. The second experiment was designed so that calves cannot adapt their

metabolism to dietary CP content whereas digestive kinetic may not be different from the first experiment. Measurements indicate that when the calf cannot adapt its metabolism to dietary CP content, there was no more difference in TEF_s and TTEF between dietary CP contents.

Conclusions

Modelling approaches for describing HP kinetics are indicative for digestive and metabolic phenomena which can affect the availability of nutrients for metabolic processes in veal calves. From results of both experiments, it appeared that metabolic adaptation of the calf to dietary CP content was a key factor for explaining HP kinetic consecutive to a meal. Variations of intensity of myofibrillar protein degradation due to dietary CP content can explain part of the differences but other metabolic pathways may be involved such as time of the day and duration of catabolic phases. Our experiments also indicate that arbitrary separation between ingestion, digestion and metabolic utilization of nutrients should be cautiously considered in modelling approaches of HP kinetic.

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Table 1. Composition of milk replacers

Diet[1]	*G76*	*G88*	*G100*	*G112*	*F76*	*F88*	*F100*	*F112*
Ingredient, g/kg								
*Skim milk powder (**SMP**)*	*214.5*	*287.0*	*359.1*	*426.6*	*181.5*	*249.3*	*319.0*	*385.0*
50%-fat enriched SMP	*369.9*	*352.1*	*333.9*	*315.3*	*375.1*	*356.4*	*338.4*	*323.7*
Lactose	*339.5*	*290.9*	*242.9*	*201.0*	*364.0*	*322.0*	*277.3*	*230.0*
Wheat starch	*30.0*	*30.0*	*30.0*	*30.0*	*30.0*	*30.0*	*30.0*	*30.0*
Lysine	—	—	—	—	*0.2*	*0.2*	—	—
Methionine	*2.0*	*2.3*	*2.6*	*2.9*	*2.0*	*2.3*	*2.5*	*2.8*
Threonine	*0.8*	*0.9*	*1.1*	*1.2*	*1.3*	*1.4*	*1.4*	*1.7*
Tryptophan	*0.2*	*0.2*	*0.3*	*0.3*	*0.3*	*0.3*	*0.3*	*0.3*
Others[2]	*43.0*	*36.6*	*30.0*	*22.6*	*45.5*	*38.1*	*31.1*	*26.5*
DM, g/kg	*954*	*954*	*953*	*959*	*962*	*960*	*962*	*960*
Chemical composition[3]*, g/kg DM*								
Ash	*64*	*65*	*67*	*64*	*64*	*63*	*63*	*64*
Crude Protein	*162*	*189*	*213*	*237*	*151*	*174*	*196*	*221*
Crude Fat	*197*	*185*	*174*	*164*	*195*	*184*	*172*	*163*
Lactose	*592*	*573*	*557*	*541*	*592*	*584*	*562*	*546*
Gross Energy, MJ/kg DM	*20.99*	*20.95*	*20.99*	*20.93*	*20.74*	*20.71*	*20.79*	*20.79*

[1] The CP content of diets G76 – G112 and F76 – F112 was determined as 76 to 112% of a reference CP content of 210 g/kg DM and 200 g/kg DM for grower and finisher diets respectively.

[2] Others correspond to minerals and vitamins, iron mixture and emulsifying agents.

[3] As measured.

Table 2. Effect of dietary CP content on HP kinetic and protein turnover (experiment 1)

	CP content of milk replacer (% of reference)					
	76	*88*	*100*	*112*	*SEM*	*Effect of CP content*
BW (kg)						
Stage 1	*69.6*	*71.2*	*72.7*	*74.2*	*2.2*	*0.20*
Stage 2	*130.9*	*135.9*	*138.2*	*138.9*	*2.8*	*0.11*
Stage 3	*208.2*	*211.9*	*216.0*	*213.0*	*3.0*	*0.68*
DMI (kg/d)						
Stage 1	*1.30*	*1.31*	*1.30*	*1.32*	*0.04*	*0.81*
Stage 2	*2.14*	*2.10*	*2.14*	*2.12*	*0.03*	*0.49*
Stage 3	*2.80*	*2.78*	*2.74*	*2.75*	*0.02*	*0.37*
ME (kJ/kg$BW^{0.85}$/d)						
Stage 1	*671*	*684*	*669*	*666*	*6*	*0.70*
Stage 2	*630*	*588*	*611*	*615*	*7*	*0.14*
Stage 3	*538*	*526*	*520*	*533*	*6*	*0.70*
TEF_s[1] (kJ/kg $BW^{0.85}$/d)						
Stage 1	*62^{a}*	*56^{a,b}*	*44^{b}*	*31^{c}*	*3*	*<0.01*
Stage 2	*55*	*43*	*44*	*38*	*3*	*0.17*
Stage 3	*51*	*36*	*48*	*39*	*3*	*0.58*
TEF_l[1] (kJ/kg $BW^{0.85}$/d)						
Stage 1	*-7^{c}*	*-4^{b,c}*	*10^{a,b}*	*22^{a}*	*4*	*<0.01*
Stage 2	*-2*	*0*	*7*	*-5*	*4*	*0.64*
Stage 3	*-15*	*6*	*-3*	*7*	*5*	*0.57*
TEF[1] (h)						
Stage 1	*55*	*52*	*54*	*52*	*3*	*0.96*
Stage 2	*53*	*43*	*50*	*34*	*4*	*0.25*
Stage 3	*37*	*42*	*45*	*47*	*2*	*0.57*
TTEF[1] (h)						
Stage 1	*7.0^{a}*	*6.4^{a,b}*	*5.9^{b,c}*	*5.5^{c}*	*0.2*	*0.04*
Stage 2	*6.6*	*7.1*	*6.2*	*5.6*	*0.2*	*0.11*
Stage 3	*7.5*	*6.5*	*6.6*	*6.3*	*0.3*	*0.64*
FDR[1] (%)						
Stage 1	*1.3^{b}*	*1.3^{b}*	*1.6^{a}*	*1.6^{a}*	*0.01*	*<0.01*
Stage 2	*1.4^{b}*	*1.4^{b}*	*1.6^{a}*	*1.6^{a}*	*0.01*	*<0.01*
Stage 3	*1.5^{b}*	*1.6^{b}*	*1.8^{a,b}*	*2.2^{a}*	*0.02*	*0.02*

BW: body weight, DMI: dry matter intake, ME: metabolizable energy, TEF_s: short term component of thermic effect of feeding, TEF_l: long term component of thermic effect of feeding, TEF: thermic effect of feeding, TTEF: time which is necessary for decreasing TEF_s for half of its maximal value, FDR: fractional degradation rate.

[1] Values are LS means, corrected for a same ME intake (kJ/kg $BW^{0.85}$/d) at each stage (672 kJ/kg $BW^{0.85}$/d during stage 1, 611 kJ/kg $BW^{0.85}$/d during stage 2 and 529 kJ/kg $BW^{0.85}$/d during stage 3).

Table 3. Effect of dietary CP content on HP kinetic and protein turnover (experiment 2)

	CP content of milk replacer (% of reference)					
	76	*88*	*100*	*112*	*SEM*	*Effect of CP content*
BW (kg)						
Grower diets	*127.7*	*128.0*	*128.0*	*127.7*	*1.9*	*0.99*
Finisher diets	*161.5*	*161.9*	*160.8*	*160.6*	*2.1*	*0.91*
DMI (kg/d)						
Grower diets	*2.26*	*2.31*	*2.30*	*2.30*	*0.02*	*0.80*
Finisher diets	*2.41*	*2.47*	*2.46*	*2.47*	*0.03*	*0.77*
TEF_s[1] (kJ/kg $BW^{0.85}$/d)						
Grower diets	*65*	*58*	*54*	*51*	*4*	*0.72*
Finisher diets	*45*	*46*	*41*	*36*	*3*	*0.52*
TTEF[1] (h)						
Grower diets	*5.8*	*6.3*	*6.3*	*5.6*	*0.2*	*0.53*
Finisher diets	*6.6*	*6.6*	*6.3*	*6.1*	*0.2*	*0.80*

BW: body weight, DMI: dry matter intake, TEF_s: short term component of thermic effect of feeding, TTEF: time which is necessary for decreasing TEF_s for half of its maximal value.

[1] Values are LS means, corrected for a same ME intake (kJ/kg $BW^{0.85}$/d) for each type of diet (721 kJ/kg $BW^{0.85}$/d for grower diets and 610 kJ/kg $BW^{0.85}$/d for finisher diets).

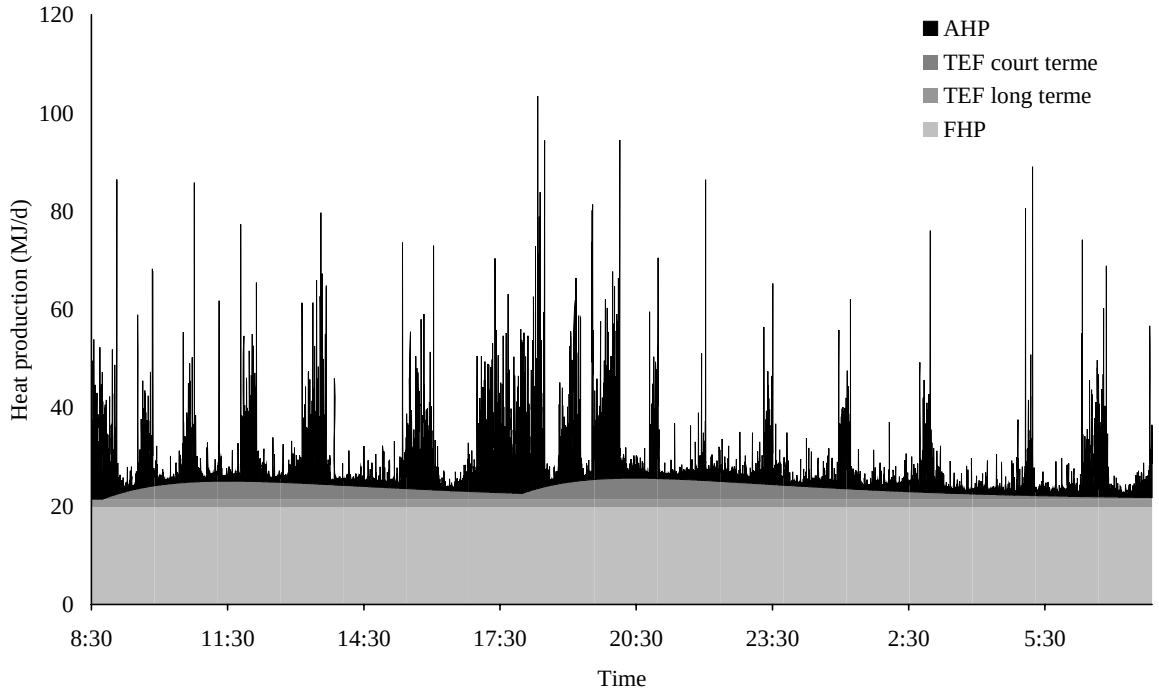

Figure 1. Partition of HP between components due to physical activity (AHP), feed intake (short and long term components of thermic effect of feeding TEF) and fasting metabolism (FHP)

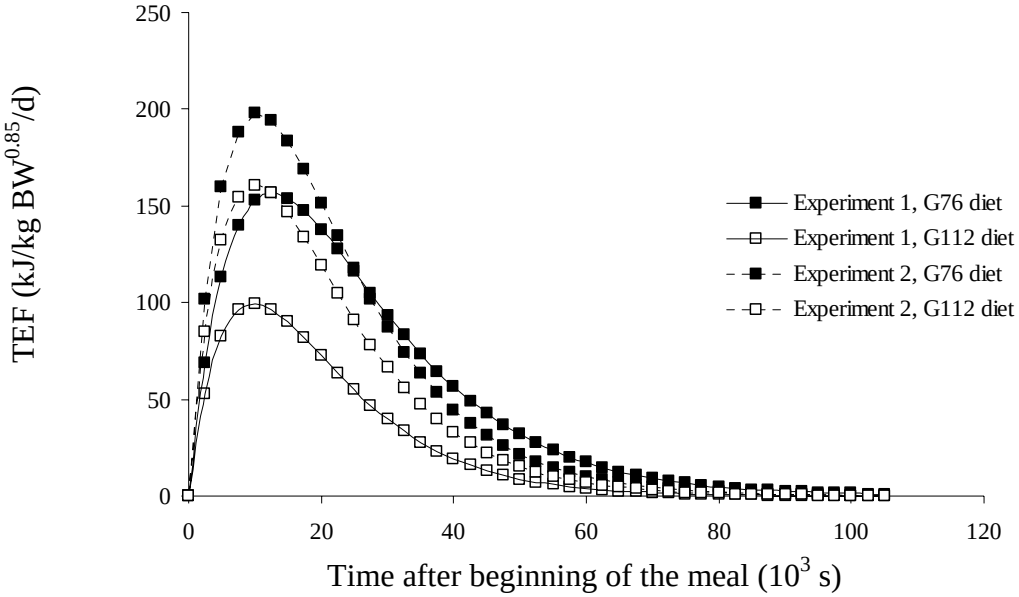

Figure 2. Effect of dietary CP content on kinetic of short term component of thermic effect of feeding (TEF)