POULTRY CRC LTD

FINAL REPORT

Sub-Project No: Poultry CRC 2.1.13

SUB-PROJECT LEADER: Isabelle Ruhnke

Sub-Project Title: The Effect of Enzymes on Grass Impaction in Free-Range Layers

DATE OF COMPLETION: 31 November 2016

ISBN 1 921010 74 6

The Effect of Enzymes on Grass Impaction in Free-Range Layers
Sub-Project No. 2.1.13

Researcher Contact Details
Name: Isabelle Ruhnke
Address: The School of Environment and Rural Science
University of New England, Armidale, NSW, Australia
Phone: 02-6773-5155
Email: iruhnke@une.edu.au

In submitting this report, the researcher has agreed to the Poultry CRC publishing this material in its edited form.

Poultry CRC Ltd Contact Details
PO Box U242
University of New England
ARMIDALE NSW 2351

Phone: 02 6773 3767
Fax: 02 6773 3050
Email: admin@poultrycrc.com.au
Website: http://www.poultrycrc.com.au

Published in 2017

Executive Summary

Range usage can frequently be associated with intestinal grass impaction (Ruhnke et al., 2015a). Fresh pasture cover and the availability of foraging material on the range are essential criteria in most accreditation guidelines (FREPA, 2015; ACO, 2013; CSIRO, 2002). However, unrestricted access to pasture can result in excessive fibre intake, reducing the intake of a balanced feed, leading to a lack of energy and nutrients such as essential amino acids. Subsequently, uncontrolled pasture intake by the hen is of major concern to the Australian free-range industry, and especially in birds with a relatively long life span, such as laying hens, pasture consumption can have a significant impact on the laying hen's health and productivity.

In order to investigate the impact of pasture and feed additives on laying hen performance and nutrient digestibility, 300 laying hens were used in this study. A general linear model was arranged to evaluate 2 time points (short term vs long term exposure to the range), 2 range types (pasture vs gravel), and 3 diets (control, multi-enzymes and organic acid).

Analysis of the performance data indicated that feed additives significantly increased the body weight of the hens. Hens that ranged on pasture were significantly heavier and produced significantly heavier eggs. Analysis of the egg quality indicated that range type significantly affected egg yolk colour (hens on pasture had darker yolk). Feed type increased liver weight significantly, with 43.1 g, 45.0 g, and 46.6 g in hens of the standard, organic acid, and multi-enzyme group respectively (P = 0.008). Range type also had a significant impact on gizzard, pancreas, and liver weight (P = 0.000; P = 0.038; and P = 0.022 respectively), and resulted in heavier organs generally, thus indicating that pasture improved gut function. Despite the enhanced development of gastrointestinal organs, the estimated ileal nutrient digestibility of crude fibre (CF), crude protein (CP), calcium and phosphorus was reduced in hens exposed to pasture. The use of feed additives improved the estimated ileal digestibility of phosphorus significant, and the estimated ileal digestibility of CF close to significant.

Table of Contents

1. Introduction

Free range farming is a rapidly growing sector of the Australian layer industry, with an estimated market share value of 49% (AECL, 2015). One of the main reason for changes in consumer's consumption is the apparent welfare benefit, and environmental impact (Blokhuis et al., 2003; Hermansen, 2003). Commonly free range laying hens have a 'meaningful and regular access on an open range on most ordinary days (FCA, 2014), where they are able to express a broad variety of behaviours, such as running, flying, and dust bathing (Lunam et al., 1996). Conversely, free range farming can be challenging in many ways. Exposing chickens which were genetically selected for closed-house production to the outdoor environment can led to a number of health, performance and welfare problems (Ruhnke at al., 2015b). For example, range usage can result in a reduced hen performance and higher mortality compared to hens housed in cages (Glatz et al., 2005). While the intensity of range usage depends on several factors such as flock size, shed design, shade coverage, and weather conditions, the use of mobile sheds and the availability of feed on the range results in the vast majority of the flock ranging during daytime. In a recent survey, 53% of all farms contacted used mobile sheds and 50% of farmers feed their layers on the range (Ruhnke et al., 2015a). Furthermore, 82% of farmers reported that >75% of the flock used the range, and hens accessed the range for >6 hours/day. Eighty-two percent of layer farmers report that their range was never stripped of vegetation. It has been reported that grass impaction can cause flock mortality up to 17 %, severe loss of body condition, and reduced hen house production (Ruhnke et al., 2015b). Consequently, the intake of pasture by the hen is of major concern to the Australian free-range industry, and especially in birds with a relatively long life span, such as laying hens, because pasture consumption can have a significant impact on the laying hen's health and productivity.

1.1 Objectives

The aim of this research was to evaluate feed enzymes and organic acids for their beneficial use on health and nutrient digestibility in free range laying hens.

In order to minimise the damage of excessive intake of viscous structural materials obtained from long grass, enzyme supplementation such as xylanases, pentosanases, hemicellulases and β-glucanases are hypothesised to be of benefit to the hen's digestive system. Modes of action may include (1) reduced viscosity of the digesta, (2) physical breakdown of fibrous feed compounds and (3) increased digestibility of the nutrients obtained with the feed.

Specific enzymes in the diet may alleviate the viscous nature of the forage material so passage time of compacted material through the digestive system is promoted. In addition, proteases may act synergetic by increasing the digestibility of proteins in the digestive tract. With a well-developed gastrointestinal system, and improved nutrient digestibility, hen health and welfare may be improved.

2. Materials and Methods

2.1 Animal Experiment

The animal experiment was reviewed and approved by the Animal Ethics Committee of the University of New England (AEC15-009). A total of 300 Lohmann Brown egg laying hens were obtained from a commercial rearing facility at the age of 16 weeks and placed in 30 pens with 10 hens/pen. After a 2-week adaption period, at the age of 18 weeks, hens were given access to the range. The total of 6 treatments were arranged 2 x 2 x 3 factorial. The range was either structured as gravel (control group) or tall fescue (*Fescue arundinacea*) (Figure 1).

Figure 1: Range design of the research experiment. The 2 x 3 factorial arrangement allowed certain hens to range on gravel, while others had access to tall fescue (*Fescue arundinacea*).

Hens were offered either a typical Australian commercial layer feed (C), a typical Australian commercial layer feed supplemented with multi enzymes (ENZ), or a typical Australian commercial layer feed supplemented with organic acids (OA). Details about the diets formulated following breeder recommendation can be obtained from Table 1. The nutrient composition of the diets is outlined in Table 2. While the basal diet (C) was supplemented with commonly used phytase/xylanse (Ronozyme WX CT 100 mg/kg and Ronozyme Hi Phos 600 FYT 60 mg/kg, the multi enzyme diet (ENZ) included phytase/xylanse/betaglucanase/xylo-glucanase/pectinase/ protease (Ronozyme Hi Phos 600 FYT 60 mg/kg, Ronozyme Multigrain 100 mg/kg, Ronozyme Pro Act 200 mg/kg and Ronozyme VP 200 mg/kg, and the diet with organic acids (OA) contained phytase/xylanse/benzoic acid/essential oils (Ronozyme WX CT 100 mg/kg, Ronozyme Hi Phos 600 FYT 60 mg/kg, and Crina Plus 300 mg/kg. All enzymes and the organic acid were provided by DSM Nutritional Products Australia Pty Ltd Wagga Wagga NSW 2650, Australia. A total of 5 replicates per treatment were investigated.

Table 1: Feed ingredients of the experimental diets

Ingredient (%)	C	ENZ	OA
Wheat	66.19	66.01	66.05
Soybean meal	14.11	14.12	14.12
Limestone	6.04	6.04	6.04
Canola Meal	3.92	3.94	3.93
Meat meal	3.76	3.76	3.76
Limestone fine grit	3.00	3.00	3.00
Canola Oil	2.21	2.25	2.23
Sodium Bicarbonate	0.20	0.20	0.20
Salt	0.17	0.17	0.17
DL-Methionine	0.16	0.16	0.16
Lysine-HCl	0.11	0.11	0.11
Free range layer premix	0.10	0.10	0.10
Choline CHL 60%	0.05	0.05	0.05
L-Threonine	0.03	0.30	0.30
Ronozyme WX CT	0.01	0.00	0.01
Ronozyme Hi Phos 600 FYT L	0.01	0.01	0.01
Crina Plus	0.00	0.00	0.03
Ronozyme Multigrain	0.00	0.01	0.00
Ronozyme ProAct	0.00	0.02	0.00
Rononzyme VP	0.00	0.02	0.00
Total	**100**	**100**	**100**

Table 2: Nutrient composition of the experimental diets

Nutrient content (%)	C	ENZ	OA
Calculated ME kcal/kg	2780.00	2780.00	2780.00
Crude protein	17.37	17.37	17.37
Lysine	0.87	0.87	0.87
Methionine	0.42	0.42	0.42
Met + Cys	0.74	0.74	0.74
Threonine	0.63	0.63	0.63
Isoleucine	0.67	0.67	0.67
Leucine	1.23	1.23	1.23
Tryptophan	0.22	0.22	0.22
Arginine	1.06	1.06	1.06
Crude fat	4.27	4.30	4.29
Crude fibre	2.90	2.90	2.90
Calcium	4.00	4.00	4.00
Available phosphorus	0.44	0.44	0.44

In order to evaluate the acute and chronic effect of pasture intake, hens were sacrificed at two time points: At the ages of 24 weeks (6 weeks after range exposure) and of 30 weeks (12 weeks after range exposure). At the beginning of the trial, the pasture was 30-50 cm of length (Figure 2 A,B). The vast majority of pasture was depleted after 6 weeks ranging activity (Figure 2C). After 12 weeks of ranging, the range was completely denuded (Figure 2D). The stocking density for the first 6 weeks of range exposure was 1.6 hens/m^2, the stocking density for the following 6 weeks of range exposure was 0.8 hens/m^2.

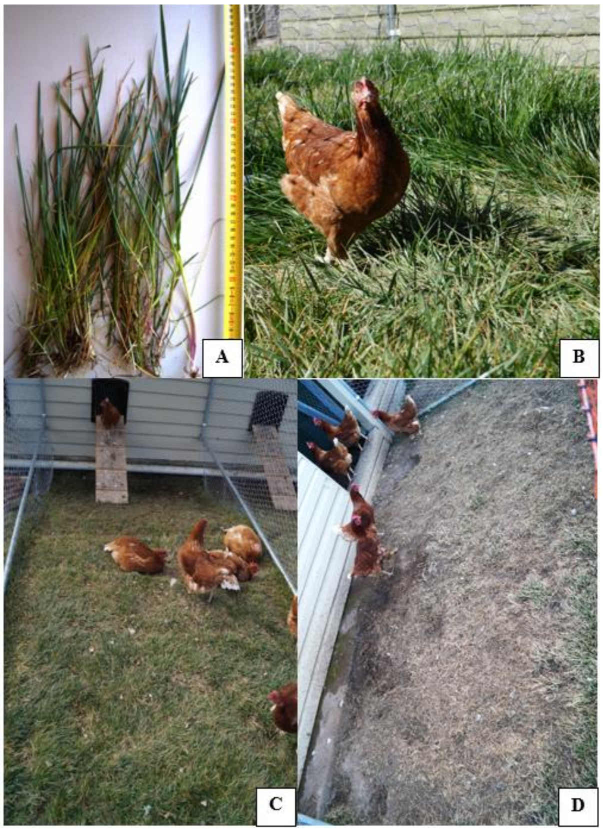

Figure 2: Pasture depletion throughout the research trial. While the initial fescue was 30-50 cm of length (A,B), the majority of fescue was ingested within 6 weeks (C). After 12 weeks of ranging, the entire range was denuded (D).

2.2 Data Collection

Performance parameters: The health status of the hens was monitored daily. The body weight of the individual hens was recorded at the beginning of the experiment, weekly, and on the day of slaughter. Feed intake was recorded weekly and on the day of slaughter. The eggs from each group were collected and their number as well as the individual egg weight was determined daily. The feed conversion ratio (FCR) was calculated as follows:

$$\mathrm{FCR} = \frac{\text{feed intake per hen per week (g)}}{\text{egg mass per hen per week (g)}}$$

Organ weight and pH: At the ages of 24 weeks (6 weeks after range exposure) and of 30 weeks (12 weeks after range exposure), five birds from each pen were randomly selected. The individual body weight of these birds was recorded, and the birds were sacrificed by stunning and cervical dislocation. Organ weight (gizzard, liver and pancreas) and digesta pH (crop and ileum) were recorded (Figure 3). The coporodeum content was pooled from 5 birds per pen in order to determine the health status of the hens indicated by evidence of parasitic eggs (coccidiosis, ascaridiosis).

Figure 3: A well-developed gizzard filled with fibrous pasture obtained from a hen that ranged on tall fescue.

Apparent ileal nutrient digestibility: The ileum content from 5 hens per pen were used for collecting digesta in order to analyze the apparent ileal nutrient digestibility. The intestinal content of the distal two-thirds of the ileum (excluding the content localized in the distal 3 cm prior to the ileocaecal junction) was sampled according to Kluth et al., (2005) and Revazvani et al., (2007). The digesta samples were pooled and freeze-dried for further analysis. One pooled sample of each replicate was used for statistical analysis, resulting in five samples for each of the 6 treatment groups. Weende analysis was performed to determine dry matter (DM), CP, CF, calcium and phosphorus (Naumann and Bassler, 2004). Total non-starch polysaccharides (NSP), soluble NSP, insoluble NSP and free sugars (mono- and disaccharides) were determined as previously described (Englyst and Hudson 1987; Theander and Westerlund 1993). Titanium dioxide (0.2%) had been implemented in the feed as an indigestible marker and was determined as previously described (Short et al., 1996). Excreta samples were collected on 3 consecutive days on time point 1, and on time point 2 respectively. Alkane analysis was used to evaluate the feed dilution due to pasture consumption. Alkane-based calculation of grass intake was performed based on Hameleers et al. (1996) with brief modification. All samples were analysed in duplicates.

Figure 4: Crop content of hens that were exposed to a pastured range (A, B, C), or a gravel range (D, E). The evidence of different digesta composition is macroscopically visible.

2.3 Statistical analysis

Performance data were analysed using a general linear model with 2 time points (short term vs long term exposure to the range) x 2 range types (pasture vs gravel) x 3 diets (control, multi-enzymes and organic acid). Means were compared using Tukey's test. For all statistical analyses, IBM SPSS Statistics Version 21 was used (IBM, Chicago, IL, USA). Statistically significant differences were set at $P < 0.05$. The pen was defined as the statistical unit.

3. Results

Performance parameters: Analysis of the performance data indicated that feed additives significantly increased the body weight of the hens. Hens fed with multi enzymes weighed on average 2.046 kg, while hens fed with the standard diet weight 2.005 kg. Hens on pasture were significantly heavier and produced significantly heavier eggs. No mortalities occurred. Age of the hens had a significant impact on egg weight, egg mass, feed intake and body weight of the hens (Table 3).

Analysis of the egg quality indicated that range type significantly affected egg yolk colour (hens on pasture had darker egg yolk). Age of the hens affected significantly albumen height, Haugh Unit, shell strength, bending, shell weight, and shell thickness (Table 4).

Organ weight and pH: Feed type increased liver weight significantly, with 43.1 g, 45.0 g, and 46.6 g in hens of the standard, organic acid, and multi-enzyme group respectively (P = 0.008). Range type also had a significant impact on gizzard, pancreas, and liver weight (P = 0.000; P = 0.038; and P = 0.022 respectively), and resulted in heavier organs generally, thus indicating that pasture improved gut function. The age of the hens had a significant impact on organ weight and pH of the digesta (Table 5); furthermore, the viscosity of the digesta was significantly affected by age (Table 6). *Coccidia* or *Ascardia* oocysts could not be detected at any time point.

Apparent ileal nutrient digestibility:

Negative CF values were obtained from hens that had access to pasture (Table 7). The ileal content of hens fed the control diet indicated an estimated -30.8 % CF digestibility, while hens of the ENZ and OA group had less CF in their ileum (-0.36 % and 0.14 %, respectively). These values indicate that a significant amount of fibre was retained in the hen's gastrointestinal tract and subsequently not taken into consideration when estimating the pasture intake based on pasture excretion. However, the estimated ileal nutrient digestibility of CF, CP, calcium and phosphorus was significantly reduced in hens exposed to pasture (P = 0.00, 0.02, 0.05, and 0.01, respectively). The use of feed additives improved the estimated ileal digestibility of phosphorus significantly (P = 0.03) and the estimated ileal digestibility of CF close to significant (P = 0.06).

Table 3: Effect of range type and feed additives on performance of egg laying free range hens from 18-29 weeks[1]

	Time point [2]		Range type[3]		Feed additives[4]			SEM[5]	P value					
	A	**C**	**G**	**P**	**C**	**ENZ**	**OA**		**T**	**R**	**F**	**TxR**	**TxF**	**F**
[6] (%)	88.5	91.7	91.4	89.0	90.7	88.4	91.0	1.10	0.181	0.370	0.595	0.968	0.902	0
	59.5^{b}	62.8^{a}	60.4^{b}	61.9^{a}	60.3	61.8	61.1	0.368	0.000	0.007	0.072	0.151	0.779	0
)	1970^{b}	2070^{a}	1996^{b}	2043^{a}	2005^{a}	2046^{b}	2008^{ab}	0.010	0.000	0.002	0.035	0.970	0.728	0
	369	402	385	386	383	383	389	5.326	0.002	0.927	0.861	0.631	0.874	0
	123^{a}	120^{b}	121	122	119	121	123	0.000	0.024	0.753	0.191	0.547	0.635	0
	2.36^{b}	2.10^{a}	2.22	2.24	2.21	2.25	2.23	0.038	0.002	0.924	0.929	0.691	0.825	0

[a b] Means in each row for each factor with different superscripts differ significantly (P<0.05)
[1] Means of 5 replicates with 10 hens per group (n = 60)/5 hens per group (n = 30)
[2] A = Acute ranging effect (hens ranged for 6 weeks): C = Chronic ranging effect (hens ranged for 12 weeks)
[3] G = Gravel range, P = Pasture range
[4] C = Control diet (standard wheat soy Australian diet supplemented with phytase/xylanse)
ENZ = Multi-enzyme diet (standard wheat soy Australian diet supplemented with phytase/xylanse/betaglucanase/xylo-glucanase/pectinase/protease)
OA = Organic acid (standard wheat soy Australian diet supplemented with phytase/xylanse/benzoic acid/essential oils)
[5]SEM = Standard error of mean
[6] Mean values were not normally distributed and non- parametric test was used to analyse data. For interaction mean values for each factor were assumed to be normally distributed.

Table 4: Effect of range type and feed additives and age on external and internal quality of egg in egg laying free range hens [1]

	Time point [2]		Range type[3]		Feed additives[4]			SEM[5]	P value					
	A	**B**	**G**	**P**	**C**	**ENZ**	**OA**		**T**	**R**	**F**	**TxR**	**TXF**	**R**
	20.5	21.2	20.6	21.1	20.7	20.7	21.1	0.250	0.198	0.256	0.726	0.177	0.698	0.
t	7.98[a]	6.82[b]	7.49	7.33	7.56	7.46	7.20	0.126	0.000	0.497	0.243	0.052	0.021	0.
	89.0	80.6	85.6	84.2	85.6	85.2	83.7	0.884	0.000	0.378	0.412	0.077	0.197	0.
(N)	50.0	44.3	46.4	47.8	47.6	47.9	45.9	0.704	0.000	0.257	0.333	0.422	0.796	0.
	0.337	0.298	0.315	0.318	0.323	0.320	0.307	0.005	0.000	0.671	0.366	0.878	0.420	0.
	5.6	5.7	4.3[a]	7.0[b]	5.8	5.7	5.6	0.193	0.416	0.000	0.620	0.132	0.947	0.
g)	5.78	6.08	5.93	5.94	5.93	6.01	5.85	0.036	0.000	0.862	0.119	0.747	0.412	0.
[6]	0.436	0.424	0.428	0.432	0.434	0.432	0.424	0.001	0.000	0.862	0.119	0.747	0.412	0.

[a b] Means in each row for each factor with different superscripts differ significantly (P<0.05)
[1] Means of 5 replicates with 5 eggs per group (n = 60)
[2] A = Acute ranging effect (hens ranged for 6 weeks): C = Chronic ranging effect (hens ranges for 12 weeks)
[3] G = Gravel range, P = Pasture range
[4] C = Control diet (standard wheat soy Australian diet supplemented with phytase/xylanse)
ENZ = Multi-enzyme diet (standard wheat soy Australian diet supplemented with phytase/xylanse/betaglucanase/xylo-glucanase/pectinase/protease)
OA = Organic acid (standard wheat soy Australian diet supplemented with phytase/xylanse/benzoic acid/essential oils)
[5]SEM = Standard error of mean
[6] Mean values were not normally distributed and non- parametric test was used to analyse data. For interaction mean values for each factor were assumed to be normally distributed.

Table 5: Effect of range type and feed additives on organs weight and pH of crop and ileum digesta of free range hens[1]

	Time point [2]		**Range[3]**		**Feed additives[4]**			**SEM[5]**	**P value**					
	A	**C**	**G**	**P**	**C**	**ENZ**	**OA**		**T**	**R**	**F**	**TxR**	**TxF**	**R**
t (g)	32.3	30.4	28.4	34.3	31.5	31.9	30.7	0.356	0.003	0.000	0.323	0.049	0.983	0.
g)	47.4	42.2	44.0	45.7	43.1	46.4	45.0	0.456	0.000	0.038	0.008	0.421	0.159	0.
nt (g)	4.04	3.54	3.71	3.88	3.73	3.81	3.80	0.039	0.000	0.022	0.432	0.422	0.296	0.
	5.00	5.33	5.16	5.16	5.15	5.25	5.09	0.031	0.000	0.962	0.112	0.108	0.011	0.
	7.35	6.90	7.17	7.09	7.13	7.18	7.08	0.030	0.000	0.178	0.316	0.444	0.217	0.

[a b] Means in each row for each factor with different superscripts differ significantly ($P<0.05$)
[1] Means of 5 replicates with 5 birds per group (n = 60)
[2] A = Acute effects (18-24 weeks), C= Chronic effect (24-29 weeks)
[3] G = Gravel range, P = Pasture range
[4] C = Control diet (standard wheat soy Australian diet supplemented with phytase/xylanse)
ENZ = Multi-enzyme diet (standard wheat soy Australian diet supplemented with phytase/xylanse/betaglucanase/xylo-glucanase/pectinase/protease)
OA = Organic acid (standard wheat soy Australian diet supplemented with phytase/xylanse/benzoic acid/essential oils)
[5]SEM = Standard error of mean

Table 6: Effect of range type and feed additives on digesta viscosity of egg laying free range

	Time point [2]		Range type[3]		Feed additives[4]			SEM[5]	P value						
	A	C	G	P	C	ENZ	OA		T	R	F	TxR	TxF	RxF	TxRxF
Crude fibre (%)	12.3[a]	-31.83[b]	16.1[a]	-32.0[b]	-30.8	-0.36	0.14	7.08	0.00	0.00	0.06	0.08	0.17	0.32	0.81
Crude protein (%)	74.9[a]	71.6[b]	75.3[a]	71.3[b]	70.1	74.7	74.7	0.79	0.02	0.02	0.13	0.86	0.56	0.59	0.77
Calcium (%)	46.0	44.9	51.2[a]	40.1[b]	40.7	42.8	52.5	2.77	0.93	0.05	0.24	0.42	0.68	0.79	0.25
Phosphorus (%)	46.4[a]	30.3[b]	33.4[b]	44.3[a]	32.3[a]	41.5[b]	42.2[b]	2.38	0.00	0.01	0.03	0.09	0.49	0.05	0.59

hens[1]

	Time point [2]		Range type[3]		Feed additives[4]			SEM[5]	P value					
	A	C	G	P	C	ENZ	OA		T	R	F	TxR	TxF	RxF
	3.36[b]	4.29[a]	3.72	3.78	3.77	3.63	3.83	1.10	0.005	0.476	0.392	0.476	0.204	0.061

[a b] Means in each row for each factor with different superscripts differ significantly (P<0.05)
[1] Means of 5 replicates with 5 birds per group (n = 60)
[2] A = Acute ranging effect (hens ranged for 6 weeks): C = Chronic ranging effect (hens ranges for 12 weeks)
[3] G = Gravel range, P = Pasture range
[4] C = Control diet (standard wheat soy Australian diet supplemented with phytase/xylanse)
ENZ = Multi-enzyme diet (standard wheat soy Australian diet supplemented with phytase/xylanse/betaglucanase/xylo-glucanase/pectinase/protease)
OA = Organic acid (standard wheat soy Australian diet supplemented with phytase/xylanse/benzoic acid/essential oils)
[5]SEM = Standard error of mean

Table 7: Indication of nutrient digestibility based on acute grass intake of free-range laying hens[1]

[a b] Means in each row for each factor with different superscripts differ significantly (P<0.05)
[1] Means of 5 replicates with 5 birds per group (n = 60)
[2] A = Acute ranging effect (hens ranged for 6 weeks): C = Chronic ranging effect (hens ranges for 12 weeks)
[3] G = Gravel range, P = Pasture range
[4] C = Control diet (standard wheat soy Australian diet supplemented with phytase/xylanse)
ENZ = Multi-enzyme diet (standard wheat soy Australian diet supplemented with phytase/xylanse/betaglucanase/xylo-glucanase/pectinase/protease)
OA = Organic acid (standard wheat soy Australian diet supplemented with phytase/xylanse/benzoic acid/essential oils)
[5]SEM = Standard error of mean

Table 8: Effect of feed additives and range on non-starch polysaccharide values in ileum content of free range hens after 6 weeks of ranging[1]

		Free Sugars								
		Rha	Fuc	Rib	Ara	Xyl	Man	Gal	Glu	Total
Feed additives[2]	**C**	0.29	0.14	0.91	1.89	2.65^{b}	6.28	11.7	11.5	34.7
	ENZ	0.40	0.16	0.11	1.48	1.04^{a}	7.05	12.4	11.0	34.3
	OA	0.34	0.12	0.06	2.48	3.66^{c}	7.08	12.9	10.8	37.5
Range type[3]	**G**	0.32	0.16	0.08	2.27	3.07	6.52	11.2	12.0	35.8
	P	0.35	0.13	0.09	1.75	2.09	6.99	13.5	10.4	35.4
SEM[4]		0.03	0.01	0.01	0.19	0.30	0.35	0.95	1.09	2.45
P value	**F**	0.54	0.71	0.48	0.177	0.00	0.61	0.85	0.97	0.88
	R	0.80	0.46	0.97	0.22	0.06	0.60	0.32	0.53	0.94
	FxR	0.87	0.61	0.43	0.89	0.44	0.88	0.76	0.95	0.96
		Insoluble Non Starch Polysaccharides (NSP)								
		Rha	Fuc	Rib	Ara	Xyl	Man	Gal	Glu	Total
Feed additives[2]	**C**	1.26	1.60	0.27	47.2	57.4	2.63	27.3	24.0	142.6
	ENZ	1.34	1.85	0.37	44.8	52.7	2.50	33.7	21.2	139.5
	OA	2.10	1.65	2.00	47.1	57.7	5.92	31.4	27.6	154.1
Range type[3]	**G**	1.79	1.59	1.65	39.0	44.5^{a}	5.21	24.0^{a}	30.1^{b}	130.5^{a}
	P	1.42	1.77	0.35	51.9	64.5^{b}	2.65	35.6^{b}	20.2^{a}	156.7^{b}
SEM[4]		0.26	0.07	0.56	2.77	3.50	1.09	2.03	1.82	6.46
P value	**F**	0.26	0.37	0.31	0.90	0.63	0.27	0.44	0.26	0.45
	R	0.53	0.28	0.31	0.02	0.00	0.29	0.00	0.00	0.04
	FxR	0.27	0.70	0.35	0.76	0.28	0.29	0.76	0.39	0.38
		Soluble Non Starch Polysaccharides (NSP)								
		Rha	Fuc	Rib	Ara	Xyl	Man	Gal	Glu	Total
Feed additives[2]	**C**	0.14	1.18	0.50	8.32	8.97	1.60	5.45	3.34	26.2
	ENZ	0.14	1.17	0.39	7.98	9.38	1.67	5.11	2.58	25.2
	OA	0.16	1.26	0.48	9.01	9.09	1.98	6.26	4.53	29.1
Range type[3]	**G**	0.19^{b}	1.36^{b}	0.51	9.82	10.1	2.29^{b}	6.80^{b}	4.72^{b}	31.9^{b}
	P	0.11^{a}	1.09^{a}	0.42	7.45	8.37	1.36^{a}	4.77^{a}	2.64^{a}	23.3^{a}
SEM[4]		0.01	0.06	0.03	0.70	0.92	0.19	0.39	0.49	2.08
P value	**F**	0.67	0.67	0.30	0.68	0.95	0.57	0.20	0.16	0.50
	R	0.00	0.03	0.20	0.10	0.36	0.02	0.00	0.03	0.03
	FxR	0.17	0.09	0.05	0.08	0.17	0.29	0.02	0.13	0.03

[a-b] Means in each row for each factor with different superscripts differ significantly ($P < 0.05$)
[1] Means of 5 replicates with 5 birds per group (n = 30)
[2] C = Control diet (standard wheat soy Australian diet supplemented with phytase/xylanse)
ENZ = Multi-enzyme diet (standard diet with phytase/xylanse/betaglucanase/xylo-glucanase/pectinase/protease)
OA = Organic acid (standard diet supplemented with phytase/xylanse/benzoic acid/essential oils)
[3] G = Gravel range, P = Pasture range
[4]SEM = Standard error of mean

4. Discussion

In Australia, the outdoor stocking density for free-rang laying hens is not prescribed by legislation or regulation. However, while the current minimum industry standard of <10,000 hens per hectare was determined by the Australian Consumer Affairs Minister (CAF, 2016) the stocking density can be frequently be 1500 hens/ha (RSPCA, 2011), or even as low as 350 hens/ha (Singh, 2014). The use of mobile sheds allows access to fresh pasture on a daily base. In order to maintain a sustainable range area and to minimize the impact of the egg production on the environment, a low stocking density is favored. Research has shown that birds given access to pasture may, in part, compensate for small deficiencies in methionine through pasture access (Moritz et al., 2005). However, constant access to pasture can result in excessive pasture intake (Ruhnke et al., 2015a) and a recent survey undertaken in Australia indicated that grass impaction is considered to be a major cause of mortality in up to 21% of free-range farms (Ruhnke et al., 2015b). In the present study, no mortalities due to grass impaction were observed. Based on observations mentioned above on commercial farms, and observations by Cronin & Singh where the overall mortality of the ranging flock was 8.3% (The University of Sydney, unpublished data) the lack of mortality in the present research study was unexpected. However, on the commercial farms, as well as at The University of Sydney, the severe grass impaction was accompanied by reduced feed intake and reduced bodyweight. In the present study, none of these parameters was negatively affected, the availability of pasture seemed beneficial rather than disadvantageous:, hens exposed to a pastured range had significantly heavier body weight ($P = 0.002$) than hens ranging on gravel. The standard body weight these Lohmann Brown laying hens referred to is 1870 g (range 1777-1964 g) at the age of 24 weeks (acute ranging effect; hens ranged for 6 weeks), and 1920 g (range 1824-2016 g) at the age of 30 weeks (chronic ranging effect; hens ranges for 12 weeks) (Lohmann, 2011). The laying hens subject to this research were heavier at both time points and in every treatment group. This is not surprising as housing in a research facility and small groups allows for more feeder space per hen, less stress and a more restricted movement area compared to commercial large scale housing systems.

No evidence of impaired health or reduced feed intake due to pasture consumption could be obtained. The impact of pasture consumption on organ weight such as significantly increased gizzard, pancreas, and liver weight relate to a more pronounced development of digestive organs, which can be used as an indicator of improved gut health. Feeding high fibre diets or providing structure in form of coarse feed particles is known to increased gizzard weights in broilers and layers (Nir et al., 1994; Engberg et al., 2002). Structural components may include seed hulls, wood shavings, or large feed particles (Starck, 1999, Hetland et al., 2003; Bjerrum et al., 2005; Amerah et al., 2008). Hens in the present study that ranged on tall fescue obviously fed on more pronounced structural components, which were also clearly visible in collected crop content (Figure 4). The significant amount of pasture intake could also be confirmed by the significantly darker egg yolk colour observed for hens that ranged on pasture compared to hens that ranged on gravel (Table 4). There findings are in agreement with the literature, where yolk color depends strongly on pasture availability and vegetative status (Đukić-Stojčić M et al., 2009; Mugani et al., 2009) Relevant oxycarotinoids such as lutein and zeaxanthin are known to be at higher levels when the protein concentration of the plant is highest, which is reflected in plant maturity. In the present study, pasture was consumed during first growth at onset and mid plant maturity. This vegetative stage is known to have highest xanthophyll levels (Nys, 2000). Zeaxanthin, lutein and α-tocopherol has been shown to accumulate in significantly higher concentration in egg yolk when hens had access to pasture, compared to hens with access to a non-pastured range (Skřivan & Englmaierová 2014).

In agreement with the heavier pancreas weight observed in the present experiment (Table 5), other studies showed that increased relative gizzard weights were often found in combination with increased relative pancreas weights (Engberg et al., 2002; Gabriel et al., 2007; Williams et al., 2008; Rougiere et al., 2009). Physiologically, the increased gizzard activity stimulates the release of cholecystokinin which reinforces pancreatic juice secretion, which can lead to an increased nutrient digestibility (Li and Owyang, 1993; Svihus et al., 2004a; Svihus, 2006). However, in the present study the estimated nutrient digestibility of CF, CP, calcium and phosphorus was significantly decreased in hens that ranged on pasture. This indicates that the overall consumption of pasture reduced the availability of nutrients despite counteraction of the gizzard and pancreas. Negative CF values obtained from hens that had access to pasture indicate that a significant amount of fibre was retained in the hen's gastrointestinal tract. This may lead to the conclusion that the method of alkane analysis in the excreta for evaluating ileal nutrient digestibility may benefit from modification for hens that have access to pasture over a prolonged time period. Various methods have been reported to estimate pasture intake (Antell & Ciszuk, 2006; Milby, 1961) and the use of alkane analysis demonstrated to be a reliable method for the detection of fibre intake in free-range broilers (Singh & Cowieson, 2013). Using this method as internal marker for estimating the intake of fescue grass *(Festuca arundinacea)* in free-range layers allowed to calculate the dilution of feed based on grass excretion and subsequently assumed grass intake. However, the CF values obtained from ileal content reveal that a significant amount of fibre was retained in the hen's gastrointestinal system. In order to quantify the amount and determine the correct ileal dilution factor for nutrient digestibility, the amount of alkanes in the ileal content rather than the excreta should be determined. In future research, analysis of the alkanes in the ileum and the excreta should be performed to allow for accurate ileal nutrient digestibility. However, Table 7 still allows to estimate differences of nutrient digestibility amongst the treatment groups and hens that were supplemented with organic acid in their diets retained close to significant less fibre in their ileum (P = 0.06; Table 7).

A benefit of organic acids and/or multienzymes in the feed could be observed on hen body weight, (Table 3), liver weight (Table 5), and phosphorus digestibility (Table 8). Supplementing feed with exogenous enzymes such as xylanases, pentosanases, hemicellulases or β-glucanases specifically targets the water-soluble NSP fraction and has been reported to reduce the viscosity of the digesta (Steenfeldt et al., 1998). However, in the present study no impact of digesta viscosity could be observed (Table 6). A reduced viscosity of the digesta has been reported to increases feed passage time in the digestive tract, resulting in an increase in feed intake (Dänicke et al., 1997; Dänicke et al., 1999b; Lazaro et al., 2003a). In the present study, no effect of enzymes or organic acids on feed intake could be observed (Table 3). An increased viscosity and subsequently increased feed passage time could have promoted the excretion of grass boluses that were coiled up in the digestive tract.

Apple cider vinegar, a fermented liquid containing acetic acid and malic acid, is commonly administered in the drinking water and has been anecdotally reported to reduce the clinical symptom of chickens suffering from grass impaction (Ruhnke et al., 2015a). It could be demonstrated, that the usage of 0.1%, 1.0%, and 10% apple cider vinegar reduced feed extract viscosity significantly (P = 0.041, P = 0.026, and P = 0.002, respectively; unpublished data). In the present study, the ileal content of hens fed the control diet indicated an estimated -30.8 % CF digestibility, while hens of the ENZ and OA group had less CF in their ileum (-0.36 % and 0.14 %, respectively). While these results were not statistically significant (P = 0.06), one can suspect a beneficial clinical outcome in hens that ranged < 6 weeks (estimated CF digestibility 12.3 %) compared to hens that ranged > 6 weeks (estimated CF digestibility -31.83 %).

Feed enzymes allow hens to utilise their full spectrum of nutrients efficiently (Cowieson & Adeola, 2005). It has been demonstrated that feed enzymes such as phytases, xylanases, β-glucanases, xylo-glucanases, pectinases, and proteases can increase protein digestibility. This is especially so in chickens that suffer a nutrient dilution due to high fibre diets and subsequently are at risk of suffering from nutrient deficiency such as insufficient essential amino acids. In these cases, proteases may play a significant contribution to overall hen health and hen welfare (Roberts et al., 2007). In the current study, the access to pasture clearly demonstrated a significantly ($P = 0.02$) reduced CP digestibility, which was also affected by the duration of ranging time ($P = 0.002$). However, while a statistically significant beneficial effect of feed additives on estimated CP digestibility could not be observed, the impact of multi-enzymes on body weight and egg weight was significant ($P = 0.035$). The feasibility of using the combination of tall fescue and multi-enzymes has previous been demonstrated in free-range broiler chickens (Buchanan et al., 2007). When 500 Ross broilers were evaluated for their nutrient utilisation with/without enzyme supplementation while foraging, enzyme inclusion enhanced broiler performance only when given access to pasture. Furthermore, Buchanan et al. (2007) support the theory that the capacity of a NSP enzyme to aid in the degradation of plant cell wall components was accentuated in early-growth forage. The composition of the same grass species varies significantly between their vegetation statuses and within the annual season (Agfact, 2003; Scott et al., 1998). While grass of the first cut was reported with a total NSP content of 37%, grass of the second cut had an increased NSP content of 43% (Bach-Knutsen, 1997). Subsequently, the nutrient values of forage can be better utilised in its early vegetation status. In the present study, the fescue consumed by the hens was in its first-cut phase with a height of 50 cm and CP values of 18.6 % dry matter (Figure 2). The vegetative status of this grass was similar to that observed on commercial farms and suggested that the quantity rather than the quality of the grass are the responsible factor for impaired health status. The true ileal digestibility would have allowed more accurate evaluation of nutrients provided by the pasture. Increased CF of the diet is known to decrease nutrient digestibility due to the barrier function of the structural components and the lack of endogenous enzymes of monogastric animals (Choct et al., 1996; Fengler & Marquardt, 1988; Kocher et al., 2000). For example, an increase of CF from 5 to 17 % due to the use of finely milled straw decreased the digestibility of CP from 73 to 63 % and the digestibility of nitrogen-free-extract from 81 to 61% (Jeroch et al., 2012). This is consistent with the findings of the present study. However, in the present study the use of multi-enzymes or organic acids improved the estimated nutrient digestibility of CF, CP and calcium numerically and estimated nutrient digestibility of phosphorus statistically significant.

In summary, the exposure of free-range hens to tall fescue (*Festuca arundinacea*) resulted in significantly heavier body weight and subsequently heavier eggs, as well as heavier gizzard, liver and pancreas weights. Despite the enhanced development of gastrointestinal organs, the estimated ileal nutrient digestibility of CF, CP, calcium and phosphorus was reduced in hens exposed to pasture. The use of feed additives improved the estimated ileal digestibility of phosphorus significant, and the estimated ileal digestibility of CF close to significant.

5. Conclusion

When evaluating nutritional parameters obtained from free range laying hens such as ileal digestibility, existing research methods need to be modified. In order to optimise the mode of action of feed additives such as organic acids and multi-enzymes in feed for free-range laying hens, the uncontrolled amount of pasture intake should be taking into account.

Acknowledgment

We thank DSM Nutritional Products and the Poultry CRC for providing financial support.

References

AECL (2015) Annual Report. Australian Egg Corporation Limited, Sydney.

ACO, Australian Certified Organic (2013) Australian Certified Organic Standard‑the requirements for organic certification, Australian Organic Ltd, Nundah, Queensland, Australia.

Agfact (2003) P2.5.6, 4th edition (http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0008/166094/p256.pdf).

Antell S & Ciszuk P (2006) Forage consumption of laying hens-the crop content as an indicator of feed intake and AME content of ingested forage. *Archiv für Geflugelkunde*, 70(4), 154-160.

Amerah AM, Ravindran V, Lentle RG & Thomas DG (2008) *Poultry Science* 87, 2320-2328.

Buchanan, NP, Kimbler, LB, Parsons, AS, Seidel, GE, Bryan, WB, Felton, EED & Moritz, JS. (2007) The effects of nonstarch polysaccharide enzyme addition and dietary energy restriction on performance and carcass quality of organic broiler chickens. *The Journal of Applied Poultry Research*, 16(1), 1-12.

Bjerrum L, Pedersen K & Engberg RM (2005) *Avian Diseases* 49, 9-15.

Blokhuis HJ, Jones RB, Geers R, Miele M & Veissier I (2003) Measuring and monitoring animal welfare: transparency in the food product quality chain. *Animal welfare* 12: 445-456.

CAF, Ministers for Consumer Affairs (2016) *Meeting of Ministers for Consumer Affairs Thursday 31 March 2016 Canberra* http://consumerlaw.gov.au/files/2016/03/CAF_Communique_March_2016.pdf

Choct M, Hughes RJ, Wang J, Bedford MR, Morgan AJ & Annison G. (1996) Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. *British Poultry Science*, 37(3), 609-621.

Cowieson AJ & Adeola O (2005) Carbohydrases, protease, and phytase have an additive beneficial effect in nutritionally marginal diets for broiler chicks. *Poultry Science* 84(12), 1860-1867.

CSIRO publishing (2002) Primary Industries Standing Committee, Model Code of Practice for the Welfare of Animals, Domestic Poultry, 4th Edition, Collingwood, Victoria, Australia

Dänicke S, Simon O, Jeroch H, & Bedford M (1997) Interactions between dietary fat type and xylanase supplementation when rye-based diets are fed to broiler chickens. 1. physicochemical chyme features. *British Poultry Science* 38(5), 537-545.

Dänicke S, Vahjen W, Simon O, & Jeroch H (1999) Effects of dietary fat type and xylanase supplementation to rye-based broiler diets on selected bacterial groups adhering to the intestinal epithelium. on transit time of feed, and on nutrient digestibility. *Poultry Science* 78(9), 1292-1299.

Đukić-Stojčić M, Perić L, Bjedov S & Milošević N (2009) The quality of table eggs produced in different housing systems.*Biotechnology in Animal Husbandry*,*25*(5-6-2), 1103-1108.

Englyst HN & Hudson GJ (1987) Colorimetric method for routine measurement of dietary fibre as non-starch polysaccharides. A comparison with gas–liquid chromatography. Food Chemistry 24, 63–76.

Engberg RM, Hedemann MS & Jensen BB (2002) The influence of grinding and pelleting of feed on the microbial composition and activity in the digestive tract of broiler chickens. *British Poultry Science* 43:569–579.

FCA (2014), Federal Court of Australia, http://www.judgments.fedcourt.gov.au/judgments/Judgments/fca/single/2014/2014fca1028

Fengler AI & Marquardt RR (1988) Water-soluble pentosans from rye: II. Effects on rate of dialysis and on the retention of nutrients by the chick. *Cereal Chemistry*, 65(4), 298-302.

FREPA (2015) Free Range Egg Standards, Free Range Egg and Poultry Australia Ltd., Benalla, Victoria. http://www.frepa.com.au/standards/egg-standards/

Gabriel I, Mallet S, Leconte M, Travel A & Lalles J. (2007) Effects of whole wheat feeding on the development of the digestive tract of broiler chickens. Anim. Feed Sci. Technol. 142:144–162.

Glatz, PC, Ru YJ, Miao ZH, Wyatt SK & Rodda BJ. (2005) Integrating poultry into a crop and pasture farming system. *International Journal of Poultry Science*, 4(4), 187-191.

Hameleers A, McNab LM & Mayes RW (1996) Use of saturated aliphatic hydrocarbons (alkanes) as markers in nutrition studies in chickens. British Poultry Science 37: S55.

Hetland H, Svihus B & Krogdahl Å (2003) *British Poultry Science* 44, 275-282.

Hermansen, JE (2003) Organic livestock production systems and appropriate development in relation to public expectations. *Livestock Production Science* 80: 3-15.

Hilmi HTA, Surakka A, Apajalahti J & Saris PEJ (2007) Identification of the most abundant Lactobacillus species in the crop of 1-and 5-week-old broiler chickens. *Applied and Environmental Microbiology* 73: 7867-7873.

Horsted KJ, Hermansen E & Ranvig H (2007) Crop content in nutrient-restricted versus non-restricted organic laying hens with access to different forage vegetations. *British Poultry Science* 48: 177-184.

Huang DS, DF Li, JJ Xing, YX Ma, ZJ Li & Lv SQ (2006) Effects of feed particle size and feed form on survival of Salmonella typhimurium in the alimentary tract and cecal S. typhimurium reduction in growing broilers. *Poultry Science* 85: 831-836.

Jeroch H, Simon A & Zentek J (2012) Geflügelernährung [German]: Ulmer: 87

Kijlstra A, Meerburg BG. & Bos AP. (2009) Food safety in free-range and organic livestock systems: Risk management and responsibility. *Journal of Food Protection* 72(12), 2629-2637.

Kluth H, Mehlhorn K & Rodehutscort K. (2005) Studies on the intestine section to be sampled in broiler studies on precaecal amino acid digestibility. *Archieves of Animal Nutrition* 59:271–279.

Kocher A, Choct M, Hughes RJ & Broz J (2000). Effect of food enzymes on utilisation of lupin carbohydrates by broilers. *British Poultry Science*, 41(1), 75-82.

Lázaro R, Garcia M, Medel P & Mateos GG. (2003) Influence of enzymes on performance and digestive parameters of broilers fed rye-based diets. *Poultry Science* 82(1), 132-140.

Li Y & Owyang C (1993) Vagal afferent pathway mediates physiological action of cholecystokinin on pancreatic enzyme secretion. *Journal of Clinical Investigation* 92:418–424.

Lohmann Tierzucht (2011) Management guide layers. https://www.scribd.com/doc/202634027/Lohmann-Brown-Classic-Management-Guide-2011-1-pdf

Lunam CA, Glatz PC & HSU Y-J (1996) The absence of neuromas in beaks of adult hens after conservative trimming at hatch. *Australian Veterinary Journal* 74: 46-49.

Milby TT (1961) Range versus confinement for growing turkeys. *Poultry Science* 40(1), 46-50.

Mugnai C, Dal Bosco A & Castellini C (2009) Effect of rearing system and season on the performance and egg characteristics of Ancona laying hens. *Italian Journal of Animal Science*, *8*(2), 175-188.

Naumann K & Bassler R (2004) Methodenbuch Band III: Die chemische Untersuchung von Futtermitteln. Melsungen [German]: Neumann-Neudamm.

Nir I., Hilley R, Shefet G & Nitsan Z (1994) Effect of grain particle size on performance. 2. Grain texture interactions. *Poultry Science* 73:781–791.

Nys Y (2000) Dietary carotenoids and egg yolk coloration-a review. *Archiv für Geflügelkunde*, *64*(2), 45-54.

Rezvani M,. Kluth H, Elwert C & Rodehutscord M (2007) Effect of ileum segment and protein sources on net disappearance of crude protein and amino acids in laying hens. *British Poultry Science* 49(1):28–36.

Roberts SA, Xin H, Kerr BJ, Russell JR & Bregendahl K. (2007) Effects of dietary fiber and reduced crude protein on nitrogen balance and egg production in laying hens. *Poultry Science* 86(8), 1716-1725.

Rougiere N, Gomez J, Mignon-Grasteau S & Carre B. (2009) Effects of diet particle size on digestive parameters in D+ and D− genetic chicken lines selected for divergent digestion efficiency. *Poultry Science* 88:1206–1215.

RSPCA (2011) RSPCA Approved Farming scheme standards Layer Hens. http://www.rspca.org.au/sites/default/files/website/what-we-do/working-with-farming-industry/RSPCALayerhensStandards.pdf

Ruhnke I, Cowling G, Sommerlad M, Swick R & Choct M (2015a) Gut impaction in free-range hens. *Proceedings of the Australian Poultry Science Symposium* 26, 242-244.

Ruhnke I, DeKoning C, Drake K, Glatz P, Walker T, Skerman A, Hunt P, Sommerlad M, Choct M, & Singh M (2015b) Free range farm demographics and practices in Australia *The 26th Australian Poultry Science Symposium* 26, 260.

Samiullah S, Roberts JR & Chousalkar KK (2014) Effect of production system and flock age on egg quality and total bacterial load in commercial laying hens. *The Journal of Applied Poultry Research* 23: 59-70.

Scott TA., Silversides FG, Classen HL, Swift ML & Bedford MR (1998) Effect of cultivar and environment on the feeding value of Western Canadian wheat and barley samples with and without enzyme supplementation. *Canadian Journal of Animal Science*, 78(4), 649-656.

Singh M & Cowieson AJ (2013) Range use and pasture consumption in free-range poultry production. *Animal Production Science* 53(11), 1202-1208.

Skřivan M & Englmaierová M (2014) The deposition of carotenoids and α-tocopherol in hen eggs produced under a combination of sequential feeding and grazing. *Animal Feed Science and Technology*, *190*, 79-86.

Starck JM (1999) Phenotypic flexibility of the avian gizzard: rapid, reversible and repeated changes of organ size in response to changes in dietary fibre content. *Journal of Experimental Biology* 202: 3171-3179.

Steenfeldt S, Müllertz A & Jensen JF (1998) Enzyme supplementation of wheat-based diets for broilers: 1. Effect on growth performance and intestinal viscosity. *Animal Feed Science and Technology* 75(1), 27-43.

Svihus B (2006) The role of feed processing on gastrointestinal function and health in poultry. in *Avian Gut Function in Health and Disease*. Vol. 28. G. C. Perry, ed. CAB Int., Wallingford, UK. 183-194.

Svihus B (2012) Gastrointestinal tract development: implications for free-range and conventional production. In Gastrointestinal tract development: implications for free-range and conventional production, *Proceedings of the 23rd Annual Australian Poultry Science Symposium*. Sydney, 19-22.

Svihus BE, Juvik H, Hetland H & Krogdahl A (2004) Causes for improvement in nutritive value of broiler chicken diets with whole wheat instead of ground wheat. *British Poultry Science* 45:55–60.

Theander O & Westerlund E (1993) Determination of individual components of dietary fiber. In *Dietary fiber in human nutrition*. (Ed. GA Spiller) 57–75 (CRC Press: Boca Raton, FL).

Williams J, Mallet S, Leconte M, Lessire M & Gabriel I (2008) The effects of fructo-oligosaccharides or whole wheat on the performance and digestive tract of broiler chickens. *British Poultry Science* 49:329–339.