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Oxidation of substrates and lipogenesis in pigs (*Sus scrofa*), mink (*Mustela vison*) and rats (*Ratus norvegicus*)[1]

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Abstract

Data from experiments with 25 growing pigs at high feeding level, with 12 adult mink on a varied energy supply and with 36 rats on a maintenance level were used in a biological model of nutrient oxidation, lipogenesis and retention at the whole body level. Nutrient oxidation was calculated from gas-exchange measurements in respiration chambers working according to indirect calorimetry principles. Lipogenesis and nutrient retention were measured by means of carbon and nitrogen balances, in accordance with the demonstrated model. The results demonstrated that growing pigs had a high level of protein retention and low protein oxidation. Digested carbohydrates were oxidized or used for lipogenesis. Oxidation of carbohydrate was the main energy source, while lipogenesis was the main source of fat retention. Independent of dietary fat level, pigs did not oxidize fat but used all dietary fat for body fat retention. The mink, being fed with high protein and fat levels but only a small amount of carbohydrate, use protein and fat as their main energy sources. Rats fed near-maintenance level used dietary carbohydrate as a main substrate for oxidation. It was demonstrated that the present model of nutrient oxidation, lipogenesis and retention in different animal species and at different dietary composition can be quantified by means of indirect calorimetry and measurements of carbon and nitrogen balances. © 1998 Elsevier Science B.V.

Keywords: Growth; Heat production; Lipogenesis; Maintenance; Nutrient oxidation

1. Introduction

Indirect calorimetry based on 24-h quantitative measurements of gas exchange in respiration chambers has been successfully used during the last century for the calculation of heat production in animals and in man. In animal science, indirect calorimetry measurements together with carbon and nitrogen balances have been used to estimate energetic values of feedstuffs and animals' requirement for nutrients and energy. Recently, measurements of gas exchange have been used to calculate the oxidation of protein (OXP), carbohydrate (OXCHO) and fat (OXF), in the intact body of different species [1–8]. Furthermore, indirect calorimetry combined with carbon and nitrogen balances has enabled us to estimate retention of

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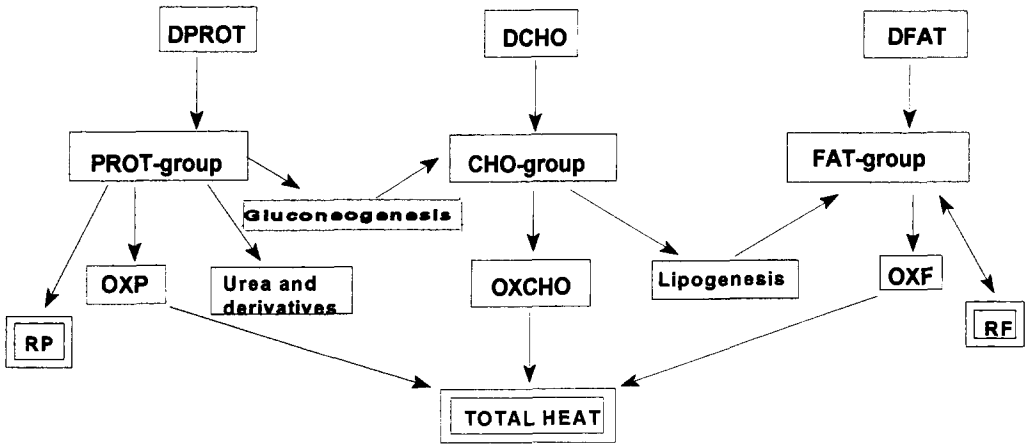

Fig. 1. Model of nutrient oxidation, lipogenesis and retention. Digested protein (DP), carbohydrate (DCHO) and fat (DFAT). Oxidized protein (OXP), carbohydrate (OXCHO) and fat (OXF). Retained protein (RP) and retained or mobilized fat (RF).

protein (RP) and fat (RF), and lipogenesis in accordance with the model shown in Fig. 1.

The oxidation and retention processes, although biochemically similar in omnivores and carnivores, depend on the quantity and quality of nutrients, which are obviously different for pigs, mink and rats. Furthermore, the abilities of animals to oxidize and accrete nutrients vary from the fast growing pigs fed on high carbohydrate diets to the strict carnivore such as mink fed on high protein and fat diets. Modern pigs fed rationally have a high protein retention (RP), a low OXP with correspondingly low gluconeogenesis. The energy requirement is mainly covered by OXCHO with no OXF and the lipogenesis is high [5]. Mink are using protein as energy sources with high OXP and a correspondingly high gluconeogenesis, while lipogenesis is low [8]. Rats are closely related to pigs from the physiological and nutritional points of view [9], but in the present study are being used with emphasis on their response to low feeding level.

In this paper, it has been attempted to indicate quantitative differences in nutrient catabolism and anabolism between species. The model has been used on data from experiments with pigs growing on high feeding level, with adult mink on a varied energy supply and with rats on maintenance level, in order to evaluate applications and limitations of the model.

2. Material and methods

2.1. Methodology in balance and respiration experiments

Balance experiments were carried out with animals kept for collection periods (4–7 days) in individual metabolic crates allowing quantitative measurements of feed intake and excretion of faeces and urine. A 24-h measurement of the gas exchange was placed in the middle of each collection period. Oxygen consumption and CO_2 production were measured by means of respiration units working in accordance with the open-air-circuit principle as discussed in details for pigs [10] and rats [11]. Chemical analyses and determination of energy in feed, faeces and urine were carried out and combined with the measured gas exchange used to calculate RP and RF.

2.2. Experiments with pigs

The data were taken from experiments with 25 pigs of Danish Landrace in which the protein and energy metabolism was measured on high feeding level in the live-weight (LW) range of 30–90 kg. The data were divided into four groups in relation to digested fat (DF) per metabolic body weight ($kg^{0.75}$), caused by different fat contents in the diets. The lowest and highest DF values (0.5 and 7.7 $g/kg^{0.75}$) were obtained

by a semipurified diet without (Group A) and with (Group D) addition of soyabean oil [12]. Intermediate values of 1.2 and 2.4 g DF/kg$^{0.75}$ were obtained by feed compounds based on barley (Group B) or maize (Group C) supplied with identical protein sources [13].

2.3. Experiments with mink

The data were taken from an experiment with 12 yearling female minks of the standard black colour type. The mink were fed a conventional mink diet mainly based on by-products of animal origin with dry matter and protein content of 315 and 176 g/kg, respectively, and a gross energy of 7.02 MJ/kg.

The balance experiments were carried out in six consecutive periods during a flush-feeding regime [14] in which the animals were kept on maintenance level in Period 1, below maintenance in periods 2 and 3, near ad libitum level in periods 4 and 5 and then again on maintenance level in Period 6 [15].

2.4. Experiments with rats

The experiment included 36 rats kept in individual metabolic crates and fed ad libitum to a LW of 75 g (Group A), 100 g (Group B) and 225 g (Group C), each group comprising 12 rats.

The diet consisted of commercial feed compound mixed with barley in following proportions: 1 : 1 in Group A and 1 : 2 in groups B and C. Thus, the protein content was 168 and 148 g/kg and gross energy 16.8 and 16.4 MJ/kg, respectively. When the stipulated LW was reached by the different groups, the feed allowance was restricted to a maintenance level and, after 7 days of habitation, the balance experiments were carried out [16].

2.5. Calculations

Retained protein was calculated from the measured nitrogen balance as the difference between ingested nitrogen (IN) and nitrogen excreted in faeces (FN) and urine (UN):

$$\mathrm{RP\,g} = (\mathrm{IN\,g} - \mathrm{FN\,g} - \mathrm{UN\,g}) \times 6.25 \text{ or } \mathrm{RP\,kJ} = \mathrm{RP\,g} \times 23.86. \quad (1)$$

The retained fat was calculated from the measured total carbon balance (RC) as the difference between ingested carbon (IC) and carbon excreted in faeces (FC) and urine (UC) and expired in CO_2 with C=CO_2, 1×0.5360. Carbon retained in nitrogen-free material was calculated as fat, assuming that glycogen depots are constant during 4–7-day measurements. With a carbon content of 52 and 76.7% in protein and fat, respectively, RF was calculated as:

$$\mathrm{RF\,g} = (\mathrm{RC\,g} - \mathrm{RP\,g} \times 0.52)/0.767 \text{ or } \mathrm{RF\,kJ} = \mathrm{RF\,g} \times 39.76. \quad (2)$$

The total heat production (HE) was calculated in accordance with the equation established by Brouwer [17], based on the measured gas exchange and nitrogen excreted in urine as:

$$\mathrm{HE\,kJ} = 16.18 \times \mathrm{O_2\,l} + 5.02 \times \mathrm{CO_2\,l} - 5.99 \times \mathrm{UN\,g}. \quad (3)$$

HE is the summation of heat produced from oxidation of protein, carbohydrate and fat and, as discussed in detail [1], it is possible to calculate the different parts, independent of the non-protein respiratory quotient being below or above 1.00 as:

$$\mathrm{OXP\,kJ} = \mathrm{UN\,g} \times 6.25 \times 18.42 \quad (4)$$

$$\mathrm{OXCHO\,kJ} = (-2.968 \times \mathrm{O_2\,l} + 4.174 \times \mathrm{CO_2\,l} - 2.446 \times \mathrm{UN\,g}) \times 17.58 \quad (5)$$

$$\mathrm{OXF\,kJ} = (1.719 \times \mathrm{O_2\,l} - 1.719 \times \mathrm{CO_2\,l} - 1.963 \times \mathrm{UN\,g}) \times 39.76. \quad (6)$$

3. Results and discussion

3.1. Experiments with pigs

The mean intake of metabolizable energy (ME), digested protein (DP), digested carbohydrate (DCHO) and digested fat (DF) during the growth period from 30 to 90 kg LW is shown in Table 1 together with the heat from oxidation of nutrients.

In the present study with pigs on a high feeding level, e.g. ME>1.2 MJ/kg$^{0.75}$ and DP in the 11–15 g/kg$^{0.75}$ range, no OXF was observed in spite of the variation in DF between 0.5 and 7.7 g/kg$^{0.75}$. The results are in agreement with [18], who demonstrated

Table 1
Intake of metabolizable energy (ME), digested protein (DP), digested carbohydrate (DCHO) and digested fat (DF) in relation to metabolic body weight ($kg^{0.75}$) and oxidized protein (OXP), carbohydrate (OXCHO) and fat (OXF) in relation to total heat production (HE) in growing pigs from 30–90 kg LW

	Group A		Group B		Group C		Group D	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM
Balances, *n*	24		83		42		18	
ME $kJ/kg^{0.75}$	1340	42	1222	104	1225	99	1419	24
DP $g/kg^{0.75}$	14.5	1.8	11.3	1.3	11.7	0.9	13.6	1.7
DCHO $g/kg^{0.75}$	59.4	1.7	51.8	2.6	48.8	2.3	48.2	0.8
DF $g/kg^{0.75}$	0.5	0.1	1.2	0.4	2.4	0.4	7.7	0.1
OXP/HE%	14.2	3.4	14.3	2.4	14.8	2.4	15.2	3.8
OXCHO/HE%	85.8	3.4	85.7	2.4	85.2	2.4	84.8	3.1
OXF/HE%	0		0		0		0	

from short-term energy balances in humans that the presence of fat in the meal did not promote fat oxidation. With no OXF, the heat production was caused mainly by OXCHO with ca. 85% while the remaining 15% originated from protein oxidation. This indicates that, with a high supply of carbohydrate in pig diets, DCHO is a major energy substrate which together with DP covers the energy requirements without any contribution from DF.

The lipogenesis was evaluated in all balance periods for each group in accordance with the model (Fig. 1). The CHO-group consisted mainly of DCHO with <5% from gluconeogenesis as DP was kept near the requirement for maximum RP, causing a low OXP and, hence, a low gluconeogenesis [19]. The FAT-group consisted of DFAT and fat from lipogenesis, being the difference between the CHO-group and OXCHO, assuming constant glycogen depots during the balance periods. With no OXF, the amount of RF was identical to the FAT-group. The values obtained for RF and the contribution from lipogenesis and DF from the diets with different fat content are demonstrated in Fig. 2.

The intake of DF was rather low in groups A, B and C with 6–22 g/day in the first balance period and 11–70 g/day in the last period, while it was 95–228 g/day in Group D. As no dietary fat was oxidized, all DF was transferred to body fat, independent of the level of DF. The amount of RF was rather identical for groups A, B and C with a final value ca. 390 g/day at 80 kg, while it increased from 95 to 500 g/day in Group D at 90 kg LW.

Lipogenesis constituted ca. 95% of RF in Group A, while it was 70–91% in Group B, and 44–82% in Group C. In Group D, with the high intake of DF, the lipogenesis only increased from 20 to 55%. It is characteristic that lipogenesis was the major source of RF when DF was low, while it decreased with increasing DF level. This may indicate that the supply of DF is a dominating factor in fat accretion, while the level of lipogenesis is adjusted according to the level of DF.

3.2. Experiments with mink

The intake of ME during restriction in periods 2 and 3 was significantly below the intake in all other periods. When feeding near ad libitum started in Period 4, the animals had an average ME intake close to 1100 $kJ/kg^{0.75}$, which was significantly above the intake in all other periods, but during the second period of refeeding the intake decreased to the same level as in periods 1 and 6 (Table 2). HE was not strongly influenced by the feed supply, but there was a tendency for a decrease during restriction in periods 2 and 3, and during refeeding in periods 4 and 5, HE was significantly higher than during the restriction periods (Table 2). The values for retained energy (RE) fluctuated from −223 $kJ/kg^{0.75}$ during the first period of restriction to 357 $kJ/kg^{0.75}$ during the first period of refeeding (Table 2), hence reflecting the varied feed supply and indicating that fat was mobilized from the body. This was also demonstrated by the animals losing weight during restriction.

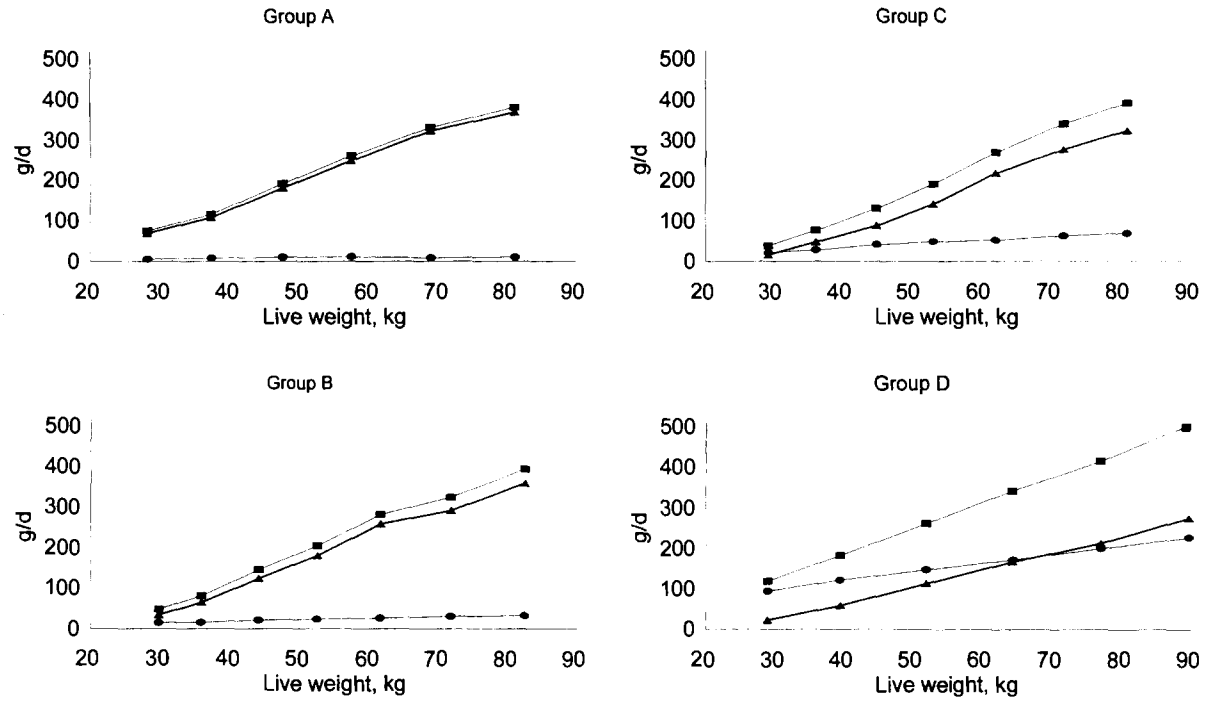

Fig. 2. Fat metabolism in pigs. (■) – Retained fat, (▲) – lipogenesis, and (●) – digested fat.

Table 2
Intake of metabolizable energy (ME), heat production (HE), retained energy (RE) and animal live weights (LW) in relation to metabolic body weight ($kg^{0.75}$), and absolute values for digested protein, fat and carbohdyrate (DP, DF and DCHO), and oxidized protein, fat and carbohydrate (OXP, OXF and (OXCHO) in mink

	Period/energy supply											
	1; maintenance		2; restricted		3; restricted		4; refeeding		5; refeeding		6; maintenance	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
ME $kJ/kg^{0.75}$	783	38.8	447	7.1	486	12.1	1122	63.0	763	39.7	710	34.6
HE $kJ/kg^{0.75}$	724	18.4	682	17.9	679	23.7	790	38.6	816	39.2	807	46.4
RE $kJ/kg^{0.75}$	78	49.6	−223	20.0	−180	19.1	357	48.0	−34	34.1	−79	47.1
LW $kg^{0.75}$	1.021	0.01	1.006	0.01	0.948	0.01	0.977	0.01	1.018	0.02	1.011	0.02
DP g	21.4	1.04	12.2	0.14	12.6	0.22	28.7	1.46	20.6	1.23	19.2	0.91
DF g	6.2	0.21	3.7	0.14	3.7	0.16	8.6	0.44	6.2	0.38	5.7	0.36
DCHO g	6.7	0.46	3.5	0.09	3.4	0.09	9.2	0.70	6.2	0.47	5.6	0.18
OXP g	20.2	1.34	12.3	0.26	12.0	0.22	25.5	1.29	19.7	1.23	18.4	0.65
OXF g	4.7	1.19	8.7	0.48	7.1	0.89	2.7	0.82	8.1	0.84	8.4	1.35
OXCHO g	9.3	1.09	5.9	0.67	7.4	1.75	10.6	2.09	7.5	1.27	7.3	1.34

During refeeding, when the animals were in positive energy balance, body weights increased again, and during Period 5 they almost reached the initial level in Period 1.

Contrary to pigs and rats, OXP was high in all periods (Fig. 3), but in relation to total HE, OXP reflected the level of feed supply, with value of 51% of HE in Period 1 decreasing to 33 and 35%

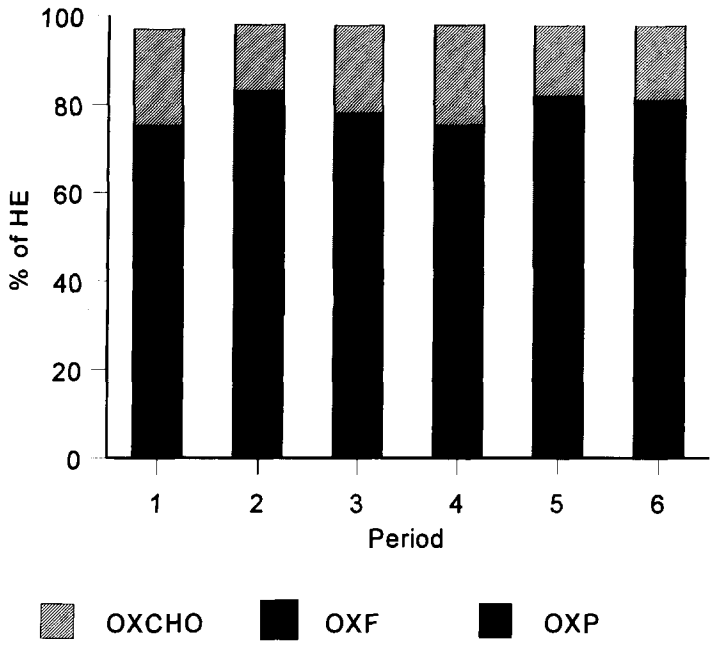

Fig. 3. Substrate oxidation in mink. Oxidized carbohydrate (OXCHO), fat (OXF) and protein (OXP) in relation to total heat production (HE).

during restriction in periods 2 and 3. The highest value (61% of HE) was recorded during the first period of refeeding (Period 4). These high values for OXP, even when the feed supply was scarce, demonstrate the profound importance of protein as an energy source for carnivorous animals. Also the pattern of fat oxidation was different from that in pigs and rats, since OXF made up a considerable part of HE in all periods (Fig. 3). Hence, the lowest value for OXF/HE (14%) was recorded during the first period of refeeding (Period 4), when a substantial energy retention occurred. High values for OXF/HE (50 and 43%, respectively) were, on the other hand, recorded when the feed supply was below the maintenance energy requirements during restriction in periods 2 and 3 (Fig. 3), hence reflecting that the energy requirement partly had to be covered by mobilization of fat from the body. The low DCHO supply was reflected by a low value for OXCHO/HE, a value that was not affected by the level of energy supply (Fig. 3). However, when DCHO was calculated in absolute values, it turned out that it exceeded the energy from DCHO (Table 2), which probably can be explained by accumulated analytical errors on the CHO fraction.

3.3. Experiments with rats

The mean intakes of ME and DP together with the heat from nutrient oxidation in rats fed near maintenance level are shown in Table 3.

The rats were in all groups able to maintain positive RP values of ca. 0.5 g/day in spite of the low intake of ME and DP. The RF values were around zero in Group A, slightly negative in Group B and positive in Group C with a mean value of 0.7 g/day. The OXP in relation to the total heat production was ca. 10–11% in Group A and B, probably being close to a minimum level of protein oxidation [5], while for the heavier rats in Group C the level of OXP was similar to the average OXP in growing pigs. OXCHO was the main source for HE with values from 80–85%, while OXF contributed with values below 10%.

The amount of DCHO was estimated from a previous experiment with rats fed an identical diet [20] and the calculation showed an intake of 3.8, 4.1 and 7.6 g/day DCHO in Group A, B and C, respectively. The values of DCHO and OXCHO were identical in Group A, with no lipogenesis, and in accordance with RF being around zero in this group. In Group B, OXCHO was above DCHO indicating a mobilization

Table 3
Intake of metabolizable energy (ME) and digested protein (DP) in relation to metabolic body weight ($kg^{0.75}$) and oxidized protein (OXP), carbohydrate (OXCHO) and fat (OXF) in relation to total heat production (HE) in rats of different LW fed at maintenance level

	Group A		Group B		Group C	
	mean	SEM	mean	SEM	mean	SEM
Balances, *n*	12		12		12	
LW g	75	0.7	112	1.4	225	2.8
ME kJ/$kg^{0.75}$	683	5.3	546	4.6	530	6.1
DP g/$kg^{0.75}$	7.0	0.05	5.7	0.05	4.9	0.05
OXP/HE%	9.7	0.20	11.4	0.53	14.7	0.38
OXCHO/HE%	80.6	1.43	85.0	0.89	79.2	0.67
OXF/HE%	9.7	1.43	3.6	1.09	6.1	0.90

of the glycogen depots simultaneously with mobilization of body fat measured as negative RF. On the contrary, in Group C the values of OXCHO were lower than DCHO. Hence, with sufficient intake of digested nutrients to cover energy requirements, surplus DCHO was used in lipogenesis, causing RF to increase to ca. 0.7 g/day.

4. Conclusions

The results demonstrated that growing pigs on a high feeding level utilized a majority of dietary protein for body protein retention and, subsequently, the level of protein oxidation was relatively low. The high level of carbohydrate in pig diets was oxidized as a main energy source, while the surplus CHO was utilized in lipogenesis processes. The amount of fat synthesized from glucose depended on supply of dietary fat. With the low DF content in a diet, up to 95% of RF constituted from lipogenesis. Since energy requirements were covered by CHO (85%) and protein (15%), there was no net oxidation of fat.

Contrary to pigs, adult mink, being strict carnivores, were fed diets with high levels of protein and fat, but a low amount of carbohydrate. The oxidation of protein and fat was the main source of energy (ca. 80%), while the low DCHO supply was reflected by low values of CHO oxidation and lipogenesis.

Rats fed at maintenance level kept slight positive RP values with OXP between 10 and 15% of total HE. Their energy requirement was mainly covered by OXCHO (80–85%) as in pigs on high feed levels, however, a slight OXF occurred (4–10%).

The results demonstrated that the present model of nutrient oxidation and retention can be quantified and verified by means of indirect calorimetry measurements and carbon and nitrogen balances. However, the method for calculation the substrate oxidation has some inborn limitations. In reality, OXP reflects the amount of deaminated protein, and not the quantitative protein oxidation, and it is not possible to distinguish between UN originating from true oxidation, gluconeogenesis or ketogenesis. Since CHO makes up a very limited part of the energy supply in carnivore diets, gluconeogenesis can be considered to be a very important pathway for providing the glucose necessary for maintaining glucose homeostatis. Furthermore, it has to be considered that the present model describes the net results of substrate oxidation without describing intermediary pathways of nutrient dynamic turnover. Therefore, in the future, experimental efforts ought to focus on measurements of the relative importance of substrate oxidation, gluconeogenesis and lipogenesis in different physiological situations. This may be achieved by a combination of our present experimental and calculation methods with use of stable isotope technique in order to study the dynamics of nutrients at the level of the whole body.

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