The bioavailability of four Zinc Oxide Sources and Zinc sulphate in broiler chickens

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LIVESTOCK RESEARCH
WAGENINGEN UR

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Wageningen UR Livestock Research
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Preface

Zinc is an essential trace element for all farm animal species. It is commonly included in animal diets as zinc oxide, zinc sulphate or organically bound zinc. Umicore Zinc Chemicals developed zinc oxide products with different mean particle sizes. Umicore Zinc Chemicals requested Wageningen UR Livestock Research to determine the bioavailability of four zinc oxide sources and zinc sulphate in broiler chickens. A precise estimate of the bioavailability of zinc sources is required both for fulfilling the zinc requirements of the animal and to reduce zinc excretion in excreta and the environment.

Summary

This study was conducted to assess the bioavailability of four zinc oxide products of Umicore Zinc Chemicals, Angleur, Belgium in broiler diets. The four zinc oxide products were AFOX coarse, AFOX fine, W78 and Pharma. The zinc oxide test products were compared with zinc sulphate (mono hydrate) and with a negative control treatment without added dietary zinc.

The production performance, zinc content in blood serum, zinc content in pancreas and tibia and apparent zinc absorbability were determined.

In total 480 Ross 308 male day-old broiler chickens were used for the study. At arrival, 480 broilers were placed in one group from 0-7 days of age. At 8 days of age, 432 broilers were selected and allocated randomly over 36 cages (12 broilers per cage). The 36 cages were allocated to one of six treatment groups. The groups (6 replicate cages each) were fed one of the experimental diets. The diets were based on wheat, corn and soybean meal. The negative control diet contained 25 mg Zn/kg from the ingredients, where the experimental diets were supplemented with 20 mg/kg zinc from the test products or from zinc sulphate (mono hydrate). Water and pelleted feed were supplied ad libitum. The lighting schedule was during the first 48 hours 16 hours of light and 8 hours of darkness. From 3 to 21 days of age the lighting schedule was 2 hours of light and 1 hour of darkness. From 22 to 28 days of age the room was continuously illuminated to stimulate feed intake prior to dissection. Each cage was provided with one feed bin and two drink cups. Two replicates per treatment were randomly allocated per tier level.

Feed intake was recorded in the period from 7 to 28 days of age and body weight of the broilers was determined at 7 and 28 days of age. At 28 days of age, blood samples were taken from 3 broilers per cage to determine zinc content in blood serum of three broilers per pen. After blood sampling, these three broilers per cage were euthanized by injection of T61 (0.1 mL/kg of BW; Intervet Nederland BV, Boxmeer, The Netherlands) and subsequently pancreas and right tibia were removed and pooled per cage to determine tibia ash, tibia zinc and pancreas zinc content. In the period from 25 to 27 days of age, faecal droppings were collected on a clean plate under the cages and pooled samples per cage were analysed for TiO_2 as marker, dry matter and Zn content. The results were used for calculation of the dry matter digestibility and apparent Zn absorbability per cage.

Zinc content in the tibia and pancreas were not significantly affected by dietary treatment. The zinc content in blood serum of broilers fed the diet supplemented with Pharma was significantly higher than Zn content in serum of broilers fed diets supplemented with AFOX coarse and AFOX fine. The Zn content in serum of broilers fed the test product W78 was in between AFOX and Pharma. The zinc supplement did not significantly affect the daily feed intake and body weight gain.

Overall it can be concluded that the tested zinc oxide supplements in a wheat - corn - soybean meal diet for male broiler chickens can substitute zinc sulphate (mono hydrate). Evaluation of the significant criteria blood serum Zn and Pancreas Zn resulted in the sequence Pharma, W78, AFOX fine and AFOX coarse from highest to lowest absorbability of Zn, respectively.

1 Introduction

Zinc is an essential trace element for all farm animal species. It is commonly included in animal diets as zinc oxide, zinc sulphate or organically bound zinc. Umicore Zinc Chemicals has developed four zinc oxide products with different mean particle sizes: AFOX coarse, AFOX fine, W78 fine and Pharma fine particles. The aim of this study was to determine the bioavailability of these products as compared to zinc sulphate by determination of apparent total tract zinc absorption (digestibility) from the digestive tract and zinc response parameters in blood and body tissues. Broiler chickens were used as target animal. A precise estimate of the bioavailability of zinc sources is required both for fulfilling the zinc requirements of the animal and to reduce zinc excretion in excreta and the environment.

On behalf of Emfema, Jongbloed et al. (2002) described the relevance of different response criteria to determine zinc bioavailability in broilers (Table 1).

Table 1
Relative importance of different response criteria to determine zinc bioavailability in poultry.

	Suboptimal supply	Above requirement
Apparent absorption of zinc	3	1
True absorption of zinc	3	3
Tibia/toe/metatarsal zinc	5	5
Pancreatic zinc	3	3
Performance	3	0
Plasma zinc	4	0

0 = low and 5 = highly responsive.

At a dietary zinc supply above requirements, the surplus of zinc would be excreted in the faeces and to a minor extent in the urine, thus reducing the observed apparent absorption of zinc. The surplus of zinc would not result in a further increase in performance of the birds and in plasma zinc level. Hence, these characteristics need to be determined using diets below zinc requirements. In this study, these criteria were used at a suboptimal supply of dietary zinc. True absorption of zinc was not determined since this would require specific techniques to distinguish between zinc from the diet and endogenous zinc secreted in the digestive tract.

1.1 Ojectives

The aim of the study was to assess the bioavailability of AFOX coarse, AFOX fine, W78 and Pharma compared to a negative control diet and to Zinc Sulphate in broilers. Therefore, the growth performance of the birds, the apparent absorption coefficient of Zn, and the content of Zn in blood serum, pancreas tissue and tibia were determined.

1.2 Sponsor

The study was carried out on request and sponsored by:

Umicore Zinc Chemicals
Rue de Chenee, 53 Bte 2
B4031 Angleur
Belgium
+32 14 24 5016
Contact person: Mr. P. Verbiest

2 Site of the trial

Wageningen UR Livestock Research
Experimental facility 161, room 25
Runderweg 2, 8219 PK Lelystad, The Netherlands

Visitors address:
Edelhertweg 15, 8219 PH Lelystad, Tel: +31 320 238238

3 Time schedule

Date of arrival broilers:	21 November 2013 (start starter period)
Date of start animal experiment:	28 November 2013 (start experimental period)
Date of end animal experiment:	19 December 2013 (end experimental period)

4 Human Resources

Responsible investigator:	T. Veldkamp Wageningen UR Livestock Research P.O. Box 65 8200 AB Lelystad, The Netherlands teun.veldkamp@wur.nl
Responsible associate investigator:	J.T.M. van Diepen Wageningen UR Livestock Research P.O. Box 65 8200 AB Lelystad, The Netherlands hans.vandiepen@wur.nl
Associate investigator:	P. Bikker Wageningen UR Livestock Research P.O. Box 65 8200 AB Lelystad, The Netherlands paul.bikker@wur.nl
Responsible feed manufacturer:	G. Beelen Research Diet Services BV P.O. Box 114 3960 BC Wijk bij Duurstede, The Netherlands
Responsible site supervisor:	A. ter Laak / G.J. Deetman Central Veterinary Institute Runderweg 2 8219 PK Lelystad, The Netherlands

5 Experimental treatments

The trial comprised five dietary treatments based on addition of different zinc sources and a negative control diet (Table 2).

Table 2
Dietary treatments

Treatment	Description	Calculated % Zn by weight[1]	Particle size[2]
NC	Negative control, no added zinc		-
$ZnSO_4$	$ZnSO_4$, 20 mg added Zn	35.5[3]	-
AFOX coarse	AFOX coarse, 20 mg added Zn from ZnO in coarse particles	73	15 microns
AFOX fine	AFOX fine, 20 mg added Zn from ZnO in fine particles	73	7 microns
W78	W78, 20 mg added Zn from ZnO	80	1 – 1.2 micron
Pharma	Pharma, 20 mg added Zn from ZnO in fine particles	80	0.5 micron

[1] Mass fraction Zn
[2] According to information provided by the sponsor.
[3] According to specification provided by the feed manufacturer.

The treatments were investigated with 480 Ross 308 male broiler chickens in battery cages from 7 – 28 days of age. Each treatment was tested with six replicate cages.

6 Experimental procedures

6.1 Test substances

Test products	: AFOX coarse
	: AFOX fine
	: W78
	: Pharma
Manufacturer	: Umicore Zinc Chemicals, Angleur, Belgium
Storage conditions	: dry warehouse
Active substance	: ZnO

6.2 Test system / broilers

Species	: Poultry, broiler chickens
Strain	: Ross 308
Gender	: Male
Source	: Probroed & Sloot, The Netherlands
Number of birds at arrival	: 480
Experimental period	: 7 – 28 days of age

6.3 Housing

A total of 480 male Ross 308 broilers chickens were used in this study from 0-28 days of age in room 25 of the experimental facility 161 of Central Veterinary Institute in Lelystad, The Netherlands. The animals were housed in one group from 0-7 days of age. After one week, a homogenous group of 432 broilers (6 treatments x 6 pens x 12 animals/cage) was allocated to groups of 12 in cages (0.45 m^2 per cage) in a climate controlled poultry house to allow for collection of excreta during day 25-28. Temperature was gradually decreased from approximately 32°C at arrival to 20°C at 18 days of age. Subsequently, this temperature was maintained until the end of the experiment at 28 days of age. During the first 48 hours the light schedule was 16 hours of light and 8 hours of darkness. From 3 to 21 days of age the light schedule was 2 hours of light and 1 hour of darkness. From 22 to 28 days of age the room was continuously illuminated to stimulate feed intake prior to dissection. Each cage was provided with one feed bin and two drink cups. Two replicates per treatment were randomly allocated per tier level.

6.4 Animals

In total 480 Ross 308 male day-old broiler chickens were obtained from a commercial hatchery. At arrival, 480 broilers were placed in one group from 0-7 days of age. At 8 days of age, 432 broilers were selected and allocated randomly over the 36 cages (12 broilers per cage). The broilers were weighed before they were placed into the cages.

6.5 Vaccination

Day-old broilers were vaccinated against IB (Infectious Bronchitis) and NCD (New Castle Disease; spray vaccination) in the hatchery. During the experiment, the broilers were vaccinated against NCD at 20 days of age according to commercial practice. Batch number and identification and expiration dates of the vaccine used are included in the study file at Wageningen UR Livestock Research.

6.6 Diets and feeding

The experimental diets were formulated and produced by Research Diet Services BV, Wijk bij Duurstede, The Netherlands. Prior to feed formulation the main feed ingredients (wheat, corn and soybean meal) were analysed for Zn content. Based on analysed Zn contents of the main feed ingredients, a starter diet was formulated to contain 34 mg/kg Zn. For the starter diet a Zn-free premix was used and 20 mg/kg $ZnSO_4$ was supplemented. The ingredient and nutrient composition of the starter diet are presented in Appendix 1. For the experimental period from 7 days of age onwards, a basal diet (based on wheat, corn, soybean meal, corn gluten meal and potato protein), adequate in mineral and nutrient content, was composed, based on analysed Zn contents of the main feed ingredients. For the experimental diets from 8 to 28 days of age, a Zn-free premix was used and the calculated Zn content of the basal diet was 25 mg/kg. The Zn requirement for broilers recommended by NRC (1994) is 40 mg/kg. The ingredient and nutrient composition are presented in Appendix 2. The basal diet was produced as one batch. This batch was divided in six sub-batches. Subsequently the zinc sources were added to supply an additional amount of 20 mg/kg Zn to the basal diet. Each diet was mixed and pelleted with steam addition (pellet diameter 2.5 mm). Water and feed were supplied ad libitum. The experimental diets were fed to the birds directly after they were placed in the cages.

7 Criteria studied

The following observations were conducted:

Production performance

The broilers were weighed per group at 0 (arrival), 7 (start of dietary treatments) and 28 (end of the experimental period) days of age. The feed consumption was measured per cage from 0 to 7 days of age and from 7 to 28 days of age. The feed conversion ratio was calculated over the respective periods of measurement.

Zinc content in blood serum

At 28 days of age blood samples were taken from 3 broilers per cage. Zinc content in blood serum of three broilers per pen was analysed. For zinc analysis, serum (0.5 mL) was diluted with 4.5 mL diluent (0.1% Triton X-100 in 0.05 % HNO_3) and measured against standards with ICP-OES (Inductively Coupled Plasma Atomic Emission Spectroscopy) (Perkin Elmer 3300DV).

Zinc content in pancreas and tibia

At 28 days of age, three birds per cage were euthanized by injection of T61 (0.1 mL/kg of BW; Intervet Nederland BV, Boxmeer, The Netherlands), and pancreas and right tibia were removed and pooled per cage. Pooled samples of pancreas were analysed for Zn content and pooled samples of tibiae were analysed for ash in fat free dry matter and Zn content. Zinc was determined with ICP AES (Perkin Elmer Optima 7300 DV) in the freshly ground pancreas samples after treatment with a mixture of perchloric acid and sulphuric acid at 300 C. Prior to analyses of tibia, pooled samples of three tibiae per cage were boiled for 10 minutes in water and cleaned of soft tissue. The tibiae were split and bone marrow was removed. Subsequently the pooled samples were grinded and dried. The pooled samples were de-fatted with an organic solvent and ashed at 825 °C. After dissolving the ash residue in HCl, zinc was analysed with ICP-AES. Zinc analyses in the pooled ash samples was conducted in duplo. Ash and Zinc content in tibia were reported on fat free dry matter base.

Dry matter digestibility and zinc absorbability

On day 25, 26 and 27 faecal droppings were collected on a clean plate under the cages and pooled samples per cage were analysed for TiO_2 and Zn content. The results were used for calculation of the dry matter digestibility and apparent Zn absorbability per cage.

Animal health observations

Health of birds was checked daily and aberrations were recorded as well as probable cause of death for lost animals. Temperature and humidity in the animal room were recorded. The data are included in the study file. Remaining birds at the end of the experiment were euthanized and offered for destruction.

8 Statistical analyses

Response parameters were statistically analyzed by ANOVA using GenStat statistical software (16th edition, VSN International Ltd., Hemel Hempstead, UK), using row as block factor and diet as treatment in the statistical model:

$Y_{ij} = \mu + block_i + treatment_j + e_{ijk}$

Where:

Y	=	Response parameter
μ	=	General mean
block	=	Row (two rows of three cage units and each cage unit has three tier levels) (i=1,2)
treatment	=	Effect of Zn test product (j=1,2,3,4,5,6)
error	=	Error term

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.

9 Results

9.1 General

The experiment was conducted according to the protocol without major problems or relevant deviations. Day-old broilers arrived healthy and starter weight was 38 g, which was slightly below the performance goals of Aviagen (breeder organization of brand Ross 308). Overall, mortality during the experiment was 4.6% and was slightly higher than in commercial practice; however no specific cause of mortality was observed. Mortality was not affected by dietary treatments.

9.2 Dietary zinc levels

Analyzed zinc levels in each of the experimental diets are presented in Table 3.

Table 3
Analyzed zinc levels in each of the experimental diets

Diet	Analysed zinc level in the diet (mg/kg)	Expected zinc supplementation (mg/kg)	Realized zinc supplementation[1] (mg/kg)
Negative Control[2]	25.1	0	0
$ZnSO_4$	42.4	20	17.3
AFOX coarse	46.8	20	21.7
AFOX fine	45.9	20	20.8
W78	43.8	20	18.7
Pharma	45.6	20	20.5

1 Calculated as the analyzed zinc content of a given diet minus zinc content of the negative control diet.
2 Calculated zinc level in negative control diet was 25 mg/kg.

The negative control (basal diet) was formulated to contain 25 mg/kg Zn. The analyzed zinc level in the negative control diet was in accordance with the calculated zinc level. From the results of the analysis it can be concluded that the mean analyzed zinc level in the Zn supplemented diets was in good agreement with expected values. The deviations of 2-3 mg/kg are within normal variation due to sampling errors and accuracy of the zinc analysis. Therefore, the calculation of apparent zinc absorption was based on an inclusion of 20 mg/kg for each of the products.

9.3 Production performance

Performance results for body weight at 28 days of age and daily feed intake, daily body weight gain and feed conversion ratio of the broilers measured from 7 to 28 days of age are presented in Table 4. Performance results were not significantly affected by dietary treatment. On average body weight of broilers at 28 days of age was 1541 g. This body weight at 28 days of age was only 0.8% below performance objectives for Ross 308 male broilers (1553 g) (Aviagen, 2012).

Table 4

Performance results for body weight (BW) at 28 days of age, daily feed intake (dFI), daily body weight gain (dBWG) and feed conversion ratio (FCR) from 7 to 28 days of age

	BW 28 d of age		dFI		dBWG		FCR	
Diet	g	rel.	g/d	rel.	g/d	rel.	g/g	rel.
Negative Control	1550	101.3	98.3	100.9	68.2	101.9	1.44	99.0
$ZnSO_4$	1530	100.0	97.4	100.0	66.9	100.0	1.46	100.0
AFOX coarse	1562	102.1	99.0	101.6	68.7	102.7	1.44	99.0
AFOX fine	1527	99.8	97.7	100.3	67.0	100.1	1.46	100.0
W78	1566	102.4	98.7	101.3	68.9	103.0	1.43	98.4
Pharma	1511	98.8	95.5	98.0	66.2	99.0	1.44	99.0
Source of variation								
P	0.481		0.448		0.413		0.437	
Lsd	65.8		3.7		3.1		0.03	

P = probability level.

Lsd = least significant difference (P<0.05).

The Lsd-value of 0.03 for feed conversion ratio (FCR) illustrates that variation in growth performance results was comparable with variation in growth performance results in experiments previously conducted in this experimental house.

9.4 Zinc content in blood serum

The zinc content was measured in serum of blood samples collected at 28 days of age. The results are presented in Table 5. The Zn content in serum of broilers fed the negative control was significantly lower than in broilers fed Zn supplemented diets. Zn content in serum of broilers fed diets supplemented with Pharma was significantly higher than Zn content in serum of broilers fed diets supplemented with AFOX coarse and AFOX fine. The Zn content in serum of broilers fed the test product W78 was in between AFOX and Pharma.

Table 5

Analysed Zinc content in blood serum at 28 days of age

	Blood serum Zn	
Diet	µg/ml	rel.
Negative Control	0.79 [d]	47.6
$ZnSO_4$	1.66 [ab]	100.0
AFOX coarse	1.49 [bc]	89.8
AFOX fine	1.46 [c]	88.0
W78	1.65 [ab]	99.4
Pharma	1.68 [a]	101.2
Source of variation		
P	< 0.001	
Lsd	0.18	

P = probability level.

Lsd = least significant difference (P<0.05).

a,b,c,d values in a column without a common superscript differ significantly (P<0.05).

9.5 Zinc content in pancreas and tibia

Zinc content in pancreas and zinc and ash content in tibia are presented in Table 6. Zinc content in pancreas tissue of broilers fed the negative control diet was significantly lower than in pancreas tissue of broilers fed the Zn supplemented diets. Within the Zn supplemented diets the type of test product did not significantly affect the zinc content in pancreas tissue of broilers. Tibia ash content in broilers fed the negative control diet was as high as the tibia ash content in broilers fed the Zn supplemented diets. This implies that the Zn content in the negative control diet was already sufficient for a maximum tibia ash response. Despite large differences between treatments, tibia Zn content was not significantly affected by dietary treatment due to the high variation between replicates.

Table 6

Analysed Zinc content in pancreas and tibia at 28 days of age

	Pancreas Zn		Tibia Zn		Tibia ash	
Diet	mg/kg dm	rel.	mg/kg ash	rel.	g/kg fat-free dm	rel.
Negative Control	66.2 [b]	66.8	80.5	60.1	550.9	100.3
$ZnSO_4$	99.1 [a]	100.0	133.9	100.0	549.4	100.0
AFOX coarse	93.7 [a]	94.6	89.0	66.5	547.5	99.7
AFOX fine	99.4 [a]	100.3	143.1	106.9	547.8	99.7
W78	91.0 [a]	91.8	135.3	101.0	546.3	99.4
Pharma	107.6 [a]	108.6	149.0	111.3	545.4	99.3
Source of variation						
P	< 0.001		0.306		0.737	
Lsd	17.1		75.5		7.9	

P = probability level.

Lsd = least significant difference (P<0.05).

a,b values in a column without a common superscript differ significantly (P<0.05).

9.6 Dry matter digestibility and apparent zinc absorbability

Dry matter digestibility of the complete diets and apparent zinc absorbability of the test products are presented in Table 7. The contribution of each of the zinc supplements to the absorbed zinc supply was calculated after correction for the basal diet. The apparent zinc absorption of the zinc supplements varied from 3.6 to 13.8%. Dry matter digestibility as well as apparent zinc absorption of the zinc supplements were not significantly affected by dietary treatment.

Table 7

Dry matter digestibility in complete diets and apparent zinc absorbability of test products

	Dry matter digestibility complete diet		Apparent zinc absorbability test product	
Diet	%	rel.	%	rel.
$ZnSO_4$	75.8	100.0	13.8	100.0
AFOX coarse	74.4	98.2	3.6	26.1
AFOX fine	75.3	99.3	10.0	72.5
W78	75.8	100.0	9.2	66.7
Pharma	76	100.3	12.5	90.6
Source of variation				
P	0.446		0.552	
Lsd	1.1		13.1	

P = probability level.

Lsd = least significant difference (P<0.05).

10 Discussion and Conclusions

The method of evaluation (Jongbloed et al., 2002) as has been used by the EMFEMA-project group was used to rank the bio-availability of the Zn sources. A ranking order is used for the different criteria as presented in Table 8.

Table 8

Relative importance of different response criteria to determine zinc bioavailability in poultry

	Suboptimal supply
Apparent absorption of zinc	3*
True absorption of zinc	3
Tibia/toe/metatarsal zinc	5
Pancreatic zinc	3
Performance	3
Plasma zinc	4

*0 = low and 5 = highly responsive.

The relative values for the different response criteria are presented in Table 9.

Table 9

Relative values for the different response criteria

	Perfor-mance	Blood serum Zn	Tibia Zn	Pancreas Zn	Absorb Zn	Evaluation Overall	Evaluation Blood serum Zn + Pancreas Zn
Diet							
$ZnSO_4$	100.0	100.0	100.0	100.0	100.0	100.0	100.0
AFOX coarse	102.2	89.8	66.5	94.6	26.1	75.6	91.9
AFOX fine	100.2	88.0	106.9	100.3	72.5	94.8	93.3
W78	102.2	99.4	101.0	91.8	66.7	93.6	96.1
Pharma	98.5	101.2	111.3	108.6	90.6	103.0	104.4

Ranking of the availability of the Zn supplements was not consistent for all response criteria but depended on the response criterion used. Moreover, this compilation of results should be interpreted carefully since the differences in performance, tibia and pancreatic zinc content and zinc absorbability between supplements were not significant. The overall results suggest that the absorbability of zinc oxide sources as AFOX fine, W78 and Pharma was close to $ZnSO_4$, whereas the absorbability of AFOX coarse may have been somewhat lower. This was largely caused by lower values for tibia zinc content and zinc absorbability. Serum zinc levels suggest somewhat lower values for both AFOX coarse and fine. Evaluation of only the significant criteria blood serum Zn and Pancreas Zn resulted in the sequence Pharma, W78, AFOX fine and AFOX coarse from highest to lowest absorbability of Zn, respectively.

Literature

Aviagen. 2012. ROSS 308 Performance objectives 2012.

Jongbloed, A.W., P.A. Kemme, G. De Groote, M. Lippens, F. Meschy. 2002. Bioavailability of major and trace minerals. EMFEMA report. International Association of the European (EU) Manufacturers of Major, Trace and Specific Feed Mineral Materials. Brussels. Belgium.

NRC. 1994. Nutrient Requirements of Poultry. 9th ed. Natl. Acad. Press, Washington, DC.

Appendices

Appendix 1 Composition of the starter diet (0 – 7 days of age) without a Zn source

DIET CALCULATION RDS

Project	:	WUR Umicore		CVB2003
Diet	:	Starter diet v3	Date 11-11-2013 15:07	Ing. G.M. Beelen

*** INGREDIENTS (in g/kg) ***

3	Wheat A5556	219,75				104 TiO2	0,00
1	Corn A5558	400,00	57	Soybean oil	33,00	120 L-lysine HCl	1,75
30	Soybeanmeal A5521	265,00				121 DL-methionine	1,70
15	Maisglutenmeel	20,00				122 L-threonine	0,10
26	Aardappeleiw. RAS<10	20,00				129 L-valine	0,00
			78	Premix (mais)	5,00		
			100	Lime fine	14,50		
			101	Monocalciumphosphate	14,50		
			103	Salt	2,00		
			105	NaHCO3	2,70		

*** CALCULATED CONTENTS (in g/kg) ***

Dry matter	871,7	Calcium	8,9		MJ/kg	kcal/kg
Ash	59,2	Phosphorus, total	6,8	AMEn German	12,77	3053
Crude protein	211,3	Phosphorus, available	4,6	ME broiler Dutch	11,84	2830
Crude fat	67,3	Phosphorus, dig pigs	3,7			
Crude fibre	27,8	Phosphorus, dig poultry	4,0	Ca:P	1,31	
Carbohydrates	503,8	Calcium/dP poultry	2,2	Ca:avP	1,94	
		Magnesium	1,5			
Starch	385,9	Potassium	7,8			
Sugars	35,5	Sodium	1,6	Zink calculated	34 mg/kg	
NDF	88,1	Chloride	2,0			
ADF	30,1	Base-excess (meq/kg)	213,2			

Aminozuren	Totaal	% v lys	Dig AA p	% v lys	Dutch recomm grower
Isoleucine	8,99	75	7,86	75	6,89
Leucine	18,74	156	16,62	159	
Lysine	12,05	100	10,47	100	10,45
Methionine	5,14	43	4,77	46	3,97
Cystine	3,57	30	2,87	27	
Meth.+Cyst.	8,71	72	7,64	73	7,63
Fenylalanine	10,74	89	9,56	91	
Tyrosine	7,98	66	7,03	67	
Fenyl.+Tyr.	18,72	155	16,58	158	
Threonine	8,11	67	6,80	65	6,79
Tryptofaan	2,40	20	2,10	20	1,67
Valine	10,16	84	8,70	83	8,36
Arginine	12,99	108	11,56	110	10,97
Histidine	5,48	45	4,78	46	
Alanine	10,67	89	8,97	86	
Asparaginezuur	20,52	170	17,88	171	
Glutaminezuur	39,53	328	35,83	342	
Glycine	8,66	72	6,92	66	
Proline	13,53	112	11,89	114	
Serine	10,59	88	9,32	89	

Appendix 2 Composition of the basal experimental diet (7 – 28 days of age) without a Zn source

DIET CALCULATION RDS

Project	:	WUR Umicore			CVB2003
Diet	:	Grower basal diet v4	Date	12-11-2013 10:21	Ing. G.M. Beelen

INGREDIENTS (in g/kg)

3	Wheat A5556	345,60				104	TiO2	2,50
1	Corn A5558	150,00	57	Soybean oil	40,00	120	L-lysine HCl	2,75
10	Soybeanmeal A5521	190,00				121	DL-methionine	1,65
15	Maisglutenmeel	50,00				122	L-threonine	0,30
26	Aardappeleiw. RAS<10	30,00				130	L-arginine	0,70
53	Maiszetmeel	150,00	78	Premix (mais)	5,00	111	KH2PO4	10,00
			117	CaCO3 zuiver	15,60			
			101	MCP 300 ppm Zn	1,50			
			103	Salt	2,20			
			105	NaHCO3	2,20			

CALCULATED CONTENTS (in g/kg)

Dry matter	873,1	Calcium	7,5		MJ/kg	kcal/kg
Ash	49,8	Phosphorus, total	5,6	**AMEn German**	**13,48**	**3221**
Crude protein	199,7	Phosphorus, available	3,7	ME broiler Dutch	12,49	2986
Crude fat	68,1	Phosphorus, dig pigs	3,0			
Crude fibre	23,2	Phosphorus, dig poultry	3,3	Ca:P	1,34	
Carbohydrates	527,2	Calcium/dP poultry	2,3	Ca:avP	2,01	
		Magnesium	1,0			
Starch	300,2	Potassium	8,9			
Sugars	28,9	Sodium	1,5	Zink calculated	25 mg/kg	
NDF	70,7	Chloride	2,2			
ADF	24,3	Base-excess (meq/kg)	230,9			

Aminozuren	Totaal	% v lys	Dig AA p	% v lys	Dutch recomm grower
Isoleucine	8,37	73	7,33	72	6,67
Leucine	18,35	161	16,28	161	
Lysine	11,41	100	10,12	100	10,11
Methionine	5,02	44	4,69	46	3,84
Cystine	3,36	29	2,73	27	
Meth.+Cyst.	8,38	73	7,42	73	7,38
Fenylalanine	10,27	90	9,17	91	
Tyrosine	7,72	68	6,85	68	
Fenyl.+Tyr.	18,00	158	16,02	158	
Threonine	7,69	67	6,57	65	6,57 **
Tryptofaan	2,18	19	1,90	19	1,62
Valine	9,49	83	8,17	81	8,09
Arginine	11,81	104	10,61	105	10,62 **
Histidine	4,81	42	4,18	41	
Alanine	10,13	89	8,55	84	
Asparaginezuur	18,02	158	15,74	155	
Glutaminezuur	38,32	336	34,85	344	
Glycine	7,81	68	6,22	61	
Proline	13,40	117	11,85	117	
Serine	9,83	86	8,79	87	

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

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Together with our clients, we integrate scientific know-how and practical experience to develop livestock concepts for the 21st century. With our expertise on innovative livestock systems, nutrition, welfare, genetics and environmental impact of livestock farming and our state-of-the art research facilities, such as Dairy Campus and Swine Innovation Centre Sterksel, we support our customers to find solutions for current and future challenges.

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