Jejunal histomorphological changes according to dietary fat consumption in broiler chickens

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The aim of this study was to determine the influence of saturation degree of dietary fat on the morphometric characteristics of jejunum in broiler chickens at 14d and its relation to fat digestibility. 160 one-day-old broilers were randomly distributed in 2 treatments (8 replicates / treatment). A basal diet was supplemented with 6% of palm oil (P, saturated) or soybean oil (S, unsaturated). A digestibility balance was carried out between 11-14d. At 14d of age, excreta samples were obtained for the analysis of lipid class composition (triglycerides, diglycerides, monoglycerides, and free fatty acids, FFA) and fatty acids (FA). Samples of the proximal portion of the jejunum of 8 chickens / treatment were also obtained, fixed in 10% buffered formalin solution and embedded in paraffin. Sections were stained with haematoxylin and eosin for morphological observations. The height of intact villi and the depth of crypts of Lieberkühn were measured. Data was analysed by ANOVA. Differences were observed in the apparent digestibility of total FA at 14d (P: 61.7% and S: 81.9%; P<0.001). The % of FFA in excreta was higher for P than for S (P: 69.52% and S: 53.25 %). Differences in the morphology of jejunum were observed. Saturation degree of dietary fat had an effect in the villi height of the proximal part of jejunum, being higher the villi in P treatment. The difference observed in the digestibility and the % of FFA can be related with the differences observed in the morphometric parameters of jejunum.

Key words: fat; jejunal histology; digestibility; lipid classes; broiler chickens

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El objetivo de este estudio fue determinar la influencia del grado de saturación de la grasa del pienso sobre las características morfométricas del yeyuno y su relación con la digestibilidad de la grasa en pollos de carne a los 14 días de edad. Se utilizaron 160 pollos de 1d de edad que fueron distribuidos al azar en 2 tratamientos (8 réplicas / tratamiento). El pienso base se suplementó con un 6% de aceite de palma (P, saturada) o aceite de soja (S, insaturada). Entre los 11-14d se realizó un balance de digestibilidad. A los 14d de edad se recogieron muestras de excreta para la determinación de las fracciones lipídicas (triglicéridos, diglicéridos, monoglicéridos y ácidos grasos libres, AGL) y de ácidos grasos (AG). Se recogieron muestras de la porción proximal del yeyuno de 8 aves / tratamiento, se fijaron en una solución de formalina tamponada al 10% y se incluyeron en parafina. Las secciones se tiñeron con hematoxilina y eosina para el estudio histológico. Se determinaron

la altura de las vellosidades intactas y la profundidad de las criptas de Lieberkünh. Los datos se analizaron mediante una ANOVA. Se observaron diferencias en la digestibilidad aparente de los AG totales a los 14d (P: 61,7% y S: 81,9%; P<0,001). El % de AGL fue superior para P que para S (P: 69,52% y S: 53,25 %). Se observaron diferencias en la morfología del yeyuno. El grado de saturación de la grasa del pienso tuvo un efecto en la altura de las vellosidades de la parte proximal del yeyuno, observándose unas vellosidades más altas en el tratamiento P. Las diferencias observadas en la digestibilidad y en el % de AGL pueden estar relacionadas con las diferencias observadas en los parámetros morfométricos en yeyuno.

Palabras clave: grasa, histología yeyunal, digestibilidad, fracciones lipídicas, pollos de carne

Introduction

Among the ingredients used in the formulation of animal diets, fats and oils are the most concentrated sources of energy. There are different factors affecting the digestibility of fats and one of the most important is the degree of saturation of fatty acids (FA) that exerts a big impact on their apparent absorption (Sklan *et al*., 1973; Renner, 1965).

Small intestine development is essential to broiler health and performance (Kawalilak *et al*., 2011), and represents the major site of nutrient digestion and absorption (Verdal et al., 2010). Jejunum has been described in broiler chickens as the major site of nutrient absorption, especially for fat (Tancharoenrat *et al*., 2014). Anatomical and histomorphological changes of the gut related to diet have been widely described in literature (Swatson *et al*., 2002; Franco *et al*., 2006; Incharoen *et al*., 2010; Laudadio *et al*., 2012) but information related to the effect of dietary fat is scarce. Intestinal development can be studied through measurements of crypt depth and villus length of the absorptive epithelium (Swatson *et al*., 2002; Franco *et al*., 2006), being a common tool in supporting the effects of nutrition on gastrointestinal physiology.

Therefore, the aim of this study was to assess the effect of saturation degree of dietary fat on the morphometric parameters of proximal jejunum in broiler chickens and its relation to fat digestibility.

Materials and methods

Birds and Housing

The study was performed at the animal experimental facilities of the *Servei de Granges i Camps Experimentals* (Universitat Autònoma de Barcelona; Barcelona, Spain) and following the European Union Guidelines for the ethical care and handling of animals under experimental conditions (2010/63/EU). A total of 160 one-day-old female broiler chickens of the Ross 308 strain were obtained from a commercial hatchery (Pondex SAU; Lleida, Spain), identified, weighed individually (initial weight: 40.54 ± 2.18 g) and housed in wire-floor cages. They were randomly assigned to one of the 2 dietary treatments (8 cages/treatment, 10 chicks/cage). Throughout the study (from hatch to 21d), feed and water were supplied for *ad libitum* consumption and animals were raised under controlled conditions of light and temperature.

Experimental diets

Diets were formulated according to requirement data from *Fundación Española para el Desarrollo de la Nutrición Animal* (FEDNA). The 2 dietary treatments were obtained including 6% of palm oil (P) or soybean oil (S) in the basal diet (Table 1). Titanium dioxide (TiO_2) was added as an inert marker for the apparent digestibility determination of fat and FA. Analytical determinations were performed according to the methods of the AOAC International (2005): dry matter (Method 934.01), ash (Method 942.05), crude protein (Method 968.06), crude fat (Method 2003.05) and crude fiber (Method 962.09). Gross energy was determined by an adiabatic calorimeter (IKA C-4000, Janke-Kunkel, Staufen, Alemania). FA content was analysed following the method of Sukhija and Palmquist (1988), adding nonadecanoic acid as an internal standard. The proximate composition of the diets is presented in Table 1.

Controls and sampling

Feed consumption and body weight (BW) were measured at 14 and at 21d. Average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) were calculated for 0-21d period.

A digestibility balance was carried out between 11d-14d. At 14d of age, samples of excreta were obtained and stored at -80ºC until analysis.

Lipid classes and fatty acid analysis

Lipid class composition (triglycerides, TAG; diglycerides, DAG; monoglycerides, MAG; and free fatty acids, FFA) of diets and excreta was determined adapting the procedure described by Darnoko *et al*., (2000) in Trullàs *et al*., (2015). FA analysis of excreta was also analysed following the method of Sukhija and Palmquist (1988).

Table 1. Ingredient and proximate composition of the experimental diets.

Item	P diet[1]	S diet[1]
Ingredient composition (%)		
Wheat	54.5	54.5
Soybean meal 47 %	35.4	35.4
Palm oil	6.00	-
Soybean oil	-	6.00
Dicalcium phosphate	0.99	0.99
Dicalcium carbonate	1.44	1.44
Sodium chloride	0.40	0.40
Vitamin and mineral premix[2]	0.40	0.40
DL-Methionine	0.23	0.23
L-Lysine	0.15	0.15
Titanium dioxide	0.50	0.50
Ethoxyquin 66%	0.02	0.02
Proximate composition (%)		
Dry matter	89.72	89.72
Crude protein	20.88	21.20
Crude fat	7.68	7.39
Crude fiber	3.37	3.18
Ash	5.64	6,30
Gross energy (Kcal/Kg)	4,207	4,259

[1] P: palm, S: soybean [2] Provides per kg of feed: vitamin A (from retinol) 10,000 IU; vitamin D3 (from cholecalciferol) 2,000 IU; vitamin E (from alfa-tocopherol) 49.5 IU; vitamin B1 2 mg; vitamin B2 5 mg; vitamin B6 4 mg; vitamin B12 4 µg; vitamin K3 5 mg; calcium pantothenate 10 mg; nicotinic acid 25 mg; folic acid 1 mg; biotin 15 µg; Fe (from FeSO4·7H2O), 60 mg; I (from $Ca(I_2O_3)_2$), 2 mg; Co (from $2CoCO_3 \cdot 3Co(OH)_2 \cdot H2O$), 0.6 mg; Cu (from $CuSO_4 \cdot 5H_2O$), 20 mg; Mn (from MnO), 150 mg; Zn (from ZnO), 125 mg; Se (from Na_2SeO_3), 0.30 mg; Mo (from $(NH_4)_6Mo_7O_{24}$), 1.2 mg.

Histomorphological Measurements

Tissue samples for histology were taken from the proximal portion of the jejunum from 8 chickens/treatment. Samples were fixed in 10% buffered formalin solution, dehydrated in a graded ethanol series and embedded in paraffin. Sections of 4 µm were stained with haematoxylin and eosin. The height of intact villi (distance from the villus tip to the villus-crypt junction) and the depth of crypts of Lieberkühn (depth of the invagination between adjacent villi) were measured under a Leica DM5000B microscope (Jenoptik, Germany) fitted to a ProgRes® CapturePro software (Jenoptik, Germany).

Statistical Analysis

The normality of the data and homogeneity of the variance were verified. Data was analysed by a one-way ANOVA (R version 3.3.1; 2016 The R foundation for Statistical Computing, Vienna, Austria). The lipid class data was analysed by a repeated measures analysis using the mixed effect linear model with the same statistical software.

Results and discussion

Productive parameters and fatty acid digestibility

The productive parameters are shown in Table 2. The trial was carried out with normality and no mortality was observed in both treatments. A difference in BW at 14d was observed, being animals fed S diet heavier than those fed P diet. However, this difference in BW was not statistically significant at 21d. No differences were obtained for ADFI, ADG and FCR.

Table 2. Effect of the dietary fat source on growth performance and feed utilization from 0-21 days.

	Treatments			
	P	S	SEM[1]	P-values
BW 0d (g)	40.4	40.7	2.18	P>0.05
BW 14d (g)	353.3	376.4	56.02	P<0.05
BW 21d (g)	816.0	827.4	84.04	P>0.05
ADFI (g/d) 0-21d	48.9	47.9	3.78	P>0.05
ADG (g/d) 0-21d	36.9	37.4	4.00	P>0.05
FCR (g/g) 0-21d	1.25	1.23	0.04	P>0.05

BW (body weight), ADFI (average daily feed intake), ADG (average daily gain), FCR (feed conversion ratio) [1]Values are means of 16 cages with 10 birds/cage until 14d, and 16 cages with 3 birds/cage from 14 to 21d.

Table 3. Apparent digestibility coefficient of fatty acids and lipid classes composition of excreta determined in broiler chickens at 14d.

	Treatments			
	P	S	*SEM*[1]	*P* - values
Apparent digestibility coefficients (%)				
Total FA	61.71	81.87	0.043	<0.001
SFA	44.50	69.88	0.052	<0.001
MUFA	76.62	83.12	0.046	<0.05
PUFA	70.25	84.75	0.039	<0.001
Lipid class composition of excreta (%)				
TAG	14.53	23.67	3.538	<0.001
DAG	9.24	15.33	1.118	<0.001
MAG	6.71	7.75	1.001	<0.01
FFA	69.52	53.25	3.745	<0.001

SFA (saturated fatty acids), MUFA (mono-unsaturated fatty acids), PUFA (poly-unsaturated fatty acids), TAG (triglycerides), DAG (diglycerides), MAG (monoglycerides), FFA (free fatty acids) [1]Values are means of 16 cages with 10 birds/cage until 14d, and 16 cages with 3 birds/cage from 14 to 21d.

Apparent digestibility of total FA, SFA, MUFA and PUFA was greater in S diet than in P diet, as expected (Table 3). This is due to the higher unsaturation degree in S diet. SFA had the lowest apparent digestibility coefficients and showed the biggest difference in apparent digestibility between treatments (in S diet the apparent digestibility coefficient of SFA was 36.32 % higher than in P diet). This difference can be explained because the absorption of saturated fatty acids varied directly with the level of unsaturated fatty acids in the mixture (Renner et al., 1961). The unsaturated fatty acids are digested and absorbed easily, meanwhile SFA needs to be emulsified before absorption (Krogdahl, 1985). The higher percentage of FFA in P diet excreta ($p<0.001$) supports the lower apparent digestibility coefficients observed in this treatment (Table 3).

Histomorphological Measurements

The means of jejunal villus height and crypt depth at 14d of age are presented in Table 4.

Table 4. Effect of the dietary fat source on jejunal mucosal morphometry of broiler chickens at 14d.

	Treatment			
	P	S	SEM[2]	p-values
Villus height (µm)	1,079.54	929.70	143.20	<0.001
Crypt depth (µm)	140.06	136.92	12.90	>0.05
Villus height:Crypt depth ratio[1]	7.70	6.72		

[1]Measured using the means of villus height and crypt depth [2]Values are means of 33 values in the case of P diet and 40 values in the case of S diet

Villus height was 13.88 % higher in chickens fed with P diet than in those fed with S diet ($P<0.001$). Crypt depth in P diet was numerically higher, but no difference was observed between treatments. Consequently, the villus height:crypt depth ratio was higher in animals fed with P diet. Despite the structural changes in the small intestine remains poorly understood, increasing the villus height suggests an increased surface area capable of greater absorption of available nutrients (Caspary, 1992). The villus crypt is considered as the villus factory and deeper crypts indicate fast tissue turnover to permit renewal of the villus as needed in response to normal sloughing or inflammation (Yason *et al*., 1987). The higher villi height together with the lower digestibility of FA observed in P treatment could be explained as an adaptive process of the intestinal absorptive surface to allow a greater absorption of nutrients (Caspary, 1992). However, information about the influence of fat in histomorphometric studies is scarce and more studies are needed to clarify better its effect.

Conclusion

It can be concluded that a high saturated dietary fat affected the morphometric characteristics of the proximal part of jejunum of 14d old broiler chickens, resulting in a higher villi height.

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