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Changes in fat and individual fatty acids digestibility during the growth of Japanese quails*

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The aim of the study was to determine changes in the digestibility of fat and individual fatty acids during the growth of Japanese quails. The study was conducted on 48 female Japanese quails (*Coturnix coturnix japonica*) from hatching to 6th week of life. All quails were fed *ad libitum* granulated commercial diets. The chemical composition and fatty acid content of the diets and faecal samples were determined and the coefficient of total tract apparent individual fatty acids digestibility and fat digestibility was calculated. There were no significant age differences in protein, fibre and ash content in faecal. Fat content decreased with the age of the birds. During the whole growth the faecal comprised mainly the C18:2n-6, 18:1n-9, C16:0, and C18:0 acids. However, the faecal fatty acids content was affected by age – in older birds more C10:0 and C14:0, and less C16:1, C18:1, C18:2 and C20:4 acids were found. The digestibility of most of fatty acids was high, except for C18:0, C20:0, and C18:1n-7. The digestibility of linoleic acid was increasing with age, from 0.70 to 0.89, while the digestibility of linoleic acid remained at similar, high level (>0.93) during the growth. C20:4n-6 was higher until 4th week of life in faecal than taken with diet. This may indicate that when quails are young this fatty acid deposited in the body comes from the conversion of C18:2n-6 and the dietary one is excreted. The high conversion on n-6 pathway, connected with high digestibility of C18:3n-3 may suggest that possible fatty acids modification, for example by addition of n-3 to the diet, should be made when birds are young, which can end up with a better n-6/n-3 ratio in body/tissues. Additionally, the SFA digestibility increases with age, so PUFA/SFA ratio could be better in young animals. However, if most of the enzymes in young animals are used for n-6 conversions, the conversion of n-3, C18:3n-3 to C20:5n3, can be inhibited.

KEY WORDS: digestibility / fat / fatty acids

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Animal tissues vary greatly in their fat and fatty acids (FA) content according to the species, age/weight of the animal, sex, genotype and external factors, especially feeding. The FA composition of animal tissues reflects the tissue fatty acids biosynthesis and fatty acids composition of diets and this relationship is stronger in monogastrics than in ruminants. Studies on pigs, cows and poultry, reviewed by Wood et al. [2008] and Woods and Fearon [2009], showed that addition of linseed oil to animal diets increases n-3 PUFA content in meat, milk and eggs. Generally, it is well known that n-3 PUFA enriched diets change fatty acids profile in tissues and the level of those changes depend on the level of PUFA n-3 in the diet and the metabolism changes of essential fatty acids in tissues. Because the levels of supplementation are not similar across studies and animals are at different stages of life, their metabolism is different what, accompanied by the differences of other conditions, makes it hard to establish an optimal level of n-3 PUFA addition to animal diet and an optimal moment of using this supplementation, based on the recent literature. At first, it seems necessary to get to know the mechanisms and the level of conversions of FA in tissues and the body of an animal to then decide which period of growth and which level of a diet supplementation can give the best results with regard to the quality of the product.

Lizardo *et al*. [2002] were the first who took efforts to study the mathematical relationship between feeding and deposition of the most important FA in backfat and offal fat of pigs. The accuracy of prediction of FA deposition in their model appeared insufficient. One of the reasons of this may be the fact the authors assumed that FA digestibility was very high, more than 85%, and was constant during the growth. Some limited studies on fat digestibility indicated that fat digestibility is affected by age [e.g. Tancharoenrat *et al.* 2013]. However, there is a lack of information on the FA digestibility dynamics during growth of animals. Thus, the aim of the study was to determine changes in the digestibility of fat and individual FA during the growth of Japanese quails.

Material and methods

Quails and diets

The working protocol was approved by the Local Ethical Commission (no 51/2011). The study was conducted on 48 female Japanese quails (*Coturnix coturnix japonica*) from hatching to the 6th week of life, randomized in 3 cages which were positioned in a room maintained at conditions appropriate for the quails – 16 birds in each cage, in a room at 22±2ºC with natural day light.

All quails were fed *ad libitum* granulated commercial diets: starter (0-2 week of life) and grower diet (2-6 week of life) prepared according to the National Research Council [NRC 1994] with regard to feeding requirements specific for the age of the quails. Granulated commercial feeds were offered dry twice a day (at 8^{00} am and 2^{30} pm). The composition of the diets is presented in Table 1. Feed allowance was changed weekly according to the body mass. The quails had free access to water throughout the whole experiment.

Table 1.Composition of the diets

Ingredient (g/kg)	Starter diet	Grower diet
Soya bean meal	390	295
Maize	250	250
Wheat	222	233
Triticale		50
Rapeseed meal	41.7	55.6
Soya oil	21.9	30.0
Potato protein	23.1	
Maize gluten	17.8	0.0
Limestone	13.4	62.6
Monocalcium phosphate additives[1]	9.0	9.5

[1]The following additives were included in the starter/grower diet (g/kg): vitamin-mineral premix 3.2/5, NaCl 2.5/2.5, sodium bicarbonate 1.4/2.1, phyzyme 0.1/0.1, ronozyme 0/0.2, methionine 1.8/1, L-lysine 0/0.4, and threonine 0/2.3.

Metabolism trial and faecal samples

The metabolism trial was carried out for three-day collection periods in the 2nd (11-14 day of life), 4th (25-28 day of life) and 6th week of life (39-42 day of life). At each point (11th, 25th and 39th day of age) 16 quails were transferred to metabolic cages. Because of the fact that quails are social birds, there were two quails in each metabolic cage – the research unit was a cage (n=8). During this trial, besides offering weighed amount of feed, total remaining feed and total faecal were collected and weighed every 8±1 hours, and the samples of faecal were collected, dried in hot air, grounded and processed for further analysis.

Performance parameters

The data of body weights and feed intake were recorded at weekly intervals. Total feed intake (FI_{total}), daily feed intake (ADFI), daily protein (DPI) and fat intake (DFI) were measured, and average daily gain (ADG) and feed conversion ratio (FCR) were calculated.

Chemical composition of diets and faecal samples

Protein, fat, fibre and ash content were determined in the diets and faecal samples according to the 954.01 [AOAC 2012a], 920.39 [AOAC 2012b], 978.10 [AOAC 2012c] and 942.05 [AOAC 2012d] method of AOAC, respectively. Energy content of the diet was calculated according to the NRC [1994] method.

Fatty acids content of diets and faecal samples

Fatty acids (FA) content was determined in the diet and faecal samples. To assess the FA content in the samples, fat was extracted according to the Folch *et al*. [1957] method, and the methyl esters of the fat were formed according to the 996.06 method of AOAC [AOAC 2012e], using $SOCl_3$ in methanol instead of BF_3. Prepared in this

way, FA methyl esters were separated by gas chromatography on Agilent 7890A gas chromatograph equipped with FID detector, a 60 m Hewlett-Packard-88 capillary column (Agilent J&W GC Columns, USA) with 0.25 mm inner diameter and 0.20 μm film thickness. A 1 μl sample was injected at a split ratio of 1:40. Helium was used as carrier gas at a flow rate 50mL/min. The injector and detector were both maintained at 260°C. Column oven temperature was programmed to increase from 140°C (held for 5 min) at a rate of 4°C/min to 190°C and then to 215°C at a rate 0.6°C/min. Individual fatty acids were identified by comparing the retention times with those of known mixtures of FA standards (Supelco 37 Component FAME Mix, cat no. 47885-U; PUFA-1 Marine Source, cat no. 47033; PUFA-2 Animal Source, cat no. 47015-U; PUFA-3 Menhaden Oil, cat no. 47085-U; Canola Oil, cat no. 4-6961; Supelco Analytical, Sigma Aldrich, USA) and quantified using undecanoic acid (C11:0) as an internal standard (cat no. 89764, Fluka, Sigma Aldrich, USA). The amount of individual fatty acids was expressed as mg FA in 1 g of faecal/diet (AOAC, 2012e).

CTTAD of fat and fatty acids

The coefficient of total tract apparent individual FA digestibility or fat digestibility (CTTAD) was calculated using the following formula:

$$CTTAD = \frac{dietFAi - faecalFAi}{dietFAi}$$

where FAi is individual fatty acid content or fat content.

CTTAD was calculated for those fatty acids which were found in the diet and faecal samples for all growth periods, and when the total amount of FA taken with the diet was higher than excreted.

Statistical analysis

The data were subjected to ANOVA with age of birds as the only factor using STATISTICA (ver. 9, StatSoft Inc., USA). The metabolic cage was considered the experimental unit. Tukey tests were calculated at a 5% significance level to compare age/growth period means for significant effects. All values are expressed as age (growth period) means with their pooled standard errors.

Results and discussion

Growth performance

The results of FI_{total}, ADFI, DPI, DFI, ADG, FCR and final body weight (BW) in three growth periods are shown in Table 2.

Nutritional value and fatty acids content of diets

Nutritional value and fatty acids content of the diets are shown in Table 3. The difference in nutritional value of the diets was planned to fulfil the requirements

Table 2. Least-squares means for final BW, FI_{total}, ADFI, DPI, DFI, ADG and FCR of Japanese quails

Item	Growth period			
	0-2 week of life	2-4 week of life	4-6 week of life	SEM
FI_{total}, g/bird	102.7[c]	174.9[b]	293.5[a]	11.5
ADFI, g/bird	7.34[c]	12.5[b]	21.0[a]	0.82
DPI, g/bird	2.0[c]	3.4[b]	4.83[a]	0.22
DFI, g/bird	0.33[c]	0.57[b]	1.14[a]	0.04
ADG[1], g/bird	-	5.8[a]	2.6[b]	0.21
FCR[1], g/g	-	2.15[b]	7.91[a]	0.31
Final BW	42.5[c]	123.8[b]	160.8[a]	4.86

[1]analysis performed for 2nd and 3rd growth period, data of initial body weight for 1st growth period were not collected;
BW – body weight, FI_{total} - total feed intake, ADFI – average daily feed intake, DPI – daily protein intake, DFI – daily fat intake, ADG – average daily gain, FCR – feed conversion ratio;
[abc]Within row means bearing different superscript differ significantly at $P<0.05$.

Table 3. Nutritional value and fatty acids content of the diets (mg/g diet)

Item	Starter diet	Grower diet
Nutritional value:		
Protein	275.3	230.4
Fat	45.3	54.4
Fibre	43	46.9
Ash	62.2	108.8
Energy, MJ	13.1	12.75
Fatty acids content:		
C4:0	0.102	0.134
C6:0	0.171	0.299
C8:0	0.286	0.369
C10:0	0.309	0.408
C12:0	0.306	0.321
C14:0	0.242	0.300
C16:0	6.329	7.433
C18:0	1.156	1.444
C22:0	0.226	0.286
C24:0	0.080	0.091
C18:1n-9	11.580	15.177
C20:1n-9	0.107	0.121
C16:1n-7	0.334	0.416
C18:1n-7	0.250	0.306
C18:2n-6	15.680	18.616
C18:3n-6	0.431	0.537
C20:3n-6	0.432	0.525
C20:4n-6	0.050	0.041
C18:3n-3	3.024	3.295
C20:3n-3	0.506	0.636
C20:5n-3	0.057	0.092

specific for the age of quails [NRC 1994]. Both diets were composed mainly of C18:2n-6, 18:1n-9, C16:0, C18:3n-3 and C18:0, and those fatty acids constituted almost 85% of all fatty acids in the diets.

Chemical composition and fatty acids content of faecal samples

Chemical composition and fatty acids content of faecal as affected by age of quails are shown in Table 4. There were no significant differences in protein, fibre and ash content in faecal. The fat content in faecal decreased however with the age of the birds. Although 23 fatty acids were identified and quantified for the diet, not all FA in the diet were detected in faecal. During the whole growth none of the C24:0, C20:1n-9, C20:3n-6, C20:3n-3, and C20:5n-3 in the diets were detected in the faecal samples. The C4:0 was found to be higher in faecal than in the diet during the whole growth, and C20:4n-6 during the growth until the 4th week of life. During the entire growth faecal mainly comprised C18:2n-6, 18:1n-9, C16:0, and C18:0.

Table 4. Effect of age on the chemical composition and fatty acids content in faecal

Item	2nd week of life	4th week of life	6th week of life	SEM
Chemical composition (mg/g)				
protein	265.3	207.5	195.7	2.56
fat	16.1[a]	11[b]	7[c]	0.16
fibre	42.6	37.5	48.3	0.41
ash	66.9	59.6	89.7	0.86
Fatty acids content (mg/g)				
C4:0	0.145	0.155	nd	0.02
C6:0	0.042	0.035	nd	0.004
C8:0	0.199	0.154	0.191	0.02
C10:0	0.077[b]	0.126[a]	0.139[a]	0.01
C12:0	0.053	0.038	0.033	0.01
C14:0	0.163[b]	0.588[a]	0.492[a]	0.05
C16:0	2.569[a]	1.918[b]	1.251[c]	0.26
C18:0	0.849	0.737	0.550	0.09
C22:0	0.221[a]	0.138[b]	0.110[b]	0.02
C18:1n-9	3.771	2.683	2.260	0.41
C16:1n-7	0.096[a]	0.024[b]	nd	0.01
C18:1n-7	0.196[a]	0.180[a]	0.102[b]	0.02
C18:2n-6	6.342[a]	3.310[b]	2.576[b]	0.59
C20:4n-6	0.203[a]	0.088[b]	nd	0.02
C18:3n-3	0.255	0.260	0.187	0.04

nd – not detected.
[abc]Within row means bearing different superscript differ significantly at $P<0.05$.

The faecal fatty acids content appeared to be affected by age. In faecal of older birds more saturated, mainly C10:0 and C14:0, and less unsaturated fatty acids, mainly from the n-7 family: C16:1, C18:1 and from the n-6 family: C18:2 and C20:4, were found.

Coefficient of total tract apparent digestibility

The CTTAD of fat and major fatty acids is given in Table 5. During the growth there was a linear increase of the fat CTTAD, as well as most of the saturated fatty

Table 5. Effect of age on the CCTAD of fat and fatty acids of Japanese quails

Item	2nd week of life	4th week of life	6th week of life	SEM
Fat	0.74[c]	0.81[b]	0.89[a]	0.02
C6:0	0.82	0.84	1.00	0.02
C8:0	0.51	0.57	0.58	0.03
C10:0	0.81[a]	0.67[b]	0.72[b]	0.03
C12:0	0.87	0.90	0.92	0.02
C16:0	0.71[c]	0.76[b]	0.86[a]	0.02
C18:0	0.44[b]	0.48[b]	0.68[a]	0.07
C22:0	0.36[c]	0.52[b]	0.68[a]	0.05
C18:1n-9	0.76[b]	0.82[ab]	0.88[a]	0.02
C16:1n-7	0.80[c]	0.94[b]	1.00[a]	0.02
C18:1n-7	0.43[b]	0.42[b]	0.72[a]	0.06
C18:2n-6	0.70[b]	0.83[a]	0.89[a]	0.03
C18:3n-3	0.94	0.93	0.95[a]	0.01

[abc]Within row means bearing different superscript differ significantly at $P<0.05$.

acids: C16:0, C18:0, and C22:0, along with the n-9 and n-7 monounsaturated fatty acids. When the digestibility of essential FAs during the growth of quails is considered, the digestibility of linoleic acid (LA, 18:2n-6) was increasing with age, from 0.7 to 0.89, while the digestibility of linolenic acid (18:3n-3) remained at similar, high level (>0.93). The n-6 metabolite of LA, arachidonic acid (AA, C20:4n-6) was found to be higher in faecal than in the diet until the 4th week of life, and then it was not found in faecal samples so it was assumed to be totally digested. The n-3 metabolite of ALA, eicosapentaenoic acid (EPA, 20:5n-3) was not found in the faecal samples during the whole growth, and it can be assumed that it was totally digested from the diet. In general, the digestibility of most of FAs was high, except for C18:0, C20:0 and C18:1n-7. On the other hand, the digestibility of all the FAs in the 6th week of life was >0.68, except for C8:0.

Digestibility coefficients of fat and fatty acids are not only important indicators of feed quality but also are important factors which can influence the deposition and further metabolism of fat and fatty acids. The effect of age on fat digestibility in broiler chicken was investigated by Tancharoenrat et al. [2013], who reported that the fat digestibility increases from 0.5 in the first week of life to 0.81 in the second and remains constant thereafter. The current results also show that the fat digestibility increases with advancing age, but the increase from the 2nd to 6th week of life was linear.

The digestibility of fatty acids is known to be influenced by many factors, like for example chain length or degree of unsaturation [Duran-Montgé *et al.* 2007]. Current knowledge on FA digestion is based on comparative studies on animals and fat sources [Blanch *et al.* 1996; Caballero *et al.* 2002; Zhang *et al.* 2011], however, none of the studies considered changes in metabolism, including FA digestion during the growth. The number of studies conducted on FA digestibility in poultry is rather limited. In the literature there is a lack of data on the influence of age on FA digestibility in any species. In this regard, the present study fills the gap.

Overall, the digestibility of most of the FA is high, more than 0.68. Duran-Montgé et al. [2007] evaluated the influence of different fat sources on FA digestibility in pigs. In their study, the majority of FAs in the basal diet, which composition was similar to the diet applied in this study, was digested at a level >75%. Similarly to this study, the digestibility of ALA was higher than LA (92 vs. 90%). However, contrary to this study, the digestibility of LA and ALA was highest when compared to the digestibility of other FAs. In this study, similar or an even higher level of digestibility was reported for C6:0, C18:1n-9 and C16:1n-7.

Also, similarly to the Duran-Montgé et al. [2007] and Zhang *et al.* [2011] studies, the digestibility of C18:0 was the lowest when compared to other FAs.

The C4:0 content was found to be higher in faecal than in the diet during the study period. This result may suggest that its deposition in the body is connected with the *de novo* synthesis and young quails gain energy from carbohydrates at a very high degree, store excess glucose as lipids and at the same time produce short chain FAs and their excessive amount is excreted.

Also C20:4n-6, the main metabolite of LA on the n-6 metabolism pathway, was higher until the 4th week of life in faecal than taken with the diet. This may indicate that when quails are young this fatty acid deposited in the body comes from conversion of LA while the dietary one is excreted.

The high conversion on n-6 pathway, connected with the high digestibility of C18:3n-3, may suggest that possible FAs modification, for example by addition of n-3 to the diet, should be made when birds are young, before the 4th week of life, which can result in a better n-6/n-3 ratio in body/tissues. Additionally, the SFA digestibility increases with age, so PUFA/SFA ratio could be more propitious in young animals. However, if most of the enzymes in young animals are used up for the n-6 conversions, the conversion of n-3, ALA to EPA, can be inhibited.

Conclusion

In conclusion, the comparison of the digestibility of fatty acids during the growth is leading to a better understanding of the FA metabolism. This is an important step to optimize and to predict the FA intake from the diet and further to create a mathematical model of the intake, deposition and conversion of FA in the quail body, as a model for other poultry species.

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