Effect of iso-energetic exchange of dietary fat and starch on growth performance and body composition of broilers; Experiment 1

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Effect of iso-energetic exchange of dietary fat and starch on growth performance and body composition of broilers

Experiment 1

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Samenvatting

De concentraties eiwit/aminozuren, vet en koolhydraten en hun ratio's kunnen het post-absorptieve energie-metabolisme en de aanzet van energie en eiwit in het lichaam beïnvloeden. In een 2x2 factorieel experiment zijn de effecten van twee ruw eiwit (hoog eiwit (HP) vs. laag eiwit (LP) concentraties; 200/190 vs. 170/160 g/kg in de groei- en eindfase) en twee vet/zetmeel concentraties; (hoog vet (HF); vet en zetmeel respectievelijk 105 en 340 g/kg) en laag vet (LF); vet en zetmeel respectievelijk 65 en 420 g/kg) op productieparameters en lichaamssamenstelling van Ross 308 vleeskuikens onderzocht in de periode van 9 tot 35 dagen leeftijd. Geconcludeerd kan worden dat de energiebron en het eiwitgehalte in iso-energetische voeders, gebalanceerd voor de eerst limiterende essentiële aminozuren, invloed hebben op groeiparameters en lichaamssamenstelling van vleeskuikens.

Summary

Dietary factors such as the concentrations of protein/amino acids, fat, and starch + sugar and their ratio, may affect the post-absorptive metabolism of energy and protein and energy deposition in the body. In a 2x2 factorial design, the effects of two dietary crude protein (high protein (HP) vs. low protein (LP) concentrations; 200/190 vs. 170/160 g/kg in grower and finisher phase) and two dietary fat/starch concentrations; (high fat (HF); fat and starch 105 and 340 g/kg, respectively) and (low fat (LF); fat and starch 65 and 420 g/kg, respectively) on growth performance and body composition of Ross 308 broilers were studied (9 to 35 d). From this experiment it can be concluded that dietary energy source and protein level in iso-energetic diets, balanced for first limiting essential amino acids, influence growth performance and body composition of broilers.

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Livestock Research Report 1061

Table of contents

Foreword

Feed4Foodure is a public-private partnership between the Dutch Ministry of Economic Affairs, a consortium of various organizations within the animal production chain and Wageningen Livestock Research. Feed4Foodure aims to contribute to sustainable and healthy livestock farming in the Netherlands, simultaneously strengthening its competitive position on the global market. The Feed4Foodure program line "More-with-Less by efficient nutrient use", aims to reduce the footprint of the Dutch livestock sector in the field of phosphate, nitrate, copper, zinc, ammonia and greenhouse gases. New nutritional models and measurement techniques will help to improve efficient use of nutrients in livestock farming.
The current report describes the first experiment in a series of three experiments that were conducted to investigate the effect of iso-energetic exchange of dietary fat and starch on growth performance and body composition of broilers. For the current study, scientists of Wageningen Livestock Research worked together with representatives from the consortium and the authors thank the industry partners of the project team for their worthwhile input.

Dr. Teun Veldkamp, project leader

Summary

Macro-nutrients such as the concentrations of protein/amino acids, fat, and starch + sugars and their ratio, may affect the post-absorptive metabolism of energy and protein and energy deposition in broilers. In a 2x2 factorial design, the effects of two dietary crude protein (high protein (HP) vs. low protein (LP) concentrations; 200/190 vs. 170/160 g/kg in grower and finisher phase) and two dietary fat/starch concentrations; (high fat (HF); fat and starch 105 and 340 g/kg, respectively) and (low fat (LF); fat and starch 65 and 420 g/kg, respectively) on growth performance and body composition of Ross 308 broilers were studied (9 to 35 d). Concentrations of apparent faecal digestible essential amino acids were similar in HP and LP diets. Feed intake of broilers fed HP diets tended ($P=0.06$) to be lower than in broilers fed LP diets. Body weight gain (BWG) of broilers fed HP diets was significantly higher than BWG of broilers fed LP diets ($P<0.001$) resulting in a significantly lower feed conversion ratio (FCR) of broilers fed HP diets ($P<0.001$). Exchange of dietary fat by starch resulted in a significantly lower FI and FCR, while BWG was not affected. The FCR was improved by substituting dietary fat by starch, the effect being more pronounced in LP than in HP diets ($P=0.012$). On d 35, the body fat content of birds fed LF diets was higher than in birds fed the HF diets ($P=0.03$). Obtained differences in nutrient deposition can be due to differences in post-absorptive metabolism and retention. Additional measurements on metabolites and hormones in the blood samples were conducted as an extra tool to explain observed differences in post-absorptive metabolism. Concentrations of glucose, insulin, non-esterified (NEFA) or 'free fatty acids, triglycerides, triiodothyronine (T3) and thyroxine (T4) were measured in blood of broilers at 35 days of age. Triglyceride concentration in the blood of broilers fed LP diets was significantly higher than in broilers fed HP diets most likely caused by increased hepatic lipogenesis and leading to an increased fat deposition. Uric acid concentrations in broilers fed HP diets was significantly higher than in broilers fed LP diets and uric acid levels in blood of broilers fed HF diets was significantly higher than of broilers fed LF diets. As a reliable biomarker for protein degradation it can be stated that protein degradation was higher in birds fed HP diets and HF diets as compared to birds fed LP diets and LF diets, respectively. Non-esterified fatty acids levels as a biomarker for lipolysis in broilers fed HF diets were significantly higher than in broilers fed LF diets. Effects of exchange of dietary fat by starch on free fatty acid concentrations are not consistent in literature. T4 levels in broilers fed HF diets was significantly higher than in broilers fed LF diets. This effect could not be confirmed by results from literature. Glucose, insulin and T3 concentrations in blood of broilers were not affected by dietary treatments in this experiment. No significant protein x fat interaction effects of dietary treatments on blood metabolites and hormones have been observed in this experiment.

1 Introduction

The subproject Feed4Foodure MMM2A quantifies the effect of nutritional interventions on the energy losses in pigs and poultry (broilers as well as layers) husbandry, and on the methane losses in ruminant nutrition. The current report describes the first in a series of three experiments that were conducted to investigate the effect of iso-energetic exchange of dietary fat and starch on the growth performance and body composition of broilers. Energy losses appear as a result of an indigestible part of dietary energy and excretion in the excreta, the synthesis and losses of endogenous protein that is excreted in the digestive tract and excreted in the excreta, energy use for different maintenance processes and subsequently reveal as heat losses and post-absorptive energy metabolism (inefficient use of energy for protein and fat deposition in the body). Poultry breeders are selecting broilers for body weight gain and breast muscle. The mean daily body weight gain has increased by 5 g and the breast meat yield has increased by 0.5% in the last decade. This selection of animals may affect the protein and fat metabolism considerably. Energy deposition is the resultant of dietary energy intake and the efficiency of utilization of energy for maintenance and for deposition of protein and fat in the body. Besides genetic factors also exogenic factors, such as climate and nutrition (feed intake and diet composition, affect the energy partitioning in the body. Literature is available on the effect of ratio of dietary macro-nutrients (protein, fat and starch + sugar) on growth performance and body composition of broilers ((Jackson et al., 1982; Laurin et al., 1985; MacLeod, 1990, 1992; Nieto et al., 1997 Collin et al., 2003; Swennen et al., 2005, 2007). In general, diets containing high concentrations of metabolisable energy or a high energy-protein ratio result in a higher fat deposition (Swennen et al., 2007). Dietary protein concentration above the protein/amino acid requirement will result in broilers with a lower body fat content and with a lower efficiency because degradation and excretion of the surplus amino acids are energy demanding processes. A reduction of the dietary protein concentration to suboptimal levels, whereby the supply of essential amino acids and/or the total of provided nitrogen are below requirement, will result in a higher fat deposition (Buyse et al., 1992). Many studies in literature were focusing on the effect of dietary protein concentration and less attention was paid on the effect of dietary fat and carbohydrate concentrations on growth performance and deposition of protein and fat in the body. Eits (2004) concluded that protein deposition increased as dietary protein concentration was increased. In case the dietary protein intake was restricted, the protein deposition in the body could not be increased by the supply of extra dietary energy. Body weight gain was significantly lower and fat deposition was significantly higher in broilers fed iso-energetic diets (low in fat or low in carbohydrate concentration) with low crude protein (12.6%) than in broilers fed diets with standard crude protein concentration (19.7%). Effects of starch + sugars in the diet on insulin levels in the blood and stimulation of protein synthesis and limitation of protein degradation have been described in literature on humans and pigs. Starch + sugar may have a protein-sparing effect in monogastrics (Fuller et al., 1977) and a higher inclusion level of starch + sugar in the diet may increase the nitrogen retention. Different studies in human suggest that the hormones glucagon and insulin play an import role; insulin decreases protein degradation and stimulates protein synthesis (Bennet *et al.,* 1990; Biolo *et al.,* 1995), while glucagon stimulates amino acid catabolism (Mallette *et al.,* 1969; Flakoll *et al.,* 1994). Rabinowitz *et al.* (1966) showed that when proteins were ingested alone, there was a large increase in plasma glucagon and a small elevated plasma insulin level. But, when proteins and carbohydrates were ingested together, insulin release was enhanced (Nuttall *et al.,* 1984). In literature related to human and pigs it is clear that there is an effect of dietary starch + sugars intake on insulin levels, which resulted in the stimulation of protein synthesis and restriction of protein degradation (Calbet *et al.,* 2002; Camp *et al.,* 2003). Camp et al. (2003) found a positive effect of higher inclusion levels of sucrose on body weight gain and feed efficiency in growing pigs. In rats, Fulks et al. (1975) found that glucose by itself inhibited protein degradation but in the absence of insulin, glucose had no significant effect on protein. Furthermore, Houston and O' Neill (1991) showed that insulin stimulated the secretion of IGF-I by chicken hepatocytes and acts synergistically with growth hormone (GH) to increase IGF-I release. The GH secretion in poultry

stimulates production and secretion of IGF 1 from the liver, which is the major source of circulating IGF 1 (Buyse et al., 2000). IGF 1 and FFA's exert a negative feedback to the hypothalamic–pituitary axis to suppress GH secretion (Buyse et al., 2000). Growth hormone (GH) has important and direct effects on the liver and adipose tissue, whereas effects on skeletal muscle are mostly mediated by IGF 1 (Scanes, 2009). Malheiros *et al.* (2003) showed that chickens on a low protein diet had decreased plasma IGF-I level. Increased plasma FFA levels were measured in broilers on a low fat (high carbohydrate) diet compared to broilers on a high fat (low carbohydrate) diet (Malheiros *et al,.* 2003). These findings contradicts with those of Tanaka *et al.* (1983), who showed that adding fat to a diet resulted in increased FFA levels. However, the diets used by Malheiros *et al.* (2003) were iso-energetically formulated. Malheiros et al. (2003) showed that a low protein diet increased fat deposition in broilers compared to chickens with a normal protein diet. The broilers fed a low protein diet had higher plasma triglyceride (TG) levels, which is also reported in other studies (Tanaka et al., 1983; Rosebrough et al., 1996; Collin et al., 2003; Swennen et al., 2005, 2007). Triglycerides are the main product of the de novo hepatic lipogenesis in the chicken.

It is therefore expected that a higher concentration of dietary starch + sugar may have a positive effect on growth performance and processing yields as higher concentrations of dietary starch + sugar will affect glucose and insulin levels in the blood. In poultry, no consistent effects were reported by exchange of dietary fat as energy source by starch + sugar. So from literature it can be concluded that exchange of dietary fat by starch + sugars affects insulin and glucose levels in the blood and nutrient metabolism in animals.

1.1 Objectives

The objective of the experiment was to study the effect of iso-energetic exchange of dietary fat and starch on growth performance, body composition, endocrine function and the intermediary metabolism of broilers.

2 Material and Methods

2.1 Experimental animals

The experiment was conducted with 1008 Ross 308 broilers. Broilers were obtained from the commercial hatchery Probroed & Sloot, Meppel, The Netherlands. Males and females were housed separately. Day-old broilers were weighed individually and sorted in weight classes. Subsequently the day-old broilers were placed in floor pens. Mean weight and variability in body weight per pen was equal in all pens. The maximum allowed difference between mean weight per pen and overall mean weight was 3%. Broilers with visual deformities were not included in the experiment. The number of broilers was 14 broilers per pen. Day-old broilers were vaccinated against IB in the hatchery and NCD (spray vaccination) at 14 days of age at the experimental facility.

2.2 Experimental treatments and design

A two level factorial experiment was conducted in which three factors were investigated at only two levels. The three factors were: dietary crude protein concentration, dietary fat/starch concentration and gender. The four experimental diets were randomly assigned within blocks of four pens situated next to each other (per gender 9 pens per diet) from 9 days of age.
The two dietary crude protein concentrations were: high dietary protein (HP) vs. low dietary protein (LP) concentrations; 200/190 vs. 170/160 g/kg in the grower and finisher phase, respectively. The two dietary fat/starch concentrations were: high dietary fat (HF) concentrations (dietary fat and starch 105 and 340 g/kg, respectively) and low dietary fat (LF) concentrations (dietary fat and starch 65 and 420 g/kg, respectively). Both experimental dietary factors (dietary crude protein and fat/starch concentration) were studied per gender. The experimental factors are summarized in Table 1.

Table 1 *Overview of the experimental factors*

Treatment-code[1]	Protein concentration (g/kg)		Fat concentration (g/kg)		Starch concentration (g/kg)	
	9-28 d of age	28-35 d of age	9-28 d of age	28-35 d of age	9-28 d of age	28-35 d of age
HP-HF	200	190	105	105	380	380
HP-LF	200	190	65	65	460	460
LP-HF	170	160	105	105	380	380
LP-LF	170	160	65	65	460	460

[1] HP (high protein), LP (low protein), HF (high fat), LF (low fat)

2.3 Experimental diets and feeding

A commercial starter diet was provided to the broilers during the starter phase from 0 to 9 d of age. The feed composition of the starter diet is presented in Appendix 1. The starter diet was fed as crumbs (2 mm) and the grower and finisher diets as pellet (3 mm). The grower and finisher diet were fed in the periods from 9 to 28 d of age and 28 to 35 d of age, respectively. The two dietary crude protein concentrations in the experimental diets in the grower and finisher phase were: high dietary protein (HP) vs. low dietary protein (LP) concentrations; 200/190 vs. 170/160 g/kg in the grower and finisher phase, respectively). The low dietary fat/starch concentrations were: high dietary fat (HF) concentrations (dietary fat and starch 105 and 340 g/kg, respectively) and low dietary fat (LF) contents (dietary fat and starch 65 and 420 g/kg, respectively). Assumptions for the feed formulation were a good pellet quality in all experimental diets to avoid differences in feed

intake due to pellet quality in broilers fed different experimental diets. Essential amino acids lysine, methionine, threonine, valine, arginine, isoleucine and tryptophan were supplemented to meet the apparent fecal digestible amino acid requirements (CVB, 2012). The restricted number of protein-rich feed ingredients (soybean meal, potato protein and corn gluten meal) should be decreased proportionally to create the diets with low dietary protein concentrations in order to avoid large differences in inclusion levels of feed ingredients between high (HP) and low (LP) protein diets. In the feed formulation it was pursued to create a difference of 3% in crude protein concentration between HP and LP diets. In the feed formulation it was pursued further to create a difference of 4% in crude fat concentration and a difference of 8% in starch concentration between high fat (HF) and low fat (LF) diets. All grower and finisher experimental diets were formulated to be iso-energetic (2925 kcal ME/kg). Feed ingredients with a high crude fiber and/or fat concentration will be exchanged by starch or feed ingredients rich in starch in order to realize iso-energetic diets. Experimental diets were also formulated to have an identical electrolyte balance (Na+K-Cl). Cellulose (Arbocel®) was used as an inert filler. In the finisher diet an inert marker (TiO_2) was included to determine fecal digestibility of nutrients in the finisher diet. Feed and nutrient composition of the grower and finisher experimental diets are presented in Table 2a and 2b, respectively.

Table 2a *Ingredient and nutrient composition of experimental grower diets in g/kg unless stated otherwise (9-28 d of age)*

	Unit/kg	HP-HF[1]		HP-LF[1]		LP-HF[1]		LP-LF[1]	
Feed ingredient									
Corn		287.2		291.2		330.8		338.0	
Wheat		250.0		250.0		250.0		250.0	
Wheat middlings		63.0		11.0		88.2		31.0	
Cellulose (Arbocel®)		31.5		5.5		44.1		15.5	
Soybean meal		198.0		198.0		122.8		135.8	
Potato protein ASH<10		20.3		24.1		12.3		13.6	
Corn gluten meal		40.6		48.2		24.6		27.2	
Corn starch		0.0		100.0		0.0		100.0	
Soy oil		73.5		34.7		76.6		37.6	
Premix (corn)[2]		5.0		5.0		5.0		5.0	
Limestone fine		11.8		11.8		12.2		12.0	
Mono-Calcium Phosphate		10.0		10.0		10.5		11.0	
Salt		1.8		1.8		0.9		0.5	
Sodium bicarbonate		2.7		2.7		3.9		3.9	
TiO2		0.0		0.0		0.0		0.0	
L-Lysine HCl		2.7		2.6		5.5		5.3	
DL-Methionine		1.5		1.5		2.7		2.7	
L-Threonine		0.4		0.2		1.9		1.8	
L-Valine		0.0		0.0		1.9		1.8	
L-Arginine		0.2		0.3		2.6		2.6	
L-Isoleucine		0.0		0.0		1.3		1.1	
L-Tryptophan		0.0		0.0		0.1		0.1	
Potassium carbonate		0.0		1.5		2.4		3.6	
Nutrient		Calc.	Ana.	Calc.	Ana.	Calc.	Ana.	Calc.	Ana.
DM		879	879	872	878	875	877	868	876
ASH		50	49	48	48	48	48	47	47
CP		200	205	200	205	170	172	170	173
CFATh		105	106	65	66	105	105	65	66
Cfib		60	52	30	27	73	61	40	33
$STARCH_{am}$		340	301	418	398	361	327	439	411
SUG		33		30		29		27	
NDF		97		80		103		85	
ADF		32		27		32		27	
Ca		7.0	7.6	7.0	7.6	7.1	7.7	7.1	7.7
P		5.9	5.9	5.4	5.5	5.9	5.8	5.5	5.6
oP		3.2		3.1		3.2		3.2	
Ca : oP		2.2		2.2		2.2		2.2	
Mg		1.4		1.2		1.3		1.2	
K		7.1	7.7	7.0	7.6	7.0	7.4	7.0	7.6
Na		1.5	1.6	1.5	1.3	1.5	1.7	1.5	1.6
Cl		2.0	2.5	2.0	2.5	2.0	2.5	2.0	2.4
EB, meq		191		189		186		188	
$ME_{broiler}$, MJ		12.2		12.2		12.2		12.2	
dLYS		9.9		9.9		9.9		9.9	
dMET		4.5		4.5		5.0		5.0	
dCYS		2.8		2.8		2.3		2.3	
dMET+CYS		7.3		7.3		7.2		7.3	
dVAL		8.1		8.2		8.0		7.9	
dARG		10.4		10.4		10.4		10.4	
dILE		7.2		7.3		6.6		6.6	
dTHR		6.5		6.4		6.4		6.5	
dTRP		1.9		1.9		1.6		1.6	
dGLY		6.4		6.3		5.0		5.0	
dSER		8.7		8.8		6.7		6.8	
dLEU		16.0		16.6		12.1		12.4	
dPHE		9.0		9.2		6.8		7.0	
dTYR		6.6		6.8		4.9		5.0	
dPHE+TYR		15.6		16.0		11.7		12.0	
dHIS		4.3		4.3		3.4		3.4	
dALA		8.6		8.8		6.6		6.7	
dASP		15.6		15.8		11.2		11.6	
dGLU		34.9		34.8		28.7		28.7	
dPRO		11.8		11.9		9.8		9.8	

[1] HP (high protein), LP (low protein), HF (high fat), LF (low fat)

[2] Composition of premix: 12,000 IE retinol, 2,400 IE cholecalciferol, 50 mg dl-a-tocopherol, 1.5 mg menadione, 2.0 mg thiamine, 7.5 mg riboflavin, 3.5 mg pyridoxine-HCl, 20 µg cyanocobalamine, 35 mg niacin, 12 mg D-pantothenic acid, 460 mg choline chloride, 1.0 mg folic acid, 0.2 mg biotin, 80 mg iron (265 mg FeSO4.H2O), 12 mg copper (48 mg $CuSO_4.5H_2O$), 85 mg manganese 85 mg (140 mg MnO), 60 mg zinc (165 mg $ZnSO_4.H_2O$), 0.4 mg cobalt (2 mg $CoSO_4.7H_2O$), 0.8 mg iodine (1.2 mg KJ), 0.15 mg selenium (0,33 mg Na_2SeO_3), 125 mg anti-oxidant Oxytrap PXN.

Table 2b *Ingredient and nutrient composition of experimental finisher diets in g/kg unless stated otherwise (28-35 d of age)*

	Unit / kg	HP-HF[1]		HP-LF[1]		LP-HF[1]		LP-LF[1]	
Feed ingredient									
Corn		304.9		310.0		346.7		349.8	
Wheat		250.0		250.0		250.0		250.0	
Sunflower meal CFib > 240		0.0		0.0		0.0		0.0	
Wheat middlings		65.0		12.0		94.0		40.0	
Cellulose (Arbocel®)		32.5		6.0		47.0		20.0	
Soybean meal		190.0		190.0		102.8		115.0	
Potato protein ASH<10		16.2		19.8		10.3		11.5	
Corn gluten meal		32.4		39.6		20.6		23.0	
Corn starch		0.0		100.0		0.0		100.0	
Soy oil		74.0		35.2		77.3		38.3	
Premix (corn)[2]		5.0		5.0		5.0		5.0	
Limestone fine		11.0		11.0		11.4		11.3	
Mono-Calcium Phosphate		8.0		8.5		8.5		9.0	
Salt		1.8		1.8		0.8		0.9	
Sodium bicarbonate		2.3		2.3		3.7		3.6	
TiO2		2.5		2.5		2.5		2.5	
L-Lysine HCl		2.6		2.6		5.6		5.4	
DL-Methionine		1.5		1.4		2.6		2.6	
L-Threonine		0.4		0.3		2.0		1.9	
L-Valine		0.0		0.0		1.9		1.9	
L-Arginine		0.1		0.3		2.7		2.8	
L-Isoleucine		0.0		0.0		1.4		1.3	
L-Tryptophan		0.0		0.0		0.2		0.2	
Nutrient		Calc.	Ana.	Calc.	Ana.	Calc.	Ana.	Calc.	Ana.
DM		878	876	871	875	874	878	867	872
ASH		49	50	48	48	47	49	46	47
CP		190	184	190	187	160	156	160	154
CFATh		105	114	65	72	105	112	65	73
Cfib		61	51	31	27	75	63	43	36
$STARCH_{am}$		348	333	426	427	368	364	447	454
SUG		33		30		28		26	
NDF		98		81		105		87	
ADF		32		27		32		27	
Ca		6.4	6.7	6.4	6.9	6.4	6.9	6.4	6.9
P		5.4	5.5	5.1	5.1	5.4	5.4	5.0	5.0
oP		2.8		2.8		2.8		2.8	
Ca : oP		2.3		2.3		2.3		2.3	
Mg		1.4		1.2		1.3		1.1	
K		7.0	6.9	7.1	6.9	7.0	6.9	7.0	6.8
Na		1.4	1.5	1.4	1.5	1.4	1.5	1.4	1.6
Cl		2.0	2.6	2.0	2.4	2.0	2.5	2.0	2.4
EB, meq		184		185		184		183	
$ME_{broiler}$ MJ		12.2		12.2		12.2		12.2	
dLYS		9.4		9.4		9.4		9.4	
dMET		4.2		4.3		4.7		4.8	
dCYS		2.7		2.6		2.2		2.2	
dMET+CYS		6.9		6.9		6.9		6.9	
dVAL		7.6		7.7		7.5		7.6	
dARG		9.9		9.9		9.9		9.9	
dILE		6.8		6.9		6.2		6.2	
dTHR		6.2		6.1		6.2		6.2	
dTRP		1.8		1.8		1.5		1.5	
dGLY		6.1		6.0		4.7		4.6	
dSER		8.2		8.3		6.2		6.3	
dLEU		14.8		15.4		11.1		11.4	
dPHE		8.4		8.6		6.2		6.4	
dTYR		6.2		6.3		4.5		4.6	
dPHE+TYR		14.6		14.9		10.7		11.0	
dHIS		4.2		4.1		3.1		3.1	
dALA		8.0		8.2		6.0		6.1	
dASP		14.7		14.8		10.0		10.4	
dGLU		33.6		33.4		27.2		27.2	
dPRO		11.3		11.4		9.3		9.3	

[1] HP (high protein), LP (low protein), HF (high fat), LF (low fat)

[2] Composition of premix: 12,000 IE retinol, 2,400 IE cholecalciferol, 50 mg dl-a-tocopherol, 1.5 mg menadione, 2.0 mg thiamine, 7.5 mg riboflavin, 3.5 mg pyridoxine-HCl, 20 µg cyanocobalamine, 35 mg niacin, 12 mg D-pantothenic acid, 460 mg choline chloride, 1.0 mg folic acid, 0.2 mg biotin, 80 mg iron (265 mg FeSO4.H2O), 12 mg copper (48 mg $CuSO_4.5H_2O$), 85 mg manganese 85 mg (140 mg MnO), 60 mg zinc (165 mg $ZnSO_4.H_2O$), 0.4 mg cobalt (2 mg $CoSO_4.7H_2O$), 0.8 mg iodine (1.2 mg KJ), 0.15 mg selenium (0,33 mg Na_2SeO_3), 125 mg anti-oxidant Oxytrap PXN.

2.4 Housing and management

In total, 1008 Ross 308 broilers (males and females) were used in the study and were placed in floor pens. A natural ventilated broiler house was used in which 6 rows of 12 floor pens (1.00 x 0.75 m) were installed. In each floor pen 14 day-old broilers were placed. The housing management, feeding and husbandry conditions are regarded as representative for a modern commercial operation in Europe. Day-old broilers were distributed among the 72 floor pens bedded with wood shavings (2 kg/m2). The maximum allowed difference between mean weight per pen and overall mean weight will be 3%. Water and feed was ad libitum available for the broilers. The feeding bins were constructed in a way that feed spillage was avoided. One day prior to placement of the broilers the rooms were pre-heated to 34°C. Temperature was decreased gradually to 22°C at 25 days of age. Lighting schedule was 0 to 4 days: 23 L(light) - 1 D(dark); 5 to 9 days: 20 L – 4 D; 10 to 30 days: 18 L – 6 D; 30 to 34 days: 20 L – 4 D and 35 days: 23 L – 1 D. Visual observation of the birds was done twice per day to check animal health. Day-old broilers were vaccinated against IB (Infectious Bronchitis) at the hatchery and at 14 days of age broilers were vaccinated against NCD (NewCastle Disease).

2.5 Observations during the study

- Prior to feed formulation, main feed ingredients were analyzed with NIR in order to formulate the experimental diets accurately according to calculations.
- Experimental diets were chemically analyzed in duplo for concentration of dry matter, crude protein (N x 6.25), crude fat, crude fiber, crude ash, starch and sugar. Minerals calcium, phosphorus, sodium, chloride and potassium were also chemically analyzed in duplo.
- Feed intake was determined per pen at 9, 18, 28 and 35 days of age.
- Body weight of broilers was determined per pen by group weighing at 9, 18, 28 and 35 days of age.
- Two broilers per pen with a body weight close to the mean body weight per pen were selected at 9, 18, 28 and 35 days of age. The selected broilers were anaesthetized and subsequently euthanized by an intravenous injection of T61 (Intervet Int.). Subsequently, the chest cavity and the abdomen were opened and the gastro-intestinal tract was ligated and removed from the bird. The content of the gastro-intestinal tract was removed and the empty gastro-intestinal tract was put together with the carcass. The two carcasses with empty gastro-intestinal tract were frozen (-20°C) per pen and were considered as one pooled sample per pen. The carcasses and gastro-intestinal tract samples were autoclaved and homogenized in a mixer and a sample was taken to analyze body composition: dry matter (ANAL-10066 ISO 1442), crude protein (ANAL-10005 NEN-ISO 937), crude fat (ANAL-10112 ISO 1443) and crude ash (ANAL-10028 NEN-ISO 936).
- Blood was sampled from six broilers per pen at 35 days of age. Blood samples were centrifuged and blood plasma was sampled in plasma-EDTA tubes. Plasma-EDTA tubes (2 x 2 ml) were stored frozen at -80°C and were subsequently chemically analyzed for glucose, insulin, non-esterified fatty acids, uric acid, triglyceride, and the thyroid hormones T3 and T4 to determine metabolite and hormone levels related to energy and nutrient metabolism.
- Excreta from the colon was collected in six anaesthetized and euthanized broilers per pen at 35 days of age for determination of nutrient digestibility. These were the same six broilers as used for blood collection. Digestibility was determined in samples of male broilers for dry matter (DM), organic matter (OM), crude protein (CP) corrected for uric acid N, crude fat (FAT) and starch + sugars.

2.6 Statistics

Response parameters were statistically analysed by ANOVA using GenStat statistical software (16th edition, VSN International Ltd., Hemel Hempstead, UK), using series of four pens situated next to each other as block factor, dietary protein concentration, dietary fat/starch concentration and the interaction between dietary protein concentration and dietary fat/starch concentration as explanatory variables according to the statistical model:

$Y = \mu + block_i + gender_j + block \times gender_{ij} + dietary\ protein\ concentration_k + dietary\ fat/starch\ concentration_l + (dietary\ protein\ concentration \times dietary\ fat/starch\ concentration)_{kl} + e_{ijkl}$

Where:

Y	=Response parameter
μ	=General mean
block (i=1..18)	=Block (four pens situated next to each other in a row)
gender	=Gender (j=1,2)
block x gender	=Effect of $block_i$ x $gender_j$
dietary protein concentration	=Effect of dietary protein concentration (k=1,2)
dietary fat/starch concentration	=Effect of dietary fat/starch concentration (l=1,2)
dietary protein concentration x dietary fat/starch concentration	=Interaction effect dietary protein $concentration_k$ x dietary fat/starch $concentration_l$
error	=Error term

Mortality data were log-transformed prior to statistical analysis.
The P-value of the treatment effect and the LSD (least significant difference (P=0.05)) were provided per response parameter. Treatment effects with a P-value ≤0.05 were considered to be statistically significant.

3 Results and Discussion

The experiment was conducted according to the protocol without major problems or relevant deviations. Day-old broilers arrived healthy. The experimental period started at 9 days of age and mean body weight of the female and male broilers was 239 g, which was slightly below the performance standards of Aviagen (breeder organization of brand Ross 308) (Aviagen, 2014). Overall, mortality during the experimental period was 2.6% and no specific cause of mortality was observed.

3.1 Growth performance

Growth performance results for the growth periods 9 to 18, 18 to 28 and 28 to 35 d of age are reported in Appendix 2, 3 and 4, respectively. Growth performance results over the period of 0 to 35 days of age are presented in Table 3.

Table 3 *Growth performance of broilers over the period 0 to 35 d of age*

			BW 35d	BW gain	FCR	Feed intake	Mortality
			g	g/d		g/d	%
Protein							
High			2264[a]	63.5	1.57[b]	99.5	4.4[a]
Low			2196[b]	61.5[b]	1.65[a]	101.3	0.8[b]
Fat							
High			2233	62.6	1.63[a]	101.6	2.5
Low			2227	62.4	1.59[b]	99.2	2.7
Gender							
Male			2347[a]	65.9[a]	1.60	105.5	2.3
Female			2112[b]	59.2[b]	1.61	95.3	2.9
Protein	**Fat**						
High	High		2277	63.9	1.58[c]	100.6	4.1
High	Low		2251	63.1	1.56[c]	98.5	4.7
Low	High		2189	61.4	1.67[a]	102.6	1.0
Low	Low		2202	61.7	1.62[b]	99.9	0.7
Protein	**Gender**						
High	Male		2394	67.2	1.56	105.0	4.2
High	Female		2134	59.8	1.57	94.1	4.6
Low	Male		2301	64.5	1.64	106.0	0.4
Low	Female		2091	58.6	1.65	96.6	1.3
Fat	**Gender**						
High	Male		2348	65.9	1.62	106.6	1.9
High	Female		2118	59.3	1.63	96.7	3.2
Low	Male		2347	65.9	1.59	104.4	2.7
Low	Female		2106	59.0	1.59	94.0	2.7
Protein	**Fat**	**Gender**					
High	High	Male	2397	67.3	1.57	105.3	3.0
High	High	Female	2156	60.4	1.59	95.9	5.3
High	Low	Male	2391	67.1	1.56	104.6	5.4
High	Low	Female	2111	59.1	1.56	92.3	3.9
Low	High	Male	2299	64.5	1.67	107.8	0.8
Low	High	Female	2079	58.2	1.67	97.5	1.1
Low	Low	Male	2303	64.4	1.61	104.2	0.0
Low	Low	Female	2102	58.9	1.63	95.7	1.4
P-values							
Protein			**0.001**	**0.001**	**<0.001**	*0.060*	**0.002**
Fat			0.760	0.760	**<0.001**	**0.010**	0.712
Gender			**<0.001**	**<0.001**	0.411	**0.001**	0.521
Protein x Fat			0.342	0.342	**0.012**	0.757	0.976
Protein x Gender			0.213	0.213	0.747	0.435	0.982
Fat x Gender			0.798	0.798	0.721	0.767	0.651
Protein x Fat x Gender			0.480	0.480	0.293	0.209	0.328

No gender by dietary treatment interactions have been observed in the experiment (Table 3). Feed intake of the birds fed HP diets tended (P=0.06) to be lower than feed intake of broilers fed LP diets. Body weight gain of broilers fed HP diets was significantly higher than body weight gain of broilers fed LP diets (63.5 vs. 61.5 g; P=0.001). Feed conversion ratio of birds fed HP diets was significantly lower than feed conversion ratio of broilers fed LP diets (1.57 vs. 1.65; P<0.001). Despite inclusion of free essential amino acids in LP diets in order to meet (CVB, 2012) apparent faecal digestible amino acid requirement values, growth performance of birds fed LP diets was lower than on HP diets. The concentration of non-essential amino acids in LP diets related to the 30 g/kg lower crude protein concentration may have been limiting body weight gain in LP diets compared to HP diets.

Feed intake of birds fed HF diets was significantly higher than feed intake of birds fed LF diets (101.6 vs. 99.2 g/d; P=0.01). Body weight gain of broilers was not affected by the dietary energy source. Feed conversion ratio of broilers fed HF diets was significantly higher than feed conversion ratio of broilers fed LF diets (1.63 vs. 1.59; P<0.001). This effect was more pronounced in LP diets than in HP diets (Protein x Fat interaction; P=0.012). It can be concluded that exchange of dietary fat by starch in LF diets resulted in a significantly lower feed intake while body weight gain was not affected. As a result feed conversion ratio was lower in birds fed LF diets. It is not clear why the positive effect of exchange of fat by starch on feed conversion was more pronounced in broilers fed LP diets than in broilers fed HP diets. In practice, an increasing trend has been observed to formulate diets with lower crude protein concentrations with supplementation of free amino acids. Results of this study implicate that it may be interesting to exchange fat by starch to a certain extent in low protein diets to improve feed efficiency. Mortality in broilers fed LP diets was significantly lower than in broilers fed HP diets (0.8 vs. 4.4%; P=0.002).

3.2 Body composition

Two broilers per pen with a body weight close to the mean body weight per pen were selected at 9, 18, 28 and 35 days of age to determine body composition for dry matter (DM), ash, crude protein (CP) and fat (Fat). The deposition of DM, Ash, CP and Fat in the periods from 9 to 18, 18 to 28 and 28 to 35 days was calculated. In Table 4 to 7 the chemical body composition of broilers at 9, 18, 28 and 35 d of age is presented.

Table 4 *Chemical body composition (dry matter (DM), ash (Ash), crude protein (CP) and crude fat (Fat)) at 9 d of age (g/kg body weight)*

			DM	Ash	CP	Fat
			g/kg	g/kg	g/kg	g/kg
Protein						
High			270	23.2	154	93.1
Low			270	23.0	154	93.3
Fat			270	23.3	154	93.5
High			270	23.0	154	92.9
Low						
Gender						
Male			267[b]	23.3	152[b]	92.3
Female			273[a]	22.9	155[a]	94.1
Protein	**Fat**					
High	High		269	23.4	153	92.8
High	Low		272	23.1	154	93.3
Low	High		271	23.1	154	94.2
Low	Low		269	22.8	154	92.5
Protein	**Gender**					
High	Male		267	23.4	152	92.2
High	Female		273	23.0	155	93.9
Low	Male		268	23.2	152	92.4
Low	Female		272	22.8	155	94.2
Fat	**Gender**					
High	Male		267	23.2[a]	152	93.2
High	Female		273	23.3[a]	155	93.8
Low	Male		268	23.4[a]	153	91.5
Low	Female		272	22.5[b]	155	94.3
Protein	**Fat**	**Gender**				
High	High	Male	264[c]	23.3	151	90.9[a]
High	High	Female	274[a]	23.4	155	94.6[a]
High	Low	Male	271[a]	23.5	153	93.5[a]
High	Low	Female	273[a]	22.7	155	93.2[a]
Low	High	Male	270[ab]	23.1	152	95.4[a]
Low	High	Female	272[a]	23.2	155	93.0[a]
Low	Low	Male	265[bc]	23.3	153	89.5[b]
Low	Low	Female	272[a]	22.4	155	95.4[a]
P-values						
Protein			0.648	0.221	0.950	0.808
Fat			0.878	0.105	0.205	0.628
Gender			**0.008**	*0.086*	**<0.001**	0.247
Protein x Fat			*0.057*	0.956	0.311	0.332
Protein x Gender			0.556	0.985	0.889	0.969
Fat x Gender			0.495	**0.010**	0.342	0.352
Protein x Fat x Gender			**0.010**	0.854	0.379	**0.011**

Until 9 days of age, broilers in all experimental groups received a similar commercial starter diet. No differences in chemical composition were observed between dietary treatments (Table 4). Dry matter and CP content in male broilers was significantly lower than in female broilers. Ash and Fat contents were not affected by gender.

Table 5 *Chemical body composition (dry matter (DM), ash (Ash), crude protein (CP) and crude fat (Fat)) at 18 d of age (g/kg body weight)*

			DM 18 d g/kg	Ash 18 d g/kg	CP 18 d g/kg	Fat 18 d g/kg
Protein						
High			291[b]	23.0[b]	164[a]	106[b]
Low			297[a]	23.4[a]	162[b]	114[a]
Fat						
High			291[b]	23.0	163	106[b]
Low			297[a]	23.3	162	113[a]
Gender						
Male			289[b]	23.2	162[b]	107[b]
Female			299[a]	23.1	164[a]	113[a]
Protein	**Fat**					
High	High		289	23.0	164	103
High	Low		293	23.0	163	109
Low	High		293	23.1	162	109
Low	Low		301	23.7	161	118
Protein	**Gender**					
High	Male		286	22.9	163	102
High	Female		296	23.1	165	110
Low	Male		292	23.6	160	111
Low	Female		302	23.2	163	116
Fat	**Gender**					
High	Male		286	23.2	162	110
High	Female		296	22.9	164	117
Low	Male		292	23.3	161	103
Low	Female		302	23.4	164	109
Protein	**Fat**	**Gender**				
High	High	Male	284	23.1	163	100
High	High	Female	294	22.9	166	106
High	Low	Male	287	22.7	162	105
High	Low	Female	298	23.3	164	113
Low	High	Male	289	23.3	161	106
Low	High	Female	298	22.9	163	113
Low	Low	Male	296	23.9	160	115
Low	Low	Female	306	23.5	163	120
P-values						
Protein			**0.001**	**0.027**	**<0.001**	**0.001**
Fat			**0.004**	*0.085*	0.226	**0.003**
Gender			**<0.001**	0.560	**0.007**	**0.014**
Protein x Fat			0.231	*0.095*	0.631	0.601
Protein x Gender			0.910	*0.098*	0.562	0.649
Fat x Gender			0.765	0.330	0.812	0.951
Protein x Fat x Gender			0.968	0.230	0.652	0.643

At 18 days of age, differences in chemical composition were observed between dietary treatments (Table 5). Dry matter, Ash and Fat content in broilers fed HP diets was significantly lower than in broilers fed LP diets. Content of CP in broilers fed HP diets was significantly higher than in broilers fed LP diets. Dry matter and Fat content in broilers fed HF diets was lower than in broilers fed LF diets. Ash and CP content were not affected by dietary fat/starch concentration. Dry matter, CP and Fat content in male broilers was significantly lower than in female broilers. Ash content was not affected by gender.

Table 6 *Chemical body composition (dry matter (DM), ash (Ash), crude protein (CP) and crude fat (Fat)) at 28 d of age (g/kg body weight)*

			DM 28 d g/kg	Ash 28 d g/kg	CP 28 d g/kg	Fat 28 d g/kg
Protein						
High			308[b]	24.0	178[a]	112[b]
Low			317[a]	24.3	174[b]	124[a]
Fat						
High			308[b]	24.0	176	112[b]
Low			317[a]	24.3	175	124[a]
Gender						
Male			307[b]	24.2	175[b]	112[b]
Female			319[a]	24.1	177[a]	124[a]
Protein	**Fat**					
High	High		304	23.9	178	105
High	Low		312	24.1	177	118
Low	High		313	24.1	175	118
Low	Low		322	24.5	174	131
Protein	**Gender**					
High	Male		304[c]	24.1	177	107
High	Female		312[b]	24.0	178	116
Low	Male		309[b]	24.3	173	117
Low	Female		325[a]	24.2	175	131
Fat	**Gender**					
High	Male		305[c]	24.2	175	109[c]
High	Female		312[b]	23.8	178	115[bc]
Low	Male		308[bc]	24.2	174	116[b]
Low	Female		325[a]	24.4	176	133[a]
Protein	**Fat**	**Gender**				
High	High	Male	303	24.4[abc]	178	104
High	High	Female	305	23.5[c]	178	107
High	Low	Male	304	23.7[bc]	176	111
High	Low	Female	319	24.4[ab]	178	125
Low	High	Male	307	24.0[abc]	173	114
Low	High	Female	319	24.1[abc]	177	122
Low	Low	Male	312	24.6[a]	173	121
Low	Low	Female	331	24.4[ab]	174	141
P-values						
Protein			**<0.001**	0.208	**<0.001**	**<0.001**
Fat			**<0.001**	0.163	*0.064*	**<0.001**
Gender			**<0.001**	0.818	**0.009**	**0.001**
Protein x Fat			0.564	0.477	0.746	0.978
Protein x Gender			**0.038**	0.894	0.405	0.180
Fat x Gender			**0.007**	0.142	0.720	**0.006**
Protein x Fat x Gender			0.486	**0.012**	0.112	0.989

At 28 days of age, differences in chemical composition were observed between dietary treatments (Table 6). Dry matter and Fat content in broilers fed HP diets was significantly lower than in broilers fed LP diets. Content of CP in broilers fed HP diets was significantly higher than in broilers fed LP diets. Ash content was not affected by dietary protein concentration. Dry matter and Fat content in broilers fed HF diets was lower than in broilers fed LF diets. Ash and CP content were not affected by dietary fat/starch concentration. Dry matter, CP and Fat content was in male broilers significantly lower than in female broilers. Ash content was not affected by gender.

Table 7 *Chemical body composition (dry matter (DM), ash (Ash), crude protein (CP) and crude fat (Fat)) at 35 d of age (g/kg body weight)*

			DM 35 d g/kg	Ash 35 d g/kg	CP 35 d g/kg	Fat 35 d g/kg
Protein						
High			323.2[b]	24.2	180.3[a]	122[b]
Low			335.7[a]	24.7	176.7[b]	138[a]
Fat						
High			325.4[b]	24.0[b]	178.8	127[b]
Low			333.5[a]	24.9[a]	178.2	133[a]
Gender						
Male			323.1[b]	24.5	177.3	125[b]
Female			335.8[a]	24.4	179.7	135[a]
Protein	**Fat**					
High	High		319.4	23.6	180.5	120
High	Low		327.0	24.9	180.2	125
Low	High		331.4	24.3	177.2	134
Low	Low		340.0	25.0	176.2	141
Protein	**Gender**					
High	Male		317.2	24.2	179.2	117
High	Female		329.2	24.3	181.4	127
Low	Male		328.9	24.9	175.5	132
Low	Female		342.5	24.4	177.9	144
Fat	**Gender**					
High	Male		315.8	23.9	177.1	119[b]
High	Female		334.9	24.0	180.6	135[a]
Low	Male		330.3	25.2	177.6	131[a]
Low	Female		336.8	24.7	178.7	136[a]
Protein	**Fat**	**Gender**				
High	High	Male	309.3	23.4	179.0	111
High	High	Female	329.4	23.8	182.0	128
High	Low	Male	325.1	24.9	179.4	124
High	Low	Female	329.0	24.8	180.9	126
Low	High	Male	322.4	24.4	175.2	126
Low	High	Female	340.4	24.3	179.3	142
Low	Low	Male	335.5	25.4	175.9	138
Low	Low	Female	344.5	24.6	176.5	145
P-values						
Protein			**<0.001**	0.298	**0.002**	**<0.001**
Fat			**0.013**	**0.031**	0.552	**0.029**
Gender			**<0.001**	0.659	*0.095*	**<0.001**
Protein x Fat			0.878	0.485	0.748	0.790
Protein x Gender			0.810	0.498	0.959	0.793
Fat x Gender			*0.053*	0.450	0.276	**0.036**
Protein x Fat x Gender			0.576	0.822	0.648	0.630

At 35 days of age, differences in chemical composition were observed between dietary treatments (Table 7). Dry matter and fat content in broilers fed HP diets was significantly lower than in broilers fed LP diets. Protein content in broilers fed HP diets was significantly higher than in broilers fed LP diets. Ash content was not affected by dietary protein concentration. Dry matter, ash and fat content in broilers fed HF diets was lower than in broilers fed LF diets. Protein content was not affected by dietary fat/starch concentration. Dry matter and fat content in male broilers was significantly lower than in female broilers. Ash and protein content were not affected by gender.

The deposition of CP and Fat in the periods from 9 to 18, 18 to 28 and 28 to 35 days is presented in Table 8.

Table 8 *Deposition of crude protein (CP) and fat (Fat) in the body in the period 9 to 18 d, 18 to 28 and 28 to 35 d of age in g per bird*

		9 – 18 d of age		18 – 28 d of age		28 – 35 d of age	
		CP g	Fat g	CP g	Fat g	CP g	Fat g
Protein							
High		79[a]	52	152[a]	93[b]	141[a]	109
Low		74[b]	56	142[b]	103[a]	134[b]	122
Fat							
High		77	52[b]	148	92[b]	137	117
Low		76	56[a]	146	104[a]	138	113
Gender							
Male		78[a]	54	153[a]	96	149[a]	121
Female		75[b]	55	142[b]	100	126[b]	109
Protein	**Fat**						
High	High	80	51	153	87	142	112
High	Low	78	54	152	100	141	105
Low	High	74	53	144	97	132	122
Low	Low	74	59	141	108	136	121
Fat	**Gender**						
High	Male	78	51	154	93[a]	147	112[ab]
High	Female	76	53	142	90[a]	127	122[a]
Low	Male	78	56	151	98[a]	151	130[a]
Low	Female	73	57	141	111[b]	125	96[b]
P-values							
Protein		**0.002**	0.109	**<0.001**	**0.014**	**0.021**	*0.068*
Fat		0.422	**0.031**	0.200	**<0.001**	0.723	0.618
Gender		**0.019**	0.726	**<0.001**	0.318	**<0.001**	0.113
Protein x Fat		0.450	0.395	0.541	0.770	0.415	0.670
Protein x Gender		1.000	0.890	0.348	0.205	0.346	0.822
Fat x Gender		0.377	0.779	0.464	**0.020**	0.295	**0.003**
Protein x Fat x Gender		0.434	0.929	0.642	0.768	0.632	0.551

Protein deposition in broilers fed HP diets was significantly higher than in broilers fed LP diets (Table 8). In the period from 18 to 28 days of age fat deposition in broilers fed HP diets was significantly lower than in broilers fed LP diets. Protein deposition was not affected by dietary fat/starch concentration whereas fat deposition in broilers fed HF diets was lower than in broilers fed LF diets up to 28 days of age. Protein deposition in male broilers was significantly higher than in female broilers. Fat deposition was not affected by gender.

3.3 Nutrient digestion

Nutrient digestibility coefficients are presented in Table 9.

Table 9 *Colonic digestibility of nutrients in the experimental diets*

		DM	DM (excl. Arbocel®)	OM	CP	Fat	Starch & Sugars
		%	%	%	%	%	%
Protein							
High		68	70	70	79	81	96
Low		68	70	70	79	82	96
Fat							
High		65[b]	68[b]	67[b]	79	82	95[b]
Low		71[a]	72[a]	73[a]	79	81	97[a]
Protein	**Fat**						
High	High	65	68	67	80	82	95
High	Low	71	72	73	79	80	97
Low	High	65	69	67	79	82	96
Low	Low	70	72	72	79	82	97
P-values							
Protein		0.638	0.627	0.715	0.791	0.420	0.559
Fat		**<0.001**	**0.004**	**<0.001**	0.718	0.645	**0.025**
Protein x Fat		0.726	0.731	0.677	0.912	0.757	0.498

Nutrient digestibility was determined at 35 days of age by collecting excreta from the colon of broilers. The nutrient digestibility was determined in male broilers only as a gender effect on digestion was not expected. Dietary protein did not affect nutrient digestibility. Dietary fat however did have an effect on the digestibility of DM, OM and starch+sugars. As Arbocel® was included as an inert filler in the experimental diets, DM digestibility was also calculated without Arbocel®. Dry matter, OM and starch+sugars digestibility in LF diets was higher than in HF diets. Also by excluding Arbocel® the DM digestibility in LF diets was higher than in HF diets. Digestibility of CP and fat were not affected by dietary treatment.

3.4 Blood metabolites and hormones

Results of the blood metabolite and hormone analyses are presented in Table 10.

Table 10 *Blood metabolites analyses at 35 d of age*

			Glucose mg/dl	Insulin *[1]	NEFA[2] mM	T3[3] ng/ml	T4[4] ng/ml	TG[5] mg/dl	UA[6] mg/dl
Protein									
High			273.4	1.089	0.3356	1.395	3.905	170.8[b]	7.87[a]
Low			280.2	1.146	0.3435	1.491	3.611	199.5[a]	6.82[b]
Fat									
High			275.0	1.053	0.4102[a]	1.509	4.353[a]	189.8	7.78[a]
Low			278.6	1.182	0.2689[b]	1.377	3.164[b]	180.5	6.90[b]
Gender									
Male			282.7[a]	1.121	0.3571	1.516	3.497	189.3	7.21
Female			270.9[b]	1.113	0.3220	1.369	4.020	181.0	7.47
Protein	**Fat**								
High	High		270.7	0.951	0.4099	1.484	4.572	169.0	8.24
High	Low		276.0	1.226	0.2614	1.305	3.239	172.7	7.49
Low	High		279.2	1.154	0.4105	1.534	4.133	210.6	7.32
Low	Low		281.2	1.138	0.2765	1.448	3.089	188.5	6.31
Protein	**Gender**								
High	Male		276.4	1.037	0.3457	1.549	3.465	176.4	7.65
High	Female		270.4	1.140	0.3256	1.241	4.346	165.2	8.08
Low	Male		289.0	1.205	0.3686	1.484	3.528	202.3	6.78
Low	Female		271.4	1.087	0.3184	1.498	3.694	196.7	6.86
Fat	**Gender**								
High	Male		279.1	0.964	0.4474	1.541	4.349	196.5	7.70
High	Female		270.9	1.141	0.3731	1.477	4.356	183.1	7.87
Low	Male		286.3	1.278	0.2669	1.492	2.644	182.2	6.73
Low	Female		270.9	1.086	0.2709	1.262	3.684	178.9	7.07
Protein	**Fat**	**Gender**							
High	High	Male	273.5	0.822	0.4525	1.555	4.604	178.5	7.97
High	High	Female	268.0	1.021	0.3673	1.412	4.540	159.5	8.51
High	Low	Male	279.3	1.193	0.2388	1.542	2.325	174.2	7.33
High	Low	Female	272.8	1.260	0.2839	1.069	4.152	170.9	7.65
Low	High	Male	284.6	1.047	0.4423	1.527	4.095	214.6	7.42
Low	High	Female	273.7	1.261	0.3788	1.541	4.172	206.6	7.22
Low	Low	Male	293.4	1.364	0.2950	1.442	2.962	190.1	6.14
Low	Low	Female	269.0	0.912	0.2580	1.455	3.215	186.8	6.49
P-values									
Protein			0.155	0.683	0.720	0.534	0.487	**0.021**	**0.006**
Fat			0.440	0.357	**<0.001**	0.396	**0.007**	0.443	**0.020**
Gender			**0.031**	0.944	0.437	0.474	*0.048*	0.396	0.529
Protein x Fat			0.728	0.302	0.743	0.765	0.732	0.291	0.732
Protein x Gender			0.224	0.430	0.495	0.302	0.398	0.819	0.636
Fat x Gender			0.449	0.191	*0.080*	0.593	0.224	0.675	0.827
Protein x Fat x Gender			0.511	0.291	0.242	0.596	0.312	0.819	0.602

[1] Since mouse insulin has been used for calibration curve construction, the unit of the insulin concentrations is "ng mouse insulin equivalent per ml plasma".

[2] Non-esterified fatty acids.

[3] triiodothyronine.

[4] thyroxine.

[5] triglyceride.

[6] uric acid.

Triglyceride concentration in the blood of broilers fed LP diets was significantly higher than in broilers fed HP diets (Table 10). The increased triglyceride levels in the plasma of low protein-fed broilers are most likely the result of a stimulated hepatic lipogenesis (Malheiros et al., 2003). Rosebrough et al. (1996, 2002) observed in related studies with broilers on a low protein diet also elevations in hepatic lipogenic enzyme activities, leading to an increased fat deposition. The increased fat deposition in this experiment at LP diets was only observed in the period from 18 to 28 days of age.

Uric acid concentrations in broilers fed HP diets was significantly higher than in broilers fed LP diets. Excess of ammonium is finally converted into uric acid for excretion (Sturkie, 2000). Therefore, plasma uric acid levels can be considered as a reliable biomarker for protein degradation. Malheiros et al. (2003) showed that lower plasma uric acid levels were derived in broilers with a low protein diet (15.8% crude protein) compared to broilers with an iso-energetic low fat or low carbohydrate (CHO) diet with a normal protein content (19.6% crude protein). These results were also observed in other studies (Rosebrough et al., 1996; Collin et al., 2003; Swennen et al., 2004, 2005, 2007). Studies with substitutions between fat and CHO at a similar protein level didn't show an effect on plasma uric acid levels (Collin et al., 2003). In the current experiment plasma uric acid levels in blood of broilers fed HF diets was significantly higher than of broilers fed LF diets.

Non-esterified fatty acids levels in broilers fed HF diets were significantly higher than in broilers fed LF diets. The plasma level of free fatty acids (FFA) is the net result of lipolysis in combination with cellular uptake of FFA (Swennen et al., 2004). Fasting induced lipolysis in adipocytes and gluconeogenesis in the liver. Lipolysis is the breakdown of lipids and involves hydrolysis of triglycerides into glycerol and free fatty acids. Therefore, FFA's are used as biomarker for lipolysis. Different studies showed that iso-energetic substitutions between protein, fat and CHO revealed no effect on FFA concentration (Malheiros et al. 2003). However, Swennen et al. (2005, 2007) measured increased FFA plasma levels in low protein fed broilers, compared to broilers with a low fat diet. The authors suggested that the low protein diet resulted in a decreased uptake of FFA due to a preference for CHO as energy source. Increased plasma FFA levels were measured in broilers on a low fat (high CHO) diet compared to broilers on a low CHO (high fat) diet (Malheiros et al,. 2003). These findings contradicts with those of the current experiment and the study of Tanaka et al. (1983), who showed that adding fat to a diet resulted in increased FFA levels.

Also T4 levels in broilers fed HF diets was significantly higher than in broilers fed LF diets. However, Carew and Alster (1997) observed that iso-energetic substitution of fat by carbohydrates did not alter thyroid hormone metabolism whereas replacing dietary protein with carbohydrates or fat was followed by an increase and a decrease in circulating T3 and T4 levels, respectively. The same results were found by Rosebrough et al. (1999) and Malheiros et al. (2003). They conclude that thyroid hormone metabolism in chickens is very sensitive to the level of dietary protein, but much less to dietary fat and carbohydrate content.

Furthermore, glucose concentration in blood of male broilers was significantly higher than in blood of female broilers. No effects of gender on glucose concentration in blood of broilers have been reported in literature.

Dietary treatments did not affect glucose, insulin and T3 levels in blood of broilers in this experiment. In general, no significant protein x fat interaction effects of dietary treatments on blood metabolites and hormones have been observed.

4 Conclusions

- Protein level and dietary energy source in iso-energetic diets balanced for most limiting essential amino acids affect growth performance and body composition of broilers.
- Lowering dietary crude protein concentration with 30 g/kg in the grower and finisher period, despite supplementation of free amino acids up to concentrations that meet CVB (2012) fecal digestible amino acid requirements, adversely affected growth performance of broilers.
- Partly substitution of fat by starch and sugars as dietary energy source improved growth performance.
- Low fat diets (higher in starch + sugars) resulted in a higher body fat content in birds at 35 days while digestion of fat was not affected by dietary treatment. The observed effects are related to differences in the post-absorptive utilization of amino acids, starch + sugar and fatty acids and retention in the body.
- Triglyceride concentration in the blood of broilers fed LP diets was significantly higher than in broilers fed HP diets most likely caused by increased hepatic lipogenesis and leading to an increased fat deposition.
- Uric acid concentrations in broilers fed HP diets was significantly higher than in broilers fed LP diets and uric acid levels in blood of broilers fed HF diets was significantly higher than of broilers fed LF diets. As a reliable biomarker for protein degradation it can be stated that protein degradation was higher in birds fed HP diets and HF diets as compared to birds fed LP diets and LF diets, respectively.
- Non-esterified fatty acids levels as a biomarker for lipolysis in broilers fed HF diets were significantly higher than in broilers fed LF diets. Effects of exchange of dietary fat by starch on free fatty acid concentrations are not consistent.
- T4 levels in broilers fed HF diets was significantly higher than in broilers fed LF diets. This effect could not be confirmed by results from literature. In literature it was stated that thyroid hormone metabolism in chickens is very sensitive to the level of dietary protein, but much less to dietary fat and carbohydrate content.
- Glucose, insulin and T3 concentrations in blood of broilers were not affected by dietary treatments in this experiment.
- No significant protein x fat interaction effects of dietary treatments on blood metabolites and hormones have been observed in this experiment.

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Appendix 1 Feed and nutrient composition of the starter diet (0-9 days of age)

Feed ingredients	Concentration	Nutrients	Units	Units / kg	
				Calculated	Analysed
Corn	325.2	DM	g	875	879
Wheat	250.0	ASH	g	61	58
Sunflower meal CFib > 240	0.0	CP	g	210	215
Wheat middlings	15.0	CFATh	g	83	86
Cellulose (Arbocel®)	7.5	Cfib	g	36	33
Soybean meal	280.0	Carbohydrates	g	481	
Potato prot. ASH<10	10.0	$STARCH_{am}$	g	360	357
Corn gluten meal	20.0	SUG	g	48	
Corn starch	0.0	NDF	g	90	
Soy oil	49.0	ADF	g	31	
Premix (corn)[1]	5.0	Ca	g	9.1	9.5
Limestone fine	14.5	P	g	7.1	7.0
Mono-Calcium Phosphate	15.0	oP	g	4.2	
Salt	2.0	Ca : oP		2.2	
Sodium bicarbonate	2.7	Mg	g	1.6	
TiO2	0.0	K	g	8.2	8.7
L-Lysine HCl	2.0	Na	g	1.6	1.7
DL-Methionine	1.9	Cl	g	2.0	2.5
L-Threonine	0.4	EB	meq	223	
L-Valine	0.0	$ME_{broiler}$	MJ	11.9	
L-Arginine	0.0	dLYS	g	10.5	
L-Isoleucine	0.0	dMET	g	4.8	
L-Tryptophan	0.0	dCYS	g	2.9	
Potassium carbonate	0.0	dMET+CYS	g	7.7	
		dVAL	g	8.5	
		dARG	g	11.7	
		dILE	g	7.7	
		dTHR	g	6.8	
		dTRP	g	2.1	
		dGLY	g	6.9	
		dSER	g	9.2	
		dLEU	g	16.0	
		dPHE	g	9.4	
		dTYR	g	6.8	
		dPHE+TYR	g	16.1	
		dHIS	g	4.8	
		dALA	g	8.7	
		dASP	g	17.6	
		dGLU	g	36.5	
		dPRO	g	11.8	

[1] Composition of premix: 12,000 IE retinol, 2,400 IE cholecalciferol, 50 mg dl-a-tocopherol, 1.5 mg menadione, 2.0 mg thiamine, 7.5 mg riboflavin, 3.5 mg pyridoxine-HCl, 20 µg cyanocobalamine, 35 mg niacin, 12 mg D-pantothenic acid, 460 mg choline chloride, 1.0 mg folic acid, 0.2 mg biotin, 80 mg iron (265 mg FeSO4.H2O), 12 mg copper (48 mg $CuSO_4.5H_2O$), 85 mg manganese 85 mg (140 mg MnO), 60 mg zinc (165 mg $ZnSO_4.H_2O$), 0.4 mg cobalt (2 mg $CoSO_4.7H_2O$), 0.8 mg iodine (1.2 mg KJ), 0.15 mg selenium (0,33 mg Na_2SeO_3), 125 mg anti-oxidant Oxytrap PXN.

Appendix 2 Growth performance of broilers from 9 to 18 days of age

			BW 18d	BW gain	FCR	Feed intake	Mortality
			g	g / d		g / d	%
Protein							
High			700	51.2	1.38[b]	70.5[b]	1.6[a]
Low			690	50.2	1.45[a]	72.7[a]	0.0[b]
Fat							
High			698	50.9	1.42	72.2	1.4
Low			693	50.5	1.41	71.0	0.3
Gender							
Male			711[a]	52.7[a]	1.40[b]	73.7[a]	0.7
Female			680[b]	48.7[b]	1.43[a]	69.5[b]	0.9
Protein	**Fat**						
High	High		706	51.7	1.36[c]	70.4	2.8
High	Low		694	50.6	1.40[bc]	70.6	0.5
Low	High		689	50.1	1.48[a]	73.9	0.0
Low	Low		691	50.3	1.42[b]	71.4	0.0
Protein	**Gender**						
High	Male		716	53.2	1.37	72.9	1.4
High	Female		684	49.1	1.39	68.1	1.9
Low	Male		705	52.2	1.43	74.5	0.0
Low	Female		675	48.3	1.47	70.8	0.0
Fat	**Gender**						
High	Male		708	52.4	1.41	73.9	0.9
High	Female		687	49.4	1.43	70.5	1.9
Low	Male		713	53.0	1.39	73.5	0.5
Low	Female		673	48.0	1.43	68.5	0.0
Protein	**Fat**	**Gender**					
High	High	Male	715	53.1	1.36	71.9	1.9
High	High	Female	697	50.3	1.37	68.9	3.7
High	Low	Male	717	53.3	1.39	73.9	1.0
High	Low	Female	672	48.0	1.41	67.3	0.0
Low	High	Male	702	51.7	1.47	75.9	0.0
Low	High	Female	677	48.5	1.49	72.0	0.0
Low	Low	Male	709	52.6	1.39	73.2	0.0
Low	Low	Female	674	48.0	1.45	69.7	0.0
P-values							
Protein			0.264	0.230	**<0.001**	**0.034**	**0.012**
Fat			0.569	0.578	0.505	0.248	*0.082*
Gender			**0.004**	**<0.001**	**0.035**	**0.001**	0.875
Protein x Fat			0.440	0.413	**0.003**	0.173	*0.082*
Protein x Gender			0.943	0.934	0.367	0.567	0.881
Fat x Gender			0.292	0.212	0.479	0.411	0.323
Protein x Fat x Gender			0.634	0.682	0.482	0.322	0.323

Appendix 3 Growth performance of broilers from 18 to 28 days of age

			BW 28d	BW gain	FCR	Feed intake	Mortality
			g	g/d		g/d	%
Protein							
High			1504[a]	80.4[a]	1.62[b]	129.5	1.2
Low			1458[b]	76.7[b]	1.71[a]	130.9	0.3
Fat							
High			1486	78.8	1.69[a]	132.6[a]	0.6
Low			1475	78.3	1.64[b]	127.9[b]	0.9
Gender							
Male			1529[a]	81.8[a]	1.68	136.7[a]	0.6
Female			1432[b]	75.2[b]	1.65	123.7[b]	0.9
Protein	**Fat**						
High	High		1510	80.4	1.63	131.0	0.6
High	Low		1497	80.3	1.60	128.1	1.8
Low	High		1461	77.2	1.74	134.2	0.6
Low	Low		1454	76.3	1.68	127.6	0.0
Protein	**Gender**						
High	Male		1555	84.0	1.63	136.2	1.2
High	Female		1452	76.8	1.60	122.9	1.2
Low	Male		1503	79.7	1.73	137.3	0.0
Low	Female		1413	73.7	1.69	124.5	0.6
Fat	**Gender**						
High	Male		1534	82.6	1.69	139.0	0.6
High	Female		1437	75.1	1.68	126.1	0.6
Low	Male		1524	81.1	1.66	134.4	0.6
Low	Female		1427	75.4	1.61	121.3	1.2
Protein	**Fat**	**Gender**					
High	High	Male	1558	84.3	1.63	136.7	1.1
High	High	Female	1463	76.6	1.64	125.2	0.0
High	Low	Male	1553	83.6	1.63	135.6	1.2
High	Low	Female	1441	76.9	1.57	120.6	2.4
Low	High	Male	1511	80.9	1.75	141.3	0.0
Low	High	Female	1412	73.5	1.73	127.1	1.1
Low	Low	Male	1494	78.6	1.70	133.3	0.0
Low	Low	Female	1413	74.0	1.65	121.9	0.0
P-values							
Protein			**0.004**	**0.002**	**<0.001**	0.360	0.180
Fat			0.491	0.620	**0.006**	**0.002**	0.630
Gender			**<0.001**	**<0.001**	0.275	**0.001**	0.631
Protein x Fat			0.849	0.728	0.438	0.206	0.180
Protein x Gender			0.655	0.579	0.723	0.865	0.664
Fat x Gender			0.996	0.402	0.144	0.919	0.664
Protein x Fat x Gender			0.566	0.680	0.676	0.285	0.195

Appendix 4 Growth performance of broilers from 28 to 35 days of age

			BW 35d	BW gain	FCR	Feed intake	Mortality
			g	g / d		g / d	%
Protein							
High			2264[a]	108.6[a]	1.77[b]	192.0	0.4
Low			2196[b]	105.4[b]	1.86[a]	196.0	0.4
Fat							
High			2233	106.7	1.84[a]	195.9	0.0
Low			2227	107.3	1.80[b]	192.1	0.7
Gender							
Male			2347[a]	116.9[a]	1.78[b]	207.7[a]	0.4
Female			2112[b]	97.1[b]	1.86[a]	180.3[b]	0.4
Protein	**Fat**						
High	High		2277	109.5	1.79	195.3	0.0
High	Low		2251	107.8	1.76	188.7	0.8
Low	High		2189	103.9	1.89	196.5	0.0
Low	Low		2202	106.9	1.84	195.5	0.7
Protein	**Gender**						
High	Male		2394	119.8	1.73	206.9	0.8
High	Female		2134	97.4	1.82	177.1	0.0
Low	Male		2301	114.0	1.83	208.5	0.0
Low	Female		2091	96.8	1.90	183.5	0.7
Fat	**Gender**						
High	Male		2348	116.2	1.81	209.5	0.0
High	Female		2118	97.2	1.88	182.3	0.0
Low	Male		2347	117.6	1.75	205.8	0.8
Low	Female		2106	97.0	1.84	178.3	0.7
Protein	**Fat**	**Gender**					
High	High	Male	2397	119.9	1.75	208.9	0.0
High	High	Female	2156	99.0	1.83	181.6	0.0
High	Low	Male	2391	119.8	1.71	204.8	1.6
High	Low	Female	2111	95.8	1.80	172.6	0.0
Low	High	Male	2299	112.5	1.87	210.1	0.0
Low	High	Female	2079	95.3	1.92	182.9	0.0
Low	Low	Male	2303	115.4	1.79	206.9	0.0
Low	Low	Female	2102	98.3	1.88	184.1	1.4
P-values							
Gender			**<0.001**	**<0.001**	**0.004**	**0.001**	0.963
Protein			**0.001**	**0.041**	**<0.001**	0.153	0.963
Fat			0.760	0.692	**0.009**	0.175	0.164
Protein x Fat			0.342	0.134	0.437	0.315	0.963
Protein x Gender			0.213	*0.091*	0.539	0.392	0.164
Fat x Gender			0.798	0.628	0.634	0.968	0.963
Protein x Fat x Gender			0.480	0.607	0.663	0.406	0.164

To explore the potential of nature to improve the quality of life

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Wageningen Livestock Research creates science based solutions for a sustainable and profitable livestock sector. Together with our clients, we integrate scientific knowledge and practical experience to develop livestock concepts for future generations.

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