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MP03-81 The 2003 Nebraska Poultry Report

Sheila Scheideler
University of Nebraska--Lincoln, spurdum2@unl.edu

Mary M. Beck
University of Nebraska-Lincoln, mbeck@poultry.msstate.edu

Curtis L. Novak
University of Nebraska--Lincoln

Leanne LaBrash
University of Nebraska--Lincoln

Danilo J. Franco
University of Nebraska--Lincoln

See next page for additional authors

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Scheideler, Sheila; Beck, Mary M.; Novak, Curtis L.; LaBrash, Leanne; Franco, Danilo J.; Jalal, Mohammad A.; Monsalve, David; and Weber, Trish, "MP03-81 The 2003 Nebraska Poultry Report" (2003). *Historical Materials from University of Nebraska-Lincoln Extension*. 1286.
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Authors

Sheila Scheideler, Mary M. Beck, Curtis L. Novak, Leanne LaBrash, Danilo J. Franco, Mohammad A. Jalal, David Monsalve, and Trish Weber

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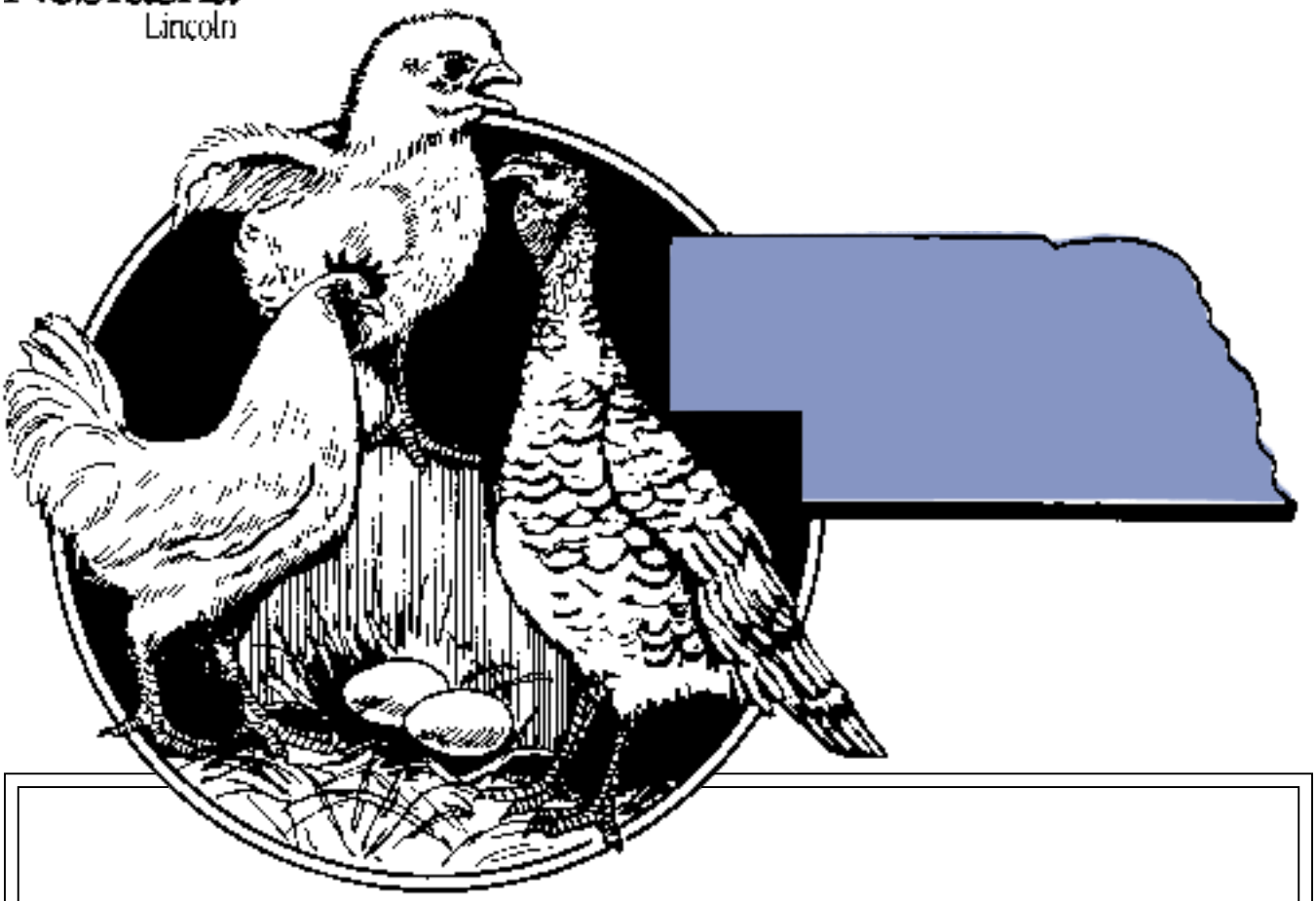

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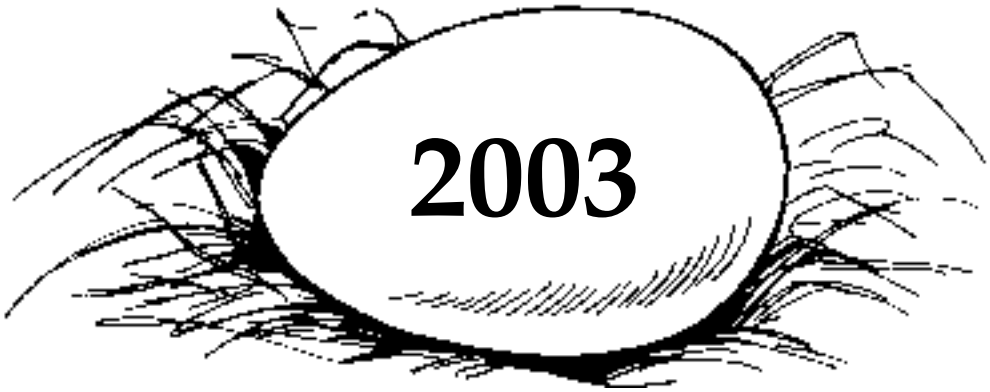

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Issued August 2003, 500

Acknowledgments

Agricultural Research Division — 207 Agricultural Hall, University of Nebraska, Lincoln, NE 68583-0702
Danisco — Finnfeeds International Limited, P.O. Box 777, Marlborough, Wiltshire, SN8 1XN, United Kingdom
Degussa-Huls Corporation — 1255 Roberts Blvd, Suite 110, Kennesaw, GA 30144-3694
Finnfeeds International, Inc. — 411 East Gano, P.O. Box 470157, St. Louis, MO 63147
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Michael Foods (M. G. Waldbaum Company) — P.O. Box 573, Wakefield, NE 68784
Midwest Poultry Consortium — 4630 Churchill Street #1, St. Paul, MN 55126
Frank E. and Inez L. Mussehl Endowment — 207 Agricultural Hall, University of Nebraska, Lincoln, NE 68583-0702
Nebraska Eggs, Ltd. — P. O. Box 216, Carroll, NE 68723-0216
Nebraska Poultry Industries — A103 Animal Sciences, University of Nebraska, Lincoln, NE 68583-0908
University of Nebraska Cooperative Extension Division
U.S. Poultry and Egg Association

NOTE: Because of budgetary constraints, the Poultry Report is published in alternate years. The next edition will appear in 2005.

Contributors

Editor
Sheila E. Scheideler
Professor
Animal Science

Mary M. Beck
Professor
Animal Science

Curtis L. Novak
Associate Professor
Animal and Poultry Science
VPI

Graduate Research Assistants and Technicians - Animal Science

Leanne LaBrash
Danilo J. Franco
Mohammad A. Jalal
David Monsalve
Trish Weber

Introduction

The Nebraska Poultry Report is produced every two years by the Animal Science Department's poultry faculty with contributions from others in the University of Nebraska who work with avian species. The purpose of the report is to make our activities known to the poultry industries in Nebraska. The majority of articles are based on on-going research but are written in a relaxed style for ease of reading. If at any time an article or piece of information is of special interest to anyone in the industry, we hope you will contact us to discuss it.

Dietary Protein, Lysine and TSAA:Lysine Ratio — Effects on Egg Components and Solids

Sheila E. Scheideler
Curtis L. Novak[1]

Introduction

Many studies have been conducted testing the effects of dietary protein, lysine and total sulfur amino acids (TSAA) on egg production parameters, but few reports can be found regarding the effects of dietary protein and amino acid intake on egg components and yields. The egg contains approximately 6 grams of protein primarily located in albumen. This protein is synthesized relatively quickly in the oviduct during albumen formation. A steady and adequate supply of protein and essential amino acids (lysine and TSAA) is needed to optimize protein formation in the oviduct. Reports have been mixed on the effect of increased lysine intake on egg weight. Scheideler et al., 1996 and Prochaska et al. (1996) reported increased egg and albumen weights with increased lysine intake in layers. Yolk weights tend to be more closely associated with TSAA intake, increasing as TSAA intake increases (Shafer et al., 1996).

Ratio of TSAA:Lysine intake also may affect the efficiency of egg protein synthesis. There have been preliminary reports of detrimental effects of high lysine intake during minimal TSAA intake on egg production and egg weights (Novak and Scheideler, 1998). The recommended ratio of TSAA:Lysine in the 1994 Nutrient Requirements of Poultry by NRC was .84. Given the benefits of increased lysine intake for albumen synthesis, the optimum level of TSAA to be fed when maintaining lysine at a beneficial level needs to be tested.

Level of protein intake (gm/day) also can affect egg protein synthesis. With the current environmental pressures to reduce N in poultry manure, producers are trying to reduce dietary protein to a minimum level adequate to maintain efficient egg production. A reduction in dietary protein from 18 to 16% can result in a 7% decrease in excreta N (Yakout, 2000). A careful balance between dietary protein and essential amino acids needs to be found to maintain optimum egg protein synthesis.

The objective of the studies reported herein was to test the main and interaction effects of dietary protein, lysine and TSAA:Lysine ratio on egg protein production.

Material and Methods

Trial One was a 2 X 4 factorial design with 2 levels of dietary lysine (800 or 900 mg/day) combined with 4 TSAA:Lysine ratios (.71, .81, .91, or 1.01) fed to DeKalb Delta white leghorn hens from 20-63 weeks of age. Three hundred and twenty hens were used for this study with 8 replicate cages (5 hens/cage) assigned to each treatment. At 35 and 55 wks of age, 3 eggs/pen were collected to determine yolk and albumen content and properties.

Trial Two was a 3 X 3 factorial arrangement of 3 levels protein (18, 16 or 14%) fed during Phase I of production (20-40 wks of age) or 16, 14.4 or 13% fed during Phase II of production (40-60 wks of age) with 3 ratios of TSAA:Lysine (.71, .81, .91) fed to Hyline W-98 white leghorn hens. Ratio of TSAA:Lysine was adjusted in each trial with the addition of dl-methionine. Four hundred thirty-two hens were randomly assigned to

(Continued on next page)

the 9 diets with 8 replicate cages (6 hens/cage) assigned to each diet. Every 5 weeks beginning at 20 wks of age until the end of the trial, 3 eggs/treatment were collected to measure fresh yolk and albumen properties.

At 60 weeks of age, 20 hens from 5 treatments (14.5% Protein at .71, .81, .91 ratios) and 16.0 and 13.8 % Protein at .81 TSAA:Lysine ratios) were euthanized and magnum tissue was excised for determination of RNA, DNA and protein content during oviposition. Total RNA, DNA and protein were assayed to determine dietary effects on the cellular working components of protein synthesis in magnum tissue.

Analysis of variance was performed by Proc Mixed procedures (Proc Mixed: SAS Institute, 1996), testing for main effects and interaction effects in each trial.

Results and Discussion

In trial one, dietary lysine significantly affected egg weight, percent albumen, yolk, albumen solids, albumen protein and yolk protein (Table 1). As lysine increased from 800 to 900 mg/day, egg wt, albumen, and albumen solids increased whereas percent yolk, albumen protein and yolk protein decreased. Ratio of TSAA:Lysine affected % albumen, yolk, albumen protein, yolk solids and yolk protein. As supplemental TSAA increased, thereby increasing the TSAA:Lysine ratio, % albumen decreased, % yolk increased, albumen protein decreased, yolk solids increased and yolk protein decreased. These data interestingly indicate a negative correlation between % solids and protein in both albumen and yolk. This is surprising, especially in the albumen. There appeared to be a strong positive effect of higher lysine on percent albumen and albumen solids and a positive effect of increased TSAA:Lysine ratio on % yolk and yolk solids.

In Trial 2, dietary protein intake significantly affected a number of egg components during the 2nd phase of egg production. The lowest levels of protein intake caused significant reductions in egg weight and egg mass, egg albumen, albumen solids, albumen protein and yolk protein. The intermediate level of protein performed similarly to the high level of protein intake, indicating that dietary protein can be lowered by as much as 2% without a negative effect on egg components. Ratio of TSAA:Lysine showed little to no effect on egg components in this trial. There was some indication of poorer egg mass (and egg production) in the low protein diets with a high TSAA:Lysine ratio. Increased TSAA:Lysine ratio also tended to decrease yolk protein as shown in Trial 1 so distinctly. It should be noted that these trials were conducted with 2 different strains — Trial 1 with DeKalb Delta hens and Trial 2 with Hyline W-98 hens, which could differentiate their responses to amino acid intake.

Table 3 gives some interesting in vitro data on the protein synthesis activity level of magnum tissue collected from hens in Trial 2 at 60 weeks of age. Level of dietary protein significantly affected amount of cellular RNA, Protein:RNA ratio and RNA:DNA ratio. RNA is basically the working machinery of the cell. It significantly increased in the magnum from hens on low protein diets indicating an adaptive response of the hens to low dietary protein — upregulating the cell to make more RNA. But the quantitative amounts of pro-

Table 1. Trial One — Dietary Lysine and TSAA:Lysine Ratio Effects on Egg Components

Lysine	TSAA	Ratio TSAA:Lysine	Egg Wt.	Egg Mass	Albumen	Yolk	Albumen Solids	Albumen Protein	Yolk Solids	Yolk Protein
Mg/kg	Mg/kg		Grams	g/hen/day	%					
800	570	0.71	58.1	48.2	60.8	27.8	11.20	9.14	50.9	17.5
800	650	0.81	58.7	48.6	60.8	27.5	11.10	9.01	51.4	17.6
800	730	0.91	59.6	50.3	60.6	28.2	11.04	8.94	52.1	16.7
800	810	1.01	59.7	47.5	60.0	27.7	11.12	10.02	53.5	17.6
900	650	0.71	59.7	48.5	62.1	27.3	11.32	10.00	51.4	17.4
900	730	0.81	60.8	49.2	61.4	26.7	11.13	10.55	51.2	17.4
900	810	0.91	60.0	51.9	60.9	27.9	11.28	6.92	51.9	14.6
900	890	1.01	60.4	50.9	60.8	27.7	11.23	6.46	52.2	15.2
SEM			0.77	1.57	0.36	0.29	0.113	0.345	0.19	0.49
Main Effects										
Lysine										
800			59.0	48.7	60.6	27.8	11.11	9.28	52.0	17.3
900			60.2	50.1	61.3	27.4	11.24	8.48	51.7	16.2
Ratio										
0.71			58.9	48.3	61.4	27.5	11.25	9.57	52.7	17.6
0.81			59.7	48.9	61.1	27.1	11.11	9.78	51.3	17.5
0.91			59.8	51.1	60.8	28.0	11.16	7.93	52.0	15.6
1.01			60.0	49.2	60.4	27.7	11.17	8.24	52.8	16.3
Statistical Probabilities										
Lysine			0.02	NS	0.03	0.10	0.10	0.004	NS	0.001
Ratio			NS	NS	0.02	0.06	NS	0.001	0.06	0.001
Lysine x Ratio			NS	NS	NS	NS	NS	0.001	NS	0.001

Table 2. Trial Two — Dietary Protein and TSAA:Lysine Effects on Egg Components (Phase 2)

Protein	Ratio TSAA:Lysine	Egg Wt.	Egg Mass	Albumen	Yolk	Albumen Solids	Albumen Protein	Yolk Solids	Yolk Protein
%		Grams	Grams	%					
16.0	.97	59.8	514	61.0	26.0	12.47	10.08	55.1	15.9
14.4	.97	59.6	51.5	60.7	26.4	12.43	10.24	55.4	15.5
13.0	.97	58.0	48.2	60.6	26.4	12.17	9.82	55.1	15.8
16.0	.85	59.2	50.8	61.3	26.0	12.69	10.64	54.9	16.01
14.4	.85	58.9	50.1	61.0	26.4	12.45	10.10	54.9	15.8
13.0	.85	58.9	49.9	60.6	26.4	12.31	9.78	55.4	15.6
16.0	.82	59.3	50.3	61.0	26.2	12.56	10.39	55.1	16.0
14.4	.82	59.9	52.3	61.1	26.1	12.71	10.28	55.0	15.9
13.0	.82	59.0	49.4	60.8	26.2	12.21	9.42	55.0	15.7
SEM		0.458	0.606	0.222	0.177	0.212	0.183	0.172	0.156
Main Effects									
Protein									
16.0%		59.4	50.8	61.1	26.1	12.57	10.37	55.0	16.0
14.4%		59.4	51.3	60.9	26.3	12.53	10.20	55.1	15.7
13.0%		58.6	49.2	60.6	26.3	12.23	9.67	55.2	15.7
Ratio									
.97		59.1	50.4	60.8	26.3	12.36	10.04	55.2	15.7
.85		59.0	50.3	61.0	26.3	12.49	10.17	55.1	15.8
.82		59.4	50.7	61.0	26.2	12.50	10.03	55.1	15.9
Statistical Probabilities									
Protein		0.06	0.001	0.03	NS	0.001	0.05	NS	0.009
Ratio		NS	NS	NS	NS	NS	NS	NS	0.09
Protein x Ratio		NS	0.03	NS	NS	NS	NS	NS	NS

Table 3. Trial Two — Dietary Protein and TSAA:Lysine Effects on Chemical Changes in the Magnum (Phase 2)

Dietary Treatment		RNA	DNA	Protein/RNA	RNA/DNA	Protein/DNA
Protein	Ratio	mg/g		mg/mg		
18.9	0.81	6.87	0.436	37.6	16.3	591
16.3	0.81	7.98	0.402	26.3	20.9	555
14.4	0.81	9.81	0.312	22.0	32.3	736
P-value		0.01	NS	0.02	0.001	NS
14.4	0.97	9.05	0.356	19.5	25.9	498
14.4	0.85	9.81	0.312	22.0	32.3	736
14.4	0.82	9.09	0.352	23.7	25.9	596
P-value		NS	NS	NS	NS	NS

tein synthesized in the magnum decreased as dietary protein decreased indicating that the adaptive response may still not be adequate in the low protein diets. This conclusion is supported by the reduced amount of albumen protein and solids in eggs from hens on the low protein diets (Table 2). Ratio of TSAA:Lysine had no effect on measurements of protein synthesis in the hens' magnum tissue.

In summary, dietary lysine and protein can significantly affect egg components. Insufficient protein intake will impair the hens' ability to adequately synthesize protein in the magnum. Increasing dietary lysine can improve proportion of albumen. TSAA needs to remain in a high ratio to lysine to maintain yolk quantity, but can negatively affect yolk protein content.

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[1]Sheila E. Scheideler, professor and poultry extension specialist, and Curtis L. Novak, former graduate student, University of Nebraska, Lincoln.

Effect of Varying Space Allowances and Metabolizable Energy Levels on Performance of Laying Hens

Mohammad A. Jalal
Sheila E. Scheideler[1]

Introduction

Most laying hens are placed in cages in today's commercial egg industry. The aim of such practice is to reduce housing, equipment and labor costs per hen and keep the eggs cleaner. It is commonly believed that decreasing cage space per hen will increase the number of eggs per cage, thus offsetting the negative effects of crowding on productivity of the individual hen, thereby increasing profitability (Adams and Craig, 1985). This view started to change in the last decade of the twentieth century, with more emphasis on animal welfare issues (Anderson *et al.*, 1995).

Cage effects on the performance of commercial laying hens are well documented. Reduced cage space allowance has been reported to decrease egg production (EP), egg weight (EW) and feed intake (FI), and to increase mortality (Bell, 1981; Cunningham, 1982, Sandoval *et al.*, 1991).

Few researchers have investigated the combined effect of cage space allowance and diet caloric content on hen and pullet performance. Jackson and Waldroup (1988) found that increased dietary nutrient density helped overcome the effects of limited feeder space associated with crowded cages, but the influence of diet was minimal when shallow cages were used and feeder space increased. Owings *et al.* (1967) reported decreased EP caused by reduced cage space allowance was partially overcome by increased dietary protein. More recently Brake and Peebles (1992) studied the effect of varying cage space allowances and lysine levels on laying hen performance. They reported no consistent effect of cage space allowance on production parameters and detected no interaction between lysine levels and cage space allowance.

New animal welfare guidelines put forth by United Egg Producers (UEP 2001) recommended a cage space allowance of 67 sq. inches per hen for smaller White Leghorn hens compared to current industry practice of 52-54 sq. inches per hen. As result of these new welfare guidelines, the objective of our research was to assess the effects of varying cage space allowances on a commercial laying hen strain fed differing levels of dietary ME.

Experimental Design

Four cage allowances (53, 64, 80 and 107 square inches per bird) were assigned to Single Comb White Leghorn hens from 50 to 65 weeks of age. Each cage space allowance was combined with three dietary ME treatments (2800, 2850, and 2900 kcal/kg) in a 3 x 4 factorial arrangement. Each treatment was randomly allotted to 6 replicate pens for a total of 72 pens. Individual pens were designated as experimental units and had varying numbers of hens; 3 (107 sq. inch), 4 (80 sq. inch), 5 (64 sq. inch), or 6 (53 sq. inch) for a total of 324 hens.

Table 1. Diet composition[1]

Ingredients	High ME	Low ME
	-------- (%) --------	
Corn	60.53	58.71
Soybean meal	23.33	21.47
Wheat midds	—	4.92
Tallow	4.25	3.00
Limestone	9.39	9.40
Dicalcium phosphate	1.70	1.66
Salt	0.40	0.40
DL-methionine	0.19	0.19
Lysine	0.06	0.09
Vitamin premix	0.08	0.08
Mineral premix	0.08	0.08
Nutrient Composition:		
M.E., kcal/kg	2900	2800
Protein, %	17.2	17.2
TSAA, %	0.73	0.73
Lysine, %	0.85	0.85
Ca, %	4.00	4.00
NPP, %	0.42	0.42

[1]Intermediate ME diet was mixed by blending equal quanitities of high and low M.E. Diets.

Experimental pens (cages) dimensions were 16 x 20 inches in a stacked deck manure belt system. The experimental design was a randomized complete block design with 6 blocks and 12 pens per block.

Diets were formulated according to the recommendation of the Hy-Line W-36 breeders' manual and to meet National Research Council (1994) nutrient requirements of laying hens for all nutrients with the exception of energy (Table 1). Diets were standard corn-soybean meal diets, isonitrogenous, and containing three varying levels of ME (2800, 2850, 2900 kcal/kg). Animal-vegetable blended fat was the source used to increase dietary energy. The intermediate diet was obtained via

Table 2. Layer production data

M.E.	Cage space allowance	Feed intake	M.E. intake	Egg production	Egg weight	Egg mass
	Sq. in/hen	g/hen/d	kcal/hen/d	%	g	g
High	107	90.54	262.57	81.89	52.16	45.79
High	80	88.72	257.29	81.18	51.88	44.71
High	64	83.87	249.23	76.04	52.25	42.36
High	53	84.99	246.46	75.92	51.15	42.33
Intermediate	107	91.84	261.76	86.91	51.90	47.73
Intermediate	80	90.33	257.45	84.49	49.94	44.57
Intermediate	64	84.03	239.49	76.58	50.94	41.25
Intermediate	53	86.20	245.68	75.44	51.07	41.07
Low	107	92.25	258.30	89.78	51.54	49.00
Low	80	89.49	250.58	79.85	51.93	44.20
Low	64	87.60	245.50	73.48	51.25	40.05
Low	53	84.56	236.77	76.66	50.84	41.33
Main Effects:						
M.E.						
High		87.04	252.39	78.76	51.85	43.55
Intermediate		88.10	251.09	80.85	50.97	43.66
Low		88.50	247.79	79.94	51.39	43.65
Cage space/hen						
107		91.55	260.88	86.19	51.86	47.51
80		89.52	255.11	81.84	51.25	44.50
64		85.19	242.74	75.37	51.48	41.22
53		85.25	242.97	76.00	51.02	41.25
Probabilities:						
M.E.		NS	NS	NS	NS	NS
Cage space		0.0002	0.0002	0.0001	NS	0.0001
ME x Cage Space		NS	NS	0.089	NS	NS

Table 3. Layer production data

M.E.	Cage space allowance	Hen weight	Body weight change
	Sq. in/hen	kg	kg
High	107	1.418	0.02920
High	80	1.398	0.02877
High	64	1.386	0.02376
High	53	1.382	0.03626
Intermediate	107	1.431	0.03177
Intermediate	80	1.370	0.03224
Intermediate	64	1.362	0.03810
Intermediate	53	1.380	0.02359
Low	107	1.403	0.03010
Low	80	1.437	0.04029
Low	64	1.378	0.02904
Low	53	1.396	0.03169
Main Effects:			
M.E.			
High		1.396	0.02950
Intermediate		1.386	0.03143
Low		1.403	0.03153
Cage space/hen			
107		1.418	0.03036
80		1.402	0.03378
64		1.375	0.02863
53		1.386	0.03052
Probabilities:			
M.E.		NS	NS
Cage space		NS	NS
M.E. x Cage space		NS	0.0137

blending high and low ME diets.

Measurements included daily feed intake, ME intake (MEI) and egg production, weekly egg weights and egg mass (EM), and biweekly hen weights (HW) and body weight change (BWC).

Data was analyzed using the Repeated Measures Analysis of SAS® software (Proc Mixed, SAS Institute, 1998) for a randomized complete block design and a 3 x 4 factorial arrangement.

Results

Variation in cage space allowance had a significant effect on feed intake (Table 2). Hens given 107 sq. inch significantly ($P<0.001$) had a greater feed intake than those housed at 64 and 53 sq. inch, but not significantly higher than those housed at 80 sq. inch. Dietary metabolizable energy level had no significant effect on feed intake. A similar effect can be observed with MEI (Table 2). Hens housed at 107 sq. inch had significantly ($P<0.001$) higher MEI than hens housed at 64 and 80 sq. inch by differences of 18.14 and 17.91 kcal/hen/day respectively.

Egg production was significantly ($P<0.0001$) improved for hen housed at 107 sq. inch compared to other densities and across all energy levels (Table 2). An interaction between dietary ME and cage space allowance was close to significance ($P<0.08$), with hens fed low ME and housed at 107 sq. inch having highest EP value of 89.78%. Egg mass exhibited a similar trend to egg production, as hens with greater cage space having a significantly ($P<0.0001$) larger EM value than those housed at 80, 64 and 53 sq. inch (Table 2). There were no significant treatment effects observed on egg weight (Table 2).

There were no significant effects of either dietary ME or cage space allowance on hen body weight (Table 3). However, there was a significant interaction effect on change in body

(Continued on next page)

weight (Table 3). Hens housed at 80 sq. inch/hen and fed low ME diet exhibited the greatest weight gain and were significantly ($P<0.05$) higher those fed other levels of ME at the same density. Hens fed the intermediate level of ME had greater BWC ($P<0.05$) than those fed high and low ME when housed at 64 sq. inch, while BWC was highest for those fed high ME at 53 sq. inch/hen.

Conclusions

It is evident that increasing cage space allowance and decreasing number of birds per pen had a positive overall effect on performance. Cage space significantly affected production parameters such as feed intake, ME intake, egg production and egg mass. There was no effect of ME levels on laying hen performance at varying cage space allowances.

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[1]Mohammad A. Jalal, graduate student, and Sheila E. Scheideler, professor and poultry extension specialist, Animal Sciences, Lincoln.

On Farm Feather Condition Survey of Commercial Laying Hens

Leanne F. LaBrash
Sheila E. Scheideler[1]

Introduction

The importance of feathering and feather cover often is overlooked in the laying hen industry. Feather synthesis, development and maintenance requirements can be easily overlooked as their contribution is not measured in classic performance criteria such as hen day egg production. Quality of feather cover warrants reconsideration as it significantly contributes to the laying hens' performance and also could influence consumers' perception of hen care. Feather cover influences laying hen performance by affecting thermoregulation, incidence of injury and behavior.

Hens with reduced feather cover experience increased heat loss. Poorly feathered hens experiencing increased heat loss will have greater maintenance energy costs as metabolic rate increases to maintain body temperature. The result is increased feed consumption in layers with increased feather loss (Ambrosen and Peterson, 1997).

Feathers also protect a hen's body from scratches, pecking, cannibalism and abrasive cage material. Deterioration of plumage that reduces body cover elicits behavior that may result in harm to the laying hen. Severe feather pecking has been shown to increase with feather damage in all ages of hens (Bilcik and Keeling, 1999) and incidence of cannibalism often parallels plumage deterioration (Ambrosen and Peterson, 1997).

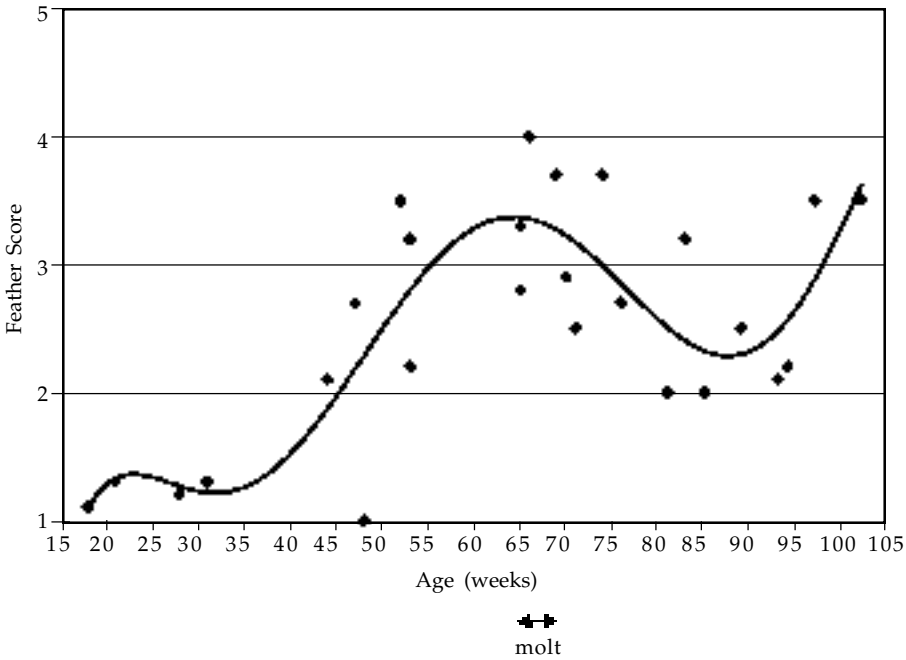

Figure 1. Feather condition curve for laying hen flocks surveyed.

Survey Methods

A survey was conducted to assess the effect of age on feather score in commercial laying hens. Flocks of Hy-Line 98, Babcock B300, and ISAWhite laying hens ranging in age from 18 to 102 weeks were feather scored. Five hens in each surveyed cage were feather scored according to a five-point scale. The five-point feather score scale was as follows (Webster and Hurnik, 1990):

1. Smooth and complete plumage.
2. Ruffled, no naked spots.
3. Naked spots up to 5 cm at widest part.
4. Naked spots greater than 5 cm wide.
5. Naked spots with injury to skin.

The five feather scores from each cage surveyed were averaged to create a cage feather score. Cage feather scores from each flock were averaged to establish an overall feather score for each flock. Flock feather scores were plotted according to age creating the feather condition curve in Figure 1.

Discussion

Hens 18 to 33 weeks of age exhibited smooth and complete plumage. At 44 to 47 weeks of age, plumage condition began to show wear and feathers became ruffled. After week 51 plumage cover further deteriorated and naked spots on hens became evident. The naked spots at this time were no larger than 5 cm at the widest part. Naked spots greater than 5 cm wide were seen starting at 66 weeks of age. Flocks were molted between 66 and 70 weeks of age and feather cover post molt improved as visible naked spots were greatly reduced. Feather scores of 2 were assigned at weeks 80 to 94. Naked spots were visible again by 97 weeks and feather cover continued to deteriorate thereafter.

In conclusion, the flocks surveyed showed a loss of feather cover over the first laying cycle. After molt hens' feather score improved but not to the same condition as at the onset of lay.

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[1]L. F. LaBrash, current graduate student and S. E. Scheideler, Professor and Poultry Extension Specialist, University of Nebraska, Lincoln.

Comparison of Traditional Fasting Molt Versus Non-fasting Low-density, Low Sodium Molt Regimes and the Cystine Requirements During Molt and Post-molt

Leanne F. LaBrash
Sheila E. Scheideler[1]

Introduction

Recent consumer pressure regarding animal care has resulted in the United Egg Producers recommending non-fasting molt procedures that allow hens access to feed during molt. Non-fasting molt is a new management tool available to address animal welfare guidelines. Non-fasting molt regimes dietary requirements and post-molt production expectations are not well defined. The objective of this study was to evaluate fasting and non-fasting molt regimes and cystine requirement to optimize second cycle egg production and re-feathering following molt.

Materials and Methods

Two hundred forty, 65-week-old ISA White laying hens were randomly assigned to 60 cages (4 hens/cage) in an augmented factorial arrangement with two molt treatments (fasting or non-fasting) and four dietary cystine levels (Table 1). Feather meal was used as the cystine source. Molt diets were corn and wheat middlings based. Non-fasted hens received a low energy (2712 kcal/kg), low sodium (0.05%) molt diet fed near 70% of ad libitum intake from 65 to 71 weeks of age. Fasted hens had feed withdrawn until 25% BW loss was achieved. After losing 25% BW, fasted hens received an intermediate molt diet until week 7. After week 7 all birds were offered a peak post-molt corn and soybean meal diet fed at 110 g/hen/d until the birds were 91 weeks of age. Photoperiod was 8 h during the 6 week of molt then increased 30 min per week following molt until a 16 h photoperiod was achieved.

Measurements

Fasted hens were weighed daily until 25% BW loss was achieved. Non-fasted hens were weighed every second day for the 6 week molt period. After the 6 week molt period all hens were weighed every two weeks. Feed intake was measured weekly. Egg production was measured daily. Tibia samples were collected for determination of bone ash at the end of the second production cycle.

Results and Discussion

Fasted hens reacted uniformly to induced molt treatment ceasing egg

Table 1. Diets.

	Low Energy, Low Na				Intermediate				Post-Molt			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
Ingredients												
Corn, yellow, %	56.75	56.77	56.77	56.80	53.85	53.59	53.32	58.86	68.89	69.96	71.04	72.11
Wheat midds, %	35.05	34.40	33.75	33.11	35.56	35.04	34.73	29.89	—	—	—	—
Soybean meal, %	—	—	—	—	—	—	—	—	15.87	14.33	12.79	11.25
Feather meal, %	1.81	2.59	3.36	4.16	0.70	1.35	2.00	2.75	0.009	0.73	1.44	2.17
Mineral premix, %	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075
Vitamin premix, %	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075
Tallow, %	0.88	0.80	0.72	0.67	3.00	3.00	3.00	1.60	2.54	2.30	2.06	1.81
Methionine, %	0.125	0.101	0.077	0.054	0.119	0.099	0.080	0.058	0.116	0.100	0.084	0.068
Lysine, %	0.582	0.545	0.508	0.472	0.649	0.618	0.586	0.566	0.306	0.317	0.328	0.338
Limestone, %	2.77	2.77	2.77	2.78	4.07	4.08	4.09	4.07	9.69	9.69	9.70	9.71
Dical. Phosphate, %	1.88	1.88	1.86	1.84	1.92	1.90	1.89	1.92	2.22	2.22	2.21	2.21
Salt, %	—	—	—	—	0.17	0.17	0.17	0.15	0.20	0.20	0.19	0.19
Nutrient Composition:												
M.E., kcal/kg	2712	2712	2712	2712	2756	2756	2756	2756	2856	2856	2856	2856
CP, %	12.34	12.91	13.55	14.03	11.80	11.98	12.39	12.58	13.54	13.15	13.42	13.54
Ca, %	1.52	1.52	1.48	1.48	1.90	2.01	1.88	1.94	4.20	4.56	4.47	4.40
P, %	0.95	0.93	0.93	0.96	0.91	0.92	0.90	0.86	0.79	0.78	0.81	0.72
Na, %	0.05	0.04	0.05	0.05	0.07	0.05	0.07	0.06	0.11	0.07	0.07	0.07
Met, %	0.29	0.29	0.26	0.26	0.28	0.26	0.26	0.31	0.31	0.28	0.27	0.27
Cys, %	0.31	0.37	0.41	0.44	0.30	0.30	0.34	0.35	0.26	0.28	0.29	0.34
TSAA, %	0.60	0.66	0.67	0.70	0.58	0.56	0.60	0.66	0.57	0.56	0.56	0.61
Lys, %	0.78	0.77	0.73	0.79	0.81	0.75	0.73	0.76	0.77	0.74	0.69	0.70
Thr, %	0.40	0.42	0.45	0.49	0.37	0.38	0.40	0.41	0.45	0.46	0.44	0.46

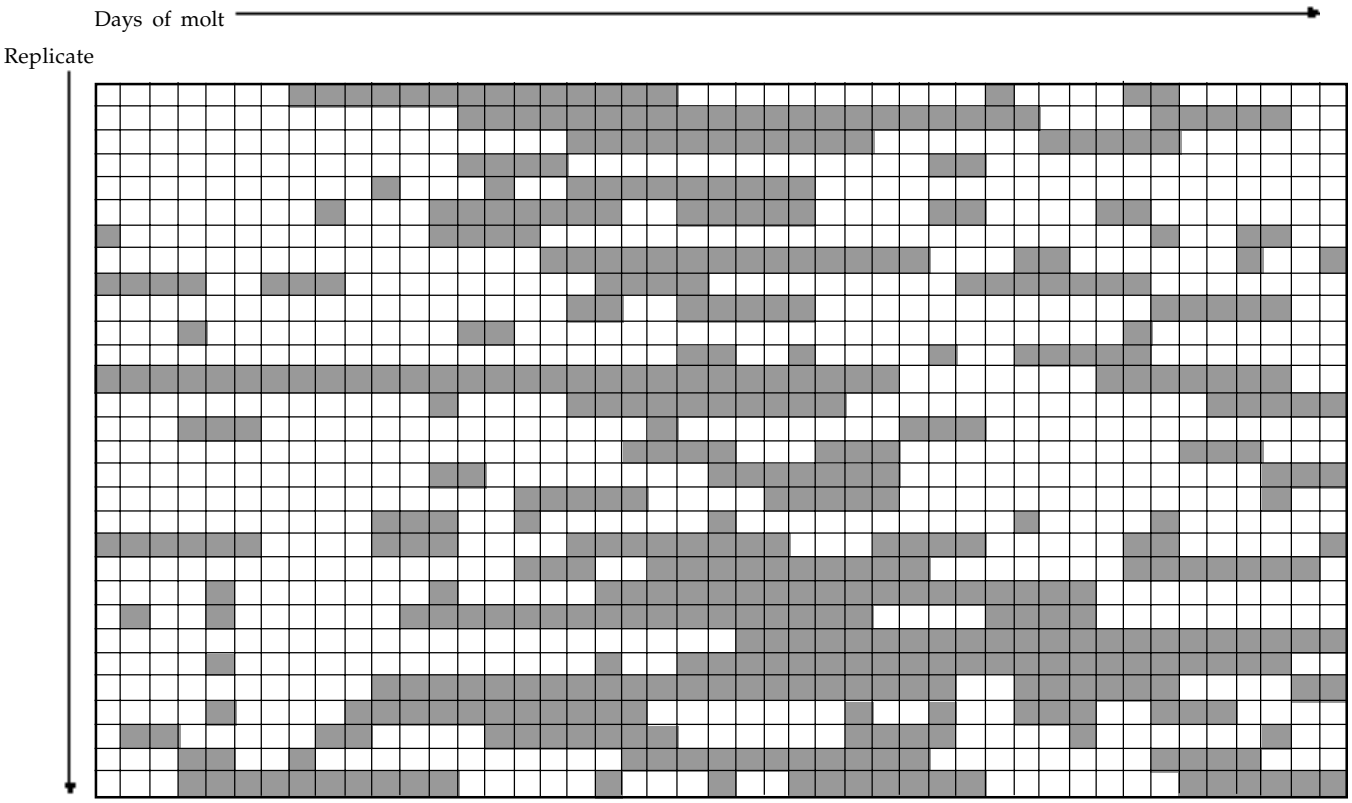

Figure 1. Non-fasted hen egg production during molt period. Open boxes represent days on which at least one egg was laid in each cage of 4 hens. Colored boxes represent days on which no eggs were laid.

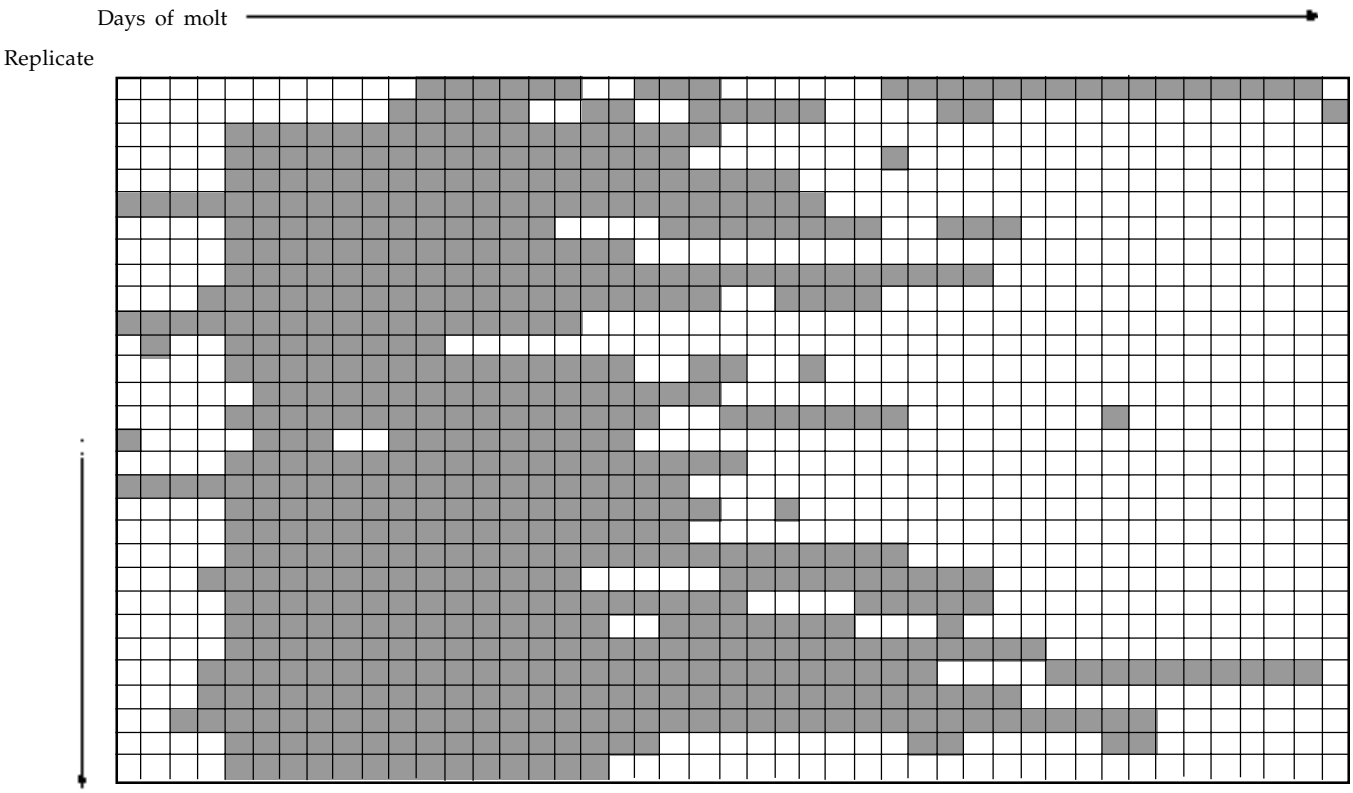

Figure 2. Fasted hen egg production during molt period. Open boxes represent days on which at leaset one egg was laid in each cage of 4 hens. Colored boxes represent days on which no eggs were laid.

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Table 2. Sulfur amino acids intake in fasted and non-fasted molt and post-molt diets.

	Fasted				Non-fasted			
	Met (mg)		Cys (mg)		Met (mg)		Cys (mg)	
Diet	Molt	Post-molt	Molt	Post-molt	Molt	Post-molt	Molt	Post-molt
I	205.95	283.33	198.22	272.69	202.09	280.97	217.87	237.87
II	195.19	262.36	208.21	279.87	193.29	258.80	252.29	251.26
III	187.38	251.36	231.09	309.98	184.69	149.86	289.79	268.46
IV	191.36	250.47	243.853	19.18	172.88	242.25	297.02	297.73

production in 3 days. Non-fasted hens did not react as consistently to induced molt and took from 3 to 33 days to cease egg production. Fasted hens remained out of lay for an average of 21 d while non-fasted hens remained out of lay for an average of 11 d (Figures 1 and 2).

Pre-molt body weights of fasted and non-fasted hens were not different (P>0.05) and the average body weight for the flock was 1.5 kg. Body weight loss during the molt period was significantly different (P<0.05) between fasted and non-fasted hens. Non-fasted hens experienced less body weight loss (10% pre-molt body weight) than the fasted hens (25% pre-molt body weight). Post-molt second production cycle body weights for the fasted and non-fasted hens were not different.

Non-fasted hens consumed 69.0 g of feed per hen per day during the molt period and fasted hens consumed 68.7 g of feed per hen per day on the molt recovery diet. During the post molt period fasted hens consumed 92.2 g of feed per day and non-fasted hens consuming 90.8 g. Total feed intake was not significantly different between the molt treatments (Figure 3).

Table 3. Egg weight (g) and post-molt methionine, cystine and TSAA levels (mg).

Diet	Egg Weight	Methionine	Cystine	TSAA
	(g)	(mg)	(mg)	(mg)
I	62.3	282.15	255.28	537.43
II	60.2	260.58	265.57	526.15
III	60.6	250.61	289.22	539.83
IV	60.2	246.36	304.46	554.82

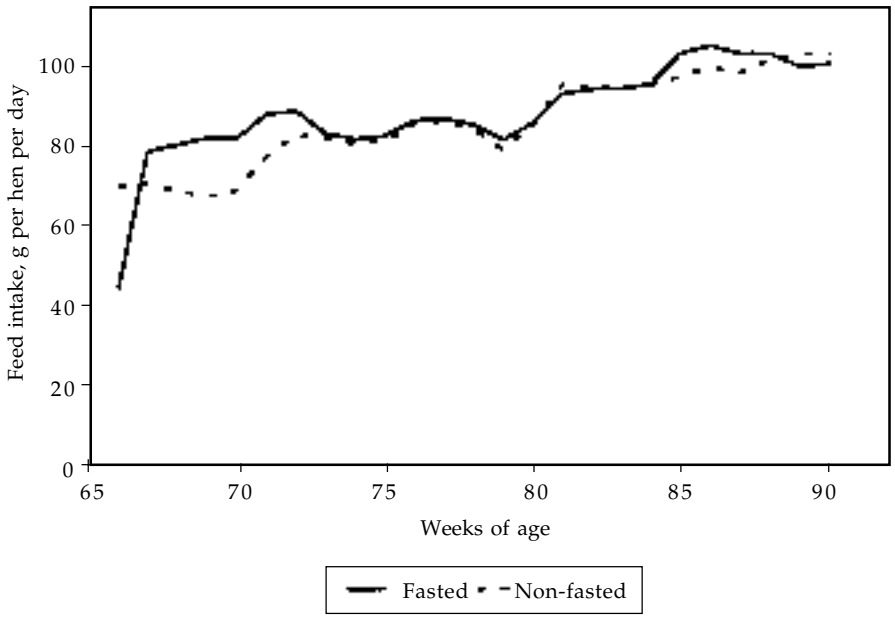

Figure 3. Feed intake.

Total eggs produced were not different for the fasted and non-fasted molt group. The fasted group and non-fasted group total hen day production was 0.77 and 0.72 respectively (Figure 4). Total eggs produced post-molt was affected by dietary cystine level. Hens consuming Diet I (282 mg Met, 255 mg Cys/hen/d) during molt and post-molt had the best hen day egg production (0.70) while hens consuming Diet IV (246 mg Met, 308 mg Cys/hen/d) had the poorest (0.62).

The NRC (1994) suggests a total of 580 mg of sulfur amino acids per day be available for white egg commercial laying hens with 300 mg of sulfur amino acids supplied as methionine. The remainder of sulfur amino acids required may be supplied by cystine. Diets fed in this trial were low in methionine and TSAA (Table 2) as feather meals methionine contribution was overestimated. Methionine levels were low enough to constitute a marginal methionine deficiency. Egg weight also was affected by the marginal methionine deficiency (Table 3). Diet I had greatest egg weight (62.3 g) and greatest methionine level (282 mg per hen per day) while Diet IV had the lowest methioinie level (246 mg/hen/d) and

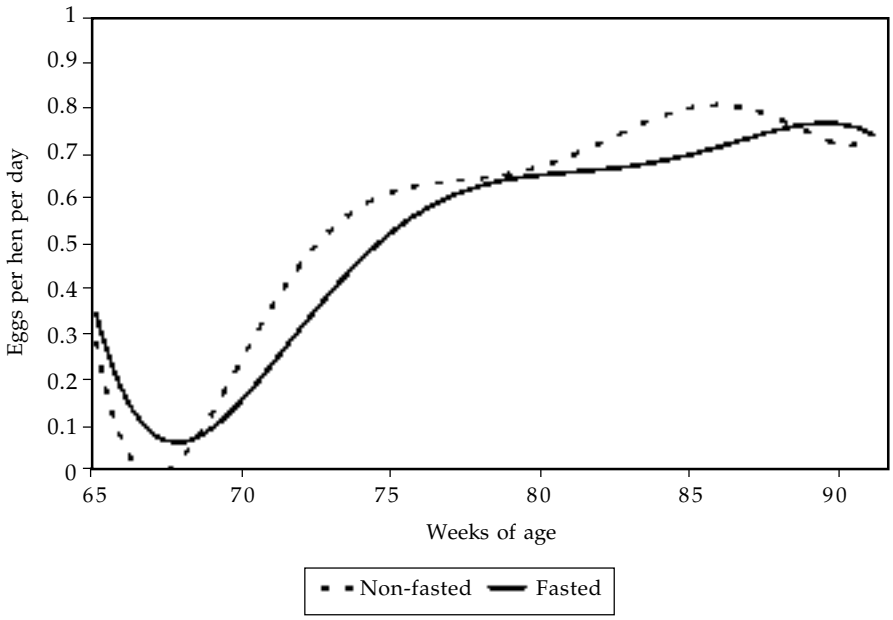

Figure 4. Hen day egg production.

an average post molt egg weight of 60.2 g.

Molt and post-molt mortality were not affected by molt treatment and there were no differences in bone ash.

Conclusion

Hens molted using a non-fasting molt regime require more time to cease egg production, do not stay out of lay as long, and lose less body weight than hens subject to a fasted molt. Hen day egg production of hens subject to a non-fasting molt was not significantly different from fasted hens. Non-fasting molt is a viable tool for molting laying hens within current animal welfare guidelines.

Increasing cystine supplementation levels beyond 255 mg/hen/d while decreasing methionine levels in molt and post molt diets had a negative effect on post molt hen day production.

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[1]Leanne F. LaBrash, current graduate student and Sheila E. Scheideler, professor and poultry extension specialist, University of Nebraska, Lincoln.

Intestinal Calcium Uptake and Reproductive Hormones Levels of Three Laying Hen Varieties After Prolonged Egg Production

Danilo J. Franco
Mary M. Beck[1]

Introduction

It is widely acknowledged that egg-shell quality is affected by many factors such as diseases, nutritional status of the flock, heat stress and age. Economic losses because of poor shell quality around the world are estimated at approximately $500 million per year (Etches, R. 1996). The deposition of calcium carbonate into the shell requires concentrations of calcium in the shell gland fluid 4 and 12 times higher than in blood; the greatest amounts of calcium are obtained from blood, bones and the gastrointestinal tract (Etches, R. 1996). In the domestic hen, the shell gland extracts 2-2.5g of calcium from the blood and transfers the element, without accumulation, to the egg over a period of 15 hours (Eastin and Spaziani, 1978). Intestinal calcium uptake plays an important role in providing the amount of calcium required to perform this task. Estradiol-17β (E_2) has a complex relationship with calcium metabolism and has been shown to increase serum calcium by increasing the density of PTH receptors in kidneys and indirectly increasing the renal activity of 1-α-hydroxylase (Castillo *et al*., 1977). The 1-α-hydroxylase is believed to be responsible for formation of 1,25-dihydroxycholecalciferol (1,25-(OH)2D3) (Martiz *et al*., 1985), which is able to mediate intestinal absorption of calcium and phosphorus (Bar *et al*., 1978) by a saturable trans-cellular route, increasing the concentration of calcium binding protein in the target cells. As the hen ages, the number of eggs produced declines along with a reduction in eggshell quality. It has been reported that E_2 implants improve calcium uptake throughout the intestine (Forman *et al*., 1996: Hansen *et al., 1998).* The increase in egg size that age brings along with insufficient calcium carbonate secretion consequently results in a reduced thickness of the eggshell (Etches, R. 1996). Numerous studies have been

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conducted to address poor eggshell quality but fewer studies have been done to compare differences in intestinal calcium uptake and eggshell quality relative to hormone profiles in older laying hens of different strains.

The objective of this study was to compare Hy-Line W-36, Brown, and W-98 hens' intestinal calcium uptake rate, eggshell quality (specific gravity and eggshell thickness), and physiological levels of reproductive hormones after prolonged egg production (more than 100 weeks of age).

Materials and Methods

As part of a larger study, these strains of Brown, W-36 and W-98 were maintained in production longer than 100 weeks and hens of each strain were randomly selected for *in vitro* calcium uptake (CaT) by intestinal duodenal cells. Blood samples were collected 4-6 hours before oviposition via brachial vein for plasma estrogen (E_2), luteinizing hormone (LH) and progesterone (P_4) determinations. The hens then were euthanized by cervical dislocation, and immediately a 3-cm segment from the mid-duodenal loop was cut into six thin slices and incubated in calcium transport buffer containing 140 mM NaCl, 4.8 mM KCl, 1.2 mM KH2PO4, 1.2 mM MgSO4, and 25 mM HEPES at 7.40 for 10 min at 37ºC (5 mM glucose and 0.5 mM $CaCl_2$ were added the same day of assay). The assay itself consisted of incubation for 4 and 9 min at 37ºC in a calcium transport buffer containing ^{45}Ca (25,000cpm/100µL). The reaction was terminated by transferring the slices at 10-second intervals to beakers containing 300 mM of mannitol. ^{45}Calcium was extracted from the tissue in 2 mL of 2.5% trichloroacetic acid (TCA) for 60 min at 37ºC. The extracts were centrifuged for 5 min at 500 x g, and 1 mL of the supernatant was placed in scintillation vials. Finally, 7 mL of scintiverse was added and the activity of ^{45}Ca was counted in a β-counter. Calcium uptake rates were expressed as nanomoles per gram of duodenal tissue per min. For eggshell quality, specific gravity (SG) and eggshell thickness were determined. Specific gravity (SG) is a non-invasive method to determine eggshell thickness and, therefore, eggshell quality. The SG of an egg is equal to the egg's density relative to water. To perform the SG determination, we immersed the eggs in a series of increasingly concentrated salt solutions until the eggs floated on the surface of one of the solutions. Every solution was checked using a hydrometer. The eggs were broken after SG determination to obtain direct measurements of eggshell thickness. Progesterone and LH were assayed by radio-immunoassay (RIA) validated for chicken at the Animal Science Physiology Laboratory, University of Nebraska-Lincoln. Estrogen concentrations were obtained by RIA using a standard kit and also by the RIA ether extraction concentration method.

The data were analyzed in an ANOVA, using a completely randomized design, and the differences among means were determined by least significant difference (LSD) with a level of significance of $\alpha = 0.05$.

Table 1. Intestinal calcium uptake.

Variable	Brown (n=15)	W-36 (n=14)	W-98 (n=15)
Calcium transport rate (nMol/g/min)	38.13±7.85 B	81.95±8.12 A	48.00±7.85 B
Total calcium uptake	869.7±44.7 B	1060.97±46.3 A	967.13±44.7 AB

The values are expressed as a mean plus minus standard error of mean, different letters represent differences among means (P<0.05) based on LSD test.

Table 2. Plasmatic concentrations of Progesterone (P4).

Variable	Browns	W-36	W-98
Progeterone (P4) Assay # 1 (pg/ml)	(n=5) 1.66±0.3 A	(n=5) 1.61±0.3 A	(n=5) 1.87±0.3 A
Progesterone (P4) Assay # 2 (pg/ml)	(n=5) 1.459±0.3 A	(n=5) 2.0±0.3 A	(n=5) 2.23±0.3 A
Progesterone (P4) Assay # 3 (pg/ml)	(n=5) 1.486±0.3 A	(n=5) 1.4±0.3 A	(n=5) 1.33±0.3 A

The values are expressed as a mean plus minus standard error of mean, different letters represent differences among means (P<0.05) based on LSD test.

Table 3. Plasmatic concentrations of Luteinizing Hormone (LH).

Variable	Browns	W-36	W-98
(LH) Assay # 1 (ng/mL)	(n=5) 3.52±0.8 A	(n=5) 2.97±0.8 A	(n=5) 4.88±0.8 A
(LH) Assay # 2 (ng/mL)	(n=5) 4.66±1.04 A	(n=5) 4.75±1.04 A	(n=5) 3.25±1.04 A
(LH) Assay # 3 (ng/mL)	(n=5) 2.68±0.5 A	(n=5) 3.15±0.6 A	(n=5) 2.71±0.5 A

The values are expressed as a mean plus minus standard error of mean, different letters represent differences among means (P<0.05) based on LSD test.

Results

Both rate of and absolute amounts of intestinal calcium taken up were higher in W-36 hens than in the other two strains (Table 1).

Plasma concentrations of both P_4 and LH were the same among the three strains (Tables 2 and 3). E_2 was problematic, in that use of a commercial kit (that at first seemed promising and was validated for chicken) proved to be unreliable. In the three assays conducted with the kit, the first indicated higher E_2 concentrations in

Table 4. Plasmatic concentrations of Estrogen, Commercial kit (E2).

Variable	Browns	W-36	W-98
Estrogen (E2) Assay # 1 (pg/mL)	(n=5) 315.35±28.1 A	(n=5) 150.02±28.1 B	(n=5) 225.42±28.1 B
Estrogen (E2) Assay # 2 (pg/mL)	(n=5) 209±22.9 A	(n=5) 193.44±22.9 A	(n=5) 192.09±22.9 A
Estrogen (E2) Assay # 3 (pg/mL)	(n=5) 144.84±12.2 A	(n=4) 119.96±13.7 AB	(n=5) 98.61±12.2 B

The values are expressed as a mean plus minus standard error of mean, different letters represent differences among means (P<0.05) based on LSD test.

Table 5. Plasmatic concentrations of Estrogen, Extraction method (E2).

Variable	Browns	W-36	W-98
Estrogen (E2) Assay # 1 (pg/mL)	(n=15) 462.39±30.3 A	(n=14) 458.73±31.4 A	(n=15) 453.07±30.3 A

The values are expressed as a mean plus minus standard error of mean, different letters represent differences among means (P<0.05) based on LSD test.

Table 6. Shell quality. (Specific gravity, Egg shell thickness).

Variable	Browns	W-36	W-98
Specific gravity	(n=71) 1075.14±0.3 A	(n=71) 1075.7±0.3 A	(n=71) 1075.21±0.3 A
Egg shell thickness (mm)	(n=63) 0.33±0.004 A	(n=63) 0.3285±0.004 A	(n=63) 0.3346±0.004 A

The values are expressed as a mean plus minus standard error of mean, different letters represent differences among means (P<0.05) based on LSD test.

W-36; the second showed no differences among strains; and the third one, that Browns had higher E_2 concentrations than the other two strains (Table 4). In addition, the absolute values of E2 were much different than those reported in the literature for laying hens, even accounting for the advanced age of these birds, and they were inconsistent between assays. To resolve the discrepancy, the blood analysis was rerun using the standard ether extraction method for E_2 assays. We believe this to be more realistic, and have chosen to base discussion and conclusions on it rather than on the other assay results (Table 5). Both specific gravity of eggs and eggshell thickness were essentially the same in all three strains (Table 6).

Discussion and Conclusions

Genetic strain has a direct effect on egg size and shell quality; in this case, eggs of Brown hens were larger than those of W-36 but not detectably larger than those of W-98. Specific gravity of W-98 eggs, however, was greater than the other two. Regardless, each hen has a physiological limit to the amount of calcium she can absorb. For any strain, as hen's age, the ability to build skeletal mass and to absorb calcium decreases. In this study, all three strains were well past the typical laying age of commercial hens, though all were still in production, laying at a rate of about ~40%. The hens had not been molted, though it is likely that many of them had experienced at least a partial, spontaneous molt.

Based on the calcium uptake data obtained, the W-36 hens retained a higher capacity at the cellular level than the other two strains, in spite of circulating E_2 that did not differ. It is of interest that the apparent advantage with regard to calcium transport ability did not transfer an advantage to the shell, since the specific gravities and shell thicknesses were all equivalent. The mechanism behind this observation warrants further investigation, with focus on estrogen-induced enzymatic (1a-hydroxylase) activity in the kidney and on 1α-hydroxylase induced estrogen receptor regulation at the gut. The skeletal integrity of hens of this age should also be examined to determine whether there is an interaction between bone calcium and the ability of the gut to transport calcium.

In conclusion, the W-36 hen, at >100 weeks of age and still in lay, appears to be more efficient with regard to the ability to sustain calcium uptake by the cells of the duodenum even though circulating E_2 and eggshell thickness are about the same as in the other two strains (W-98 and Brown).

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[1]Danilo J. Franco, graduate student, and Mary M. Beck, professor, Animal Sciences, University of Nebraska, Lincoln.

Effect of Heat Stress on Productive Parameters Observed With Three Varieties of Laying Hens

Danilo J. Franco
Mary M. Beck[1]

Introduction

During summer high environmental temperatures can be extremely hazardous for laying hens, not only due to increased mortality, but also because of a reduction in the number and quality of the eggs produced during heat stress. Whereas studies on alleviation of heat stress have focused on costly management adjustments, genetic improvement of heat tolerance may provide a low-cost solution, particularly attractive to developing countries with hot climates. Genetic variation of susceptibility to heat stress (HS) has been shown to exist between breeds (Fox, 1951, 1980) as well as among sire and dam families (KheirEldin and Shaffner, 1954) for mortality rates as well as for the bird's ability to control increases in body temperature (Lee *et al*., 1945). Genotype by environment interaction usually is described as a situation in which different genotypes (breeds, lines, or strains) respond differently to contrasting environments (Sheridan, 1990). During heat stress, blood flow to unfeathered extremities (comb and leg) and other tissues such as tongue, larynx and trachea increases to aid loss of heat by radiation, conduction and convection (Nolan and Sturkie 1978, Van Kampen 1984, Richards, S, 1971). Birds exhibit a variety of panting patterns to loose heat as water vapor (Richards, 1970). The objective of this study was to evaluate the productive performance of three varieties of Hy-line laying hens (Brown, W-98, and W-36), when exposed to heat stress.

Materials and Methods

Thirty hens of each strain (Brown, W-36, W-98) at 45 weeks of age, were allowed to acclimatize for one week at 22ºC, and then were exposed to heat stress (35ºC) for two weeks with an additional week at 22ºC to recover. Egg production, feed intake, egg-shell quality, and mortality were measured. Reproductive hormone levels were obtained from plasma collected the first day of exposure to HS and at day twelve after exposure, to evaluate adaptation to higher temperatures. The data for production parameters were analyzed using the SAS program 1999 version 8.0 as repeated measure ANOVA in a 3 x 3 factorial experiment, with strain as one factor and experimental phase as the other. The reproductive hormone data were analyzed using the SAS program 1999 version 8.0 as repeated measure ANOVA in a 3 x 2 factorial experiment, with strain as one factor and phase during HS as the other.

Results

Although overall egg production and feed intake were lower during HS compared with the phases before and after HS ($P<0.05$), egg production for the strain W-98 was not reduced

Table 1. Egg production, feed intake, and egg weight parameters for strain during each phase of the experiment

	Hy-Line Brown			Hy-Line W-98			Hy-Line W-36		
Variable	Before HS	During HS	After HS	Before HS	During HS	After HS	Before HS	During HS	After HS
Egg production, %	66.35±4.9 A	34.42±3.8 B	49.32±4.99 C	47.14±4.9 A	50.62±3.8 AB	63.21±4.9 B	50.53±4.9 A	38.03±3.8 B	50.39±4.9 A
Feed intake, g/hen/day	96.45±2.7 A	41.92±2.3 B	92.63±2.7 A	90.72±2.7 A	58.78±2.3 B	96.97±2.7 C	72.11±2.7 A	46.53±2.3 B	81.72±2.7 C
Egg weight, g	63.16±1.1 A	58.82±1.4 B	58.12±1.1 B	60.58±1.1 A	55.43±1.4 B	57.67±1.1 AB	58.34±1.1 A	47.46±1.4 B	49.32±1.1 B
Yolk weight, g	16.58±0.3 A	16.45±0.2 A	14.08±0.3 B	16.41±0.3 A	16.36±0.2 A	15.11±0.3 B	16.51±0.3 A	15.71±0.2 A	12.74±0.3 B
Shell weight, g	7.61±0.1 A	6.84±0.2 B	7.73±0.1 A	8.05±0.1 A	6.97±0.2 B	8.14±0.1 A	7.67±0.1 A	5.82±0.2 B	7.02±0.1 C
Albumen weight, g	38.79±0.8 A	35.47±1.3 B	36.27±1.2 A	35.26±0.9 A	33.01±1.3 A	34.14±1.1 A	33.79±0.8 A	26.45±1.3 B	28.61±1.4 B

The values are expressed as a mean plus minus standard error of mean, different letters represent differences among means ($P<0.05$) within each strain based on LSD test.

Table 2. Specific gravity and Haugh unit parameters for strain during each phase of experiment

Variable	Hy-Line Brown			Hy-Line W-98			Hy-Line W-36		
	Before HS	During HS	After HS	Before HS	During HS	After HS	Before HS	During HS	After HS
Specific gravity	1.0801 A	1.0721 B	1.0858 C	1.0836 A	1.0755 B	1.0862 A	1.0826 A	1.0714 B	1.0868 C
Haugh unit	80.45 A	82.11 A	79.48 A	73.04 A	72.25 A	79.61 B	85.37 A	76.93 B	81.41 C

The values are expressed as a mean, different letters represent differences among means (P<0.05) within each strain based on LSD test.

Table 3. Mortality rate observed during the experiment.

Variable	Hy-Line Brown	Hy-Line W-98	Hy-Line W-36
Morality, %	50	6	10

during HS, which could indicate a higher resistance to HS by this strain (Table 1). Egg weight as well as weight and quality of eggshell were affected during HS. The egg weight value was lower during HS than during the phase before and after HS ($P < 0.05$) for all of the strains (Table 1). Specific gravity for Brown, W-98, and W-36 were lower during HS than during the phases before and after HS ($P < 0.05$) (Table2). The albumen weight (Table 1) also was reduced mainly in the Brown and W-36, which showed differences between the phase before and during HS ($P < 0.05$).

The yolk weight was reduced but only during the phase after HS ($P < 0.05$) (Table 1). The Brown hens were more susceptible to HS with a higher mortality rate (Table 3).

The ability of the hens to acclimate to HS was evidenced by an increased in the levels of reproductive hormones (LH, P_4, and E_2) twelve days after exposure (Table 4). The quality of egg albumin measured by Haugh units was significantly reduced during HS only in W-36 hens, but an increase was observed after HS for all the strains except the Brown (Table 2).

Discussion and Conclusions

The results indicate that the Hy-Line Brown strain was more susceptible to HS, with 50% mortality, compared to the other two strains (W-36, 10%; W-98, 6%). This is not surprising, given that the body weight of the Brown hens is considerably greater (1,906.9 Kg vs. 1,444.8 Kg (W-36) and 1,301.6 Kg (W-98)), and that their feather cover was considerably greater. The results of the production variables were generally as expected, with overall decreases in all three strains during heat stress. However, between strains, there were several observations worth nothing. First, the W-98 hens actually increased egg production over the 4 week study, including during HS, in contrast to both Browns and W-36.

During the week of recovery, the W-36s were able to recover to pre-HS production levels, but the Browns were not. Based on observations, it appears that W-98 hens are much less susceptible to HS than either the W-36 or Brown Hy-Line hens with regard to both livability and egg production.

Physiologically, it appears that the Brown hens are the most susceptible, showing the highest mortality rate and lowest production parameters during heat stress. The acclimation of the survivor hens was substantiated by the increase in reproductive hormone levels after 12 days of heat exposure, which can indicate important physiological

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Table 4. Changes in reproductive hormone levels after twelve days of HS exposure.

Variable	Hy-Line Brown		Hy-Line W-98		Hy-Line W-36	
	1st day of HS	Day 12 of HS	1st day of HS	Day 12 of HS	1st day of HS	Day 12 of HS
P_4 ng/mL	N=10 0.19±0.1 A	N=10 1.53±0.4 B	N=10 0.27±0.1 A	N=10 0.757±0.3 A	N=10 0.365±0.1 A	N=10 0.86±0.3 A
LH ng/mL	N=10 4.88±0.5 A	N=10 5.29±0.8 AB	N=10 4.23±0.5 A	N=10 5.75±0.8 B	N=10 4.16±0.5 A	N=10 6.96±0.8 B
E_2 pg/mL	N=10 51.82±11.7 A	N=10 139.9±19.1 B	N=10 67.9±11.7 A	N=10 126.17±19.1 B	N=10 36.9±12.4 A	N=10 139.9±19.1 B

The values are expressed as a mean plus minus standard error of mean, different letters represent differences among means (P<0.05) based on LSD test.

changes in the regulation of the secretion of reproductive hormones during HS.

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[1]Danilo J. Franco, graduate student and Mary M. Beck, Professor, Animal Sciences, University of Nebraska, Lincoln.

Role of Alpha-galactosidase in Corn-Soy Based Layer Rations

Sheila E. Scheideler
Trish Weber[1]

Introduction

Soybean meal is a component of nearly every poultry ration formulated for the laying hen in the Midwest. It is the major source of highly available protein for poultry but unfortunately is not a great source of metabolizable energy. Gross energy of soybean meal actually exceeds the gross energy of corn, but due to complex carbohydrates, the actual energy in soybean meal available to poultry (metabolizable energy) is less than corn. Oligosaccarides staffinose and raffinose are carbohydrates in soybean meal which are not easily digested by poultry due to the inherent absence of galactosidase in the hen's digestive tract. It is hypothesized that the addition of alpha-galactosidase in the hen's diet could improve digestibility of soybean meal's carbohydrate fraction. However little research data is available in laying hens at present to support this hypothesis.

The objective of the research reported herein was to test the effectiveness of supplementing an alpha-galactosidase to laying hen diets giving an energy value to the enzyme during ration formulation.

Experimental Design and Measurements

Four diets were fed to Hy-Line W-36 pullets from 20 to 50 weeks of age. The experimental design was a 2 x 2 factorial arrangement of 2 basal diets (Control or Negative Control) with or without enzyme supplementation. The enzyme utilized in this study was Loders-Croklaan's alpha-galactosidase at an inclusion rate of .04% of the ration. Diets were reformulated every 3 weeks by a commercial egg industry modeling program to meet the hens' nutrition needs according to feed intake, rate of egg production, egg weight and body weight gain. Diet formulations are shown in Table 1 for the various feeding periods. The enzyme was given an energy value in the formulation matrix allowing for less total ME in the negative control diets with the expectation of more energy available from the soybean meal in the ration (estimated to be 248kcal/kg more from soybean meal).

Each of the 4 diets was fed to 18 replicate cages with six hens/cage (54 sq.in./hen). Measurements during the trial included daily egg production and feed intake. Hen body weight, egg weight and specific gravity were measured every third week and a nutrient digestibility study was conducted at the end of the trial.

Statistical analysis of the data was conducted using Proc Mixed procedure from SAS testing for main effects of diet and enzyme supplementation.

Results and Discussion

Table 1 shows the experimental diets utilized from 23 to 40 weeks of age. The price differential between the control diets and the negative control diets ranged from $9.12 to $4.23 during this time. The price difference was due to the energy value contribution predicted from the addition of alpha-galactosidase in the negative control rations. Less energy from fat

Table 1. Experimental diets.

Series	C (23-25 wks)		D (26-28 wks)		E (29-31 wks)		F (32-34 wks)		G (35-37 wks)		H (38-40 wks)	
Ingredient	Control	(-)Control	Control	(-)Control	Control	(-)Control	Control	(-)Control	Control	(-)Control	Control	(-)Control
Corn	53.3	56.5	51.1	52.9	50.4	52.4	52.0	54.4	53.7	56.0	54.9	57.10
Soybean meal	29.6	28.0	30.4	29.9	30.7	30.2	30.1	29.4	28.8	28.2	27.9	27.4
Tallow	4.0	2.83	4.0	4.0	5.0	4.0	5.0	3.44	4.9	3.2	4.7	3.1
Corn oil	0.54	—	1.28	—	0.63	0.08	0.21	0.08	—	—	—	—
Large particle limestone	5.19	5.19	5.80	5.80	5.80	5.80	4.9	4.9	5.5	5.5	5.5	5.44
Fine limestone	4.31	4.32	4.50	4.50	4.50	4.50	4.9	4.9	4.2	4.3	4.3	4.25
Dicalcium phosphate	2.34	2.34	2.30	2.30	2.30	2.30	2.27	2.26	2.2	2.2	2.1	2.12
Salt	0.42	0.42	0.41	0.41	0.41	0.41	0.40	0.40	0.39	0.39	0.38	0.38
Methionine	0.17	0.18	0.15	0.15	0.14	0.14	0.15	0.16	0.15	0.15	0.14	0.14
Mineral premix	0.06	0.06	0.06	0.06	0.06	0.06	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin premix	0.06	0.06	0.06	0.06	0.06	0.06	0.05	0.05	0.05	0.05	0.05	0.05
$/ton	$111.65	$102.53	$117.14	$108.90	$115.85	$109.62	$112.52	$107.37	$109.22	$104.78	$107.61	$103.38
Calculated Analysis:												
M.E. (Kcal/lb)	1,316	1,309	1,321	1,314	1,321	1,313	1,323	1,316	1,322	1,316	1,325	1,318
Protein, %	18.6	18.14	18.72	18.73	18.8	18.8	18.66	18.62	18.2	18.15	17.87	17.86
Calcium, %	4.2	4.2	4.47	4.47	4.51	4.51	4.28	4.28	4.26	4.25	4.23	4.22
aP, %	0.53	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.51	0.51	0.49	0.49
Methionine, %	0.48	0.49	0.46	0.46	0.46	0.46	0.46	0.47	0.45	0.45	0.44	0.44
TSAA, %	0.80	0.80	0.78	0.78	0.78	0.78	0.78	0.78	0.76	0.76	0.75	0.75

Table 2. Egg production data by periods.

			Feed Intake				Egg Production			
Diet[1]	M.E.	Enzyme	Early (20-28 wks)	Peak (29-40 wks)	Post (41-49 wks)	Mean	Early (20-28 wks)	Peak (29-40 wks)	Post (41-49 wks)	Mean
1	Control	(-)	80.0[a]	102.4[a]	104.7[a]	95.7	71.8[a]	94.1[a]	84.7[a]	83.5
2	(-) Control	(-)	78.5[b]	100.5[b]	101.3[b]	93.5	67.8[b]	91.1[b]	84.1[a]	81.0
3	Control	(+)	79.2[b]	100.4[b]	102.4[b]	94.1	69.6[a]	95.4[a]	87.9[a]	84.3
4	(-) Control	(+)	80.6[a]	100.8[b]	102.8[ab]	94.8	70.7[a]	93.3[a]	87.4[a]	83.8
Probabilities										
Diet			0.05	0.08	0.04	NS	0.02	NS	NS	NS
ME			NS	NS	NS	NS	NS	NS	NS	NS
ENZ			NS	NS	0.06	NS	NS	NS	NS	NS
ME x ENZ			0.01	0.05	0.02	NS	NS	0.10	NS	NS
Period			0.001	0.001	0.001	NS	0.001	0.001	0.001	0.001
Diet x Period			NS	NS	NS	NS	NS	NS	NS	0.001

Table 3.Egg measurements by periods.

			Egg Weight				Egg Specific Gravity			
Diet	M.E.	Enzyme	Early (20-28 wks)	Peak (29-40 wks)	Post (41-49 wks)	Mean	Early (20-28 wks)	Peak (29-40 wks)	Post (41-49 wks)	Mean
1	Control	(-)	51.0	57.5	59.4	56.0	1.0846	1.0829	1.0810	1.083
2	(-) Control	(-)	51.3	57.1	58.9	55.8	1.0848	1.0834	1.0826	1.084
3	Control	(+)	51.5	57.2	59.4	56.0	1.0853	1.0835	1.0820	1.084
4	(-) Control	(+)	51.1	56.8	59.1	55.7	1.0855	1.0836	1.0823	1.084
Probabilities										
Diet						NS				NS
Period						.0001				.0001
Diet x Period						NS				NS

[1]Each diet fed to 18 replicate cages with 6 Hy-Line W-36 hens per replicate cage (54 sq. in./hen) from 17-49 weeks of age.

sources was needed in these diets. The negative control rations do not include the cost of the enzyme addition.

Table 2 shows the dietary effects on feed intake and egg production broken down into 3 periods of lay: 1. Early (20-28 weeks of age), 2. Peak (29-40 weeks of age) and 3. Post Peak (41-49 weeks of age). Feed intake was consistently affected by diet. This is surprising as one would expect the hens to respond to the negative control lower ME diets with increased feed intake to meet their energy needs. In fact, the opposite was observed in this trial as hens consumed less of the negative control ration compared to hens on the control ration. Supplementation of enzyme significantly

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reduced feed intake in hens fed the control diets but not in hens fed the negative control diets (as shown by the significant ME x Enzyme effects ($p<.05$) during each period of egg production. Feed intake would be expected to increase as the hens aged. Early egg production was significantly affected by diet. Hens fed the negative control diet had lower egg production compared to the control fed hens. Supplementation of the enzyme improved egg production of hens on the negative control diets during all 3 periods of egg production. This affect was only significant during the peak egg production period.

Table 3 presents the egg weight and egg specific gravity data. Diet and enzyme supplementation had no significant effects on egg weight or egg specific gravity during any time period of egg production. Egg weight increased and specific gravity decreased as the hens aged as would be expected.

In conclusion, this study supports the hypothesis that addition of an alpha-galactosidase enzyme improves metabolizable energy available from soybean meal. Hens performed very well on diet formulations reducing the energy available from fat sources and relying on more energy from soybean meal when alpha-galactosidase was added to the rations. Cost savings in ration formulation can be substantial with addition of alpha-galactosidase to layer rations and equal performance to control diets.

Ackowledgements

Funding for this research was provided by Loders-Croklaan, 800 East Roosevelt Road, Bldg D, Suite 102, Glen Ellyn, IL. 60137-5864.

[1]Sheila E. Scheideler, Professor and Extension Poultry Specialist, and Trish Weber, Agricultural Research Technician, Animal Science, Lincoln.

Key Points in Mixing and Delivery of Microingredients in Feed Formulations

David Monsalve[1]

Introduction

Microingredients in animal feeds are components incorporated at inclusion levels below 500 ppm. The addition of microingredients directly into the main mixer, with or without prior premixing can affect the final quality of the feed. Sometimes this step can even cause economic losses to the poultry producer. We need to keep in mind standards goals in premixing and final feed mixing. Data to assist in accurate feed mixing include: make a correct evaluation of our mixer (determine periodically the coefficient of variation), checking the mixing time for different types of formula, checking physical properties of the ingredients (hygroscopicity, angle of repose, static chargeability, particle size, among others), and the effect of sequence of ingredients addition to the mixer, type of carrier, type of binding agents etc.

A better way to include a microingredients without compromising their effectiveness can be reached through a premixing step; this is a pre-step to final feed mixing. Reasons for premixing vary from convenience to an essential step to optimize microingredient particle distribution in the feed. For example, some commercial enzymes or vitamins, depending upon the concentration, can be added in the final feed in a ratio that ranges from 40 to 100 g per metric ton feed.

This review will be focused on some important aspects to be considered when adding microingredients to poultry diets.

1. Liquid binders:

Liquid binders act as antistatic agents, preventing particles from desegregating and attaching to the equipment, act as anti-dust agent, reducing losses through the dust control systems and reducing occupational hazards such as smell, irritation, allergies and possible dust explosion. For example, rice hulls, by themselves are a poor carrier since their shiny and slick surfaces are not able to hold active ingredients, therefore disqualifying it as a premix carrier. However, the addition of a liquid binder to rice hulls improves drastically their holding capacity. Liquid binders also allow more active ingredients to be adsorbed by the carrier, turning it into an adequate carrier.

2. Carriers and microingredients:

Type of Carriers: Carrier is defined as a substance capable of accepting and holding a fine powder without segregation or separation over time. We can divide carriers into two major classes: Organic (rice hulls, wheat bran), Inorganic (limestone, dicalcium phosphate).

Particle Size: The carrier particle size should be large enough to produce a premix with good flowability and low dust, and small enough to hold the active ingredients without segregation.

Bulk Density: Limestone is the most common carrier for trace mineral premixes while rice hulls are the most common for vitamin, enzyme and drug feed additive premixes. The benefits of dense premixes, such as increasing throughput in the premix plant, allow more pallets to be stacked on top of each other and flow faster in

the microproportioners at the feed plant.

Moisture: Premix ingredients should have a moisture ≤ 5 % to reduce the potential of reduction-oxidation reactions. Rice hulls and limestone have a standard moisture ≤ 5 %. Other organic carriers such as wheat bran, and corn cobs have a moisture > 12% which should be dried to <5% before they can be considered acceptable carriers.

Fat Content: Carriers should have ≤ 4% fat content, since fat oxidation destroys several vitamins, and enzymes. Inorganic carriers have no fat, rice hulls have 2-4% fat.

3. Premix Flowability:

Premix flowability has become a critical control point (CCP) in many feed mills. Good flowability reduces labor and improves accuracy and throughput. In fact, a premix with good flowability acquires this characteristic due to large dense particle size of active ingredients and carriers. Several other factors impact flowability such as moisture and chemical reactions. Hygroscopic microingredients, such as choline, spray dried vitamins, and hydrated trace elements, as well as carriers with 10% moisture, can cause the premix to cake (lump) and turn dark. This problem can be alleviated by reformulating with less hygroscopic components or by adding a free flow agent such hydrophilic silica absorbing the moisture from the microingredients or carriers and allowing the premix to flow more freely.

4. Particle Size and Number of Particles:

Particle size is critical to producing a quality premix and obtaining adequate distribution in the feed. Particle size impacts flowability, stability, static charge and biological response.

Flowability: generally speaking, the smaller the particle size, the poorer the flowability and vice versa.

Stability: the smaller the particle size, the larger the total product surface area, the higher the exposure to oxidation and therefore the greatest potential for enzyme and vitamins loss.

Biological response: the active ingredient particle size should be determined by the fortification level in feeds. The less active ingredient per ton of feed, the smaller the particle size must be to make sure there is an adequate distribution of particles in the feed, and the active microingredient reaches its optimum response.

5. Premix Operation:

Charging the Mixer: The sequence of charging ingredients into the mixer has a major impact on the final quality of the premix produced. The first ingredient charged into the mixer should be the carrier, usually an organic byproduct in the case of vitamin or drug premixes and an inorganic in the case of trace mineral premixes. Oil then is added through spray nozzles and mixed. Finally, the active components are charged into the mixer. There is no specific order for charging active ingredients. The batch is mixed depending on the type of mixer, type of formula following data record for good manufacturing practices plan (GMP's).

Premix Cleaning: GMP's for drug feed additives require very specific cleaning procedures for mixers, conveyors, bins, etc., to prevent contamination from batch to batch. Premix plants dedicated to non-drug premixes have less stringent GMP's that don't require as frequent cleaning of equipment. However, even equipment dedicated to non-drug premixes should be scraped and swept regularly to remove scale build up, especially in the mixer.

Premix Quality Control: The many microingredients handled at a premix plant and the cost of individual microingredient assays have become part of quality assurance practices, during manufacturing. It is not practical due to delivery constraints, nor economical to assay every active ingredient in every premix batch.

Mixing Time: Mixing time varies depending on the type of mixer used. Undermixing will cause poor dispersion of active ingredients throughout the mix, measured by a high coefficient of variation among samples of the same batch.

Over mixing sometimes is blamed for demixing; individual particles with static charges repel each other, causing segregation and poor distribution throughout the premix. Particles with static charges also are attracted to the metal parts of the mixer, and there is an accumulation and deposit of static particles on the shaft and walls of the mixer forming scales.

Mixer Test: Mixers should be tested before or immediately after installation. Quality assurance practices such as quantifying the variation of a nutrient or ingredient in the feed product can help to test the mixer regularly depending on specific needs. Several factors can contribute to poor mixer performance: shafts get out of alignment, ribbons and pads wear out, gates start leaking, changes in mixing time and batch size.

A mixer test involves:

— Perform a normal mixer batch run, including sequence of ingredient addition, mixing time.
— At the end of the batch run, collect 10 samples from the batch. Depending on the type of mixing equipment used, sampling can be done in a number of ways.

Each group of 10 samples from each mixing batch is assayed for a typical active ingredient. This marker must be indicative of the uniformity of other microingredients in the premix. A marker must be a typical microingredient of the premixes made in that mixer (Vitamin A in vitamin

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premixes; copper in trace mineral premixes; or a specific drug in drug blends). The marker is assayed by equipment that consistently gives a very low analytical error (high performance liquid chromatography, gas chromatography, atomic absorption, etc.). The coefficient of variation for each set of 10 samples is indicative of the mixer performance and distribution of the microingredient assayed throughout the mix. A coefficient of variation below 10% is indicative of an adequate distribution of the active ingredient.

6. Quality assurance- GMP's:

Allow for continuous checks throughout the process to assure meeting product specifications when the manufacturing process is completed. Quality control is still conducted to verify the GMP procedures. A few common quality control procedures include:

- Physical examination of every batch for appearance (color, texture) against standard.
- Number of batches assayed on a rotating basis of 1 active ingredient per month.
- Complete assay of one premix at random every month.
- Retain batch samples for 2 years.
- Every batch has an individual batch sheet and is individually charged.
- Complete traceability of raw materials for every batch.

Summary

Adequate monitoring of every step on the mixing process is very important in order to get an optimal ingredient distribution in the feed.

References

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[1]David Monsalve, graduate student, Animal Sciences, University of Nebraska, Lincoln.

Supplementation of Pullet and Layer Diets With a Multi-Enzyme Product

Sheila E. Scheideler
Trish Weber[1]

Introduction

The use of exogenous enzymes in poultry rations has gained considerable application in practical poultry diet formulation. With the introduction of phytase enzymes to the feed market, nutritionists have become more comfortable with the capabilities of feed enzymes and where they might fit in a feed management program. The main function of exogenous enzymes is to supplement and complement digestive activity in the chicken aiding in its digestion of common feed ingredients such as corn and soybean meal, to provide nutrients for growth and egg production.

Avizyme 1500 is a microbial multi-enzyme package with amylase, protease and xylanase activity. It is added to poultry diets to improve starch and protein digestion of corn and soybean meal. Several studies have been conducted in our lab testing the effectiveness of Avizyme 1500 in laying hen corn-soy diet (Scheideler, et al., 1998 and Gomez et al., 1999). These studies only tested the effectiveness in layer rations after hens had come into egg production, showing positive effects of Avizyme 1500 supplementation on egg production parameters. Avizyme 1500 can be formulated into the ration in 2 ways - "over the top," which means the enzyme is added without any adjustments in dietary metabolizable energy (M.E.) or nutrients supplied; or "specified down" in which credit is given to the enzyme for potential energy supplied. We were interested in the potential beneficial effects of Avizyme 1500 supplementation in pullet and/or layer diets on pullet weight gain and subsequent effects on rate of egg production in either an "over the top" or "specified down" formulation scheme for diet M.E.

Experimental Design

A 2 X 3 factorial arrangement of 2 levels M.E. (Control or Low) with 3 Avizyme 1500 supplementation programs: Program 1. No Avizyme 1500, 2. Avizyme in Pullets only (day old - 16 wks of age) and 3. Avizyme in both Pullet and Layer Diets (day old - 40 wks of age). Diets were primarily corn-soybean meal based with some wheat midds and added fat to meet energy needs (Table 1). Pullets were fed 4 diets: Starter from 0-6 weeks; Grower from 6-12 weeks; Developer from 12-16 weeks and then Layer from 16-40 weeks. The six dietary treatments were each

Table 1. Avizyme 1500 in pullet and layer diets — diet composition.

Ingredients	Starter (0-6 wks)		Grower (6-12 wks)		Developer (12-16 wks)		Layer (16-40 wks)	
	Control	Low ME	Control	Low ME	Control	Low ME	Control	Low ME
	(%)							
Corn	62.2	64.8	67.0	70.2	74.6	70.3	47.7	50.2
Soybean meal	31.3	30.9	25.9	25.4	20.7	21.4	29.6	29.2
Wheat midds	—	—	—	—	—	4.10	3.9	4.7
Tallow	2.16	—	2.9	0.24	0.63	—	4.6	2.9
Corn oil	—	—	—	—	—	—	1.0	1.0
Limestone	1.33	1.35	1.43	1.43	1.51	1.66	9.2	9.2
Dicalcium phosphate	1.97	1.96	1.85	1.84	1.77	1.71	1.95	1.90
Salt	0.49	0.49	0.45	0.45	0.45	0.49	0.47	0.47
DL-methionine	0.20	0.20	0.18	0.18	0.16	0.14	0.22	0.22
Lysine	0.13	0.13	0.05	0.06	0.03	—	—	—
Mineral premix	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Nutrients								
M.E., kcal/kg	2995	2905	3080	2970	3030	2940	2900	2770
Protein, %	20.5	20.5	18.2	18.2	16.2	16.2	18.5	18.5
TSAA, %	0.80	0.80	0.72	0.72	0.65	0.65	0.77	0.77
Lysine, %	1.10	1.10	0.90	0.90	0.75	0.77	0.95	0.95
Ca, %	1.00	1.00	1.00	1.00	1.00	1.05	4.0	4.0
aP, %	0.45	0.45	0.42	0.42	0.40	0.40	0.45	0.45

Table 2. Avizyme 1500 in pullet and layer diets — pullet production data.

M.E.	Avizyme 1500	Pullet Body Weight 2	4	6	8	10	12	16	16	C.V.[a]	Avg. Feed Intake
		Weeks									
		(Kg)								(%)	(g/pullet/day)
High	No	0.240	0.351	0.393	0.589	0.799	0.978	1.082	1.140	7. 38	37.00
High	Yes	0.240	0.351	0.396	0.598	0.808	0.979	1.089	1.157	7.44	37.00
Low	No	0.230	0.349	0.379	0.579	0.784	0.957	1.070	1.131	7.29	37.80
Low	Yes	0.242	0.352	0.393	0.590	0.795	0.973	1.085	1.146	7.32	38.00
Main Effects											
M.E.											
High		0.240	0.351	0.395	0.593	0.804	0.978	1.086	1.148	7.41	37.00
Low		0.240	0.350	0.386	0.586	0.789	0.965	1.078	1.140	7.31	37.90
Avizyme 1500											
No		0.232	0.350	0.386	0.584	0.791	0.967	1.076	1.135	7.33	37.40
Yes		0.241	0.351	0.394	0.594	0.802	0.970	1.087	1.151	7.31	37.50
Probabilities											
M.E.		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	NS	0.05
Avizyme		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	NS	NS
M.E. x Avizyme		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	NS	NS

[a]Coefficient of variation for body weight wtihin a replicate pen.

assigned to 8 pens each starting with 10 pullet chicks/cage during the pullet stage and then at time of transfer to the layer cages, only 8 hens/cage (64 sq.in/hen) were transferred.

Measurements during the pullet stage included weekly feed intake, monthly pullet body weights, and cage uniformity of body weight as measured by coefficient of variation. At 16 weeks of age, one pullet was sampled for determination of tibia ash. At 12 weeks of age a nutrient retention study was conducted using the chromic oxide marker method (Scott, et. al, 1976) to determine effects of diet on retention of N, Ca, P and dietary M.E. Pullets were transferred to the layer stage of the study at 16 weeks of age. During the layer stage, daily egg production and feed intake were recorded. Bi-weekly, one day of egg production was sampled to determine egg weight and specific gravity of eggs. Hens were weighed monthly and uniformity of body weight was calculated. A nutrient retention study similar to the one conducted at 12 weeks in the pullets was conducted at 30 weeks of age to determine dietary effects on N, Ca, P retention and M.E..

The trial concluded at 40 weeks of age. At the conclusion, one hen was sampled from each pen to determine tibia ash. Statistical analysis of the

(Continued on next page)

Table 3. Avizyme 1500 in pullet and layer diets — layer production data.

M.E.	Pullet	Layer	Feed Intake	Hen Wt.	C.V.[a]	Egg Production	Egg Wt.	Egg Mass	Specific Gravity
			(g/hen/day)	(kg)	(%)	(%)	(g)	(g/hen/day)	
High	No	No	94.5	1.526	8.07	80.6	54.2	48.0	1.0864
High	Yes	No	99.1	1.510	8.39	79.3	53.8	47.1	1.0856
High	Yes	Yes	97.2	1.512	7.38	81.0	53.5	47.8	1.0860
Low	No	No	93.3	1.462	7.43	81.2	53.1	47.3	1.0864
Low	Yes	No	96.2	1.477	6.82	80.7	54.0	47.8	1.0861
Low	Yes	Yes	96.2	1.515	7.22	79.5	54.6	48.2	1.0858
Main Effects									
M.E.									
High			96.9	1.516	8.08	80.3	53.9	47.7	1.0860
Low			95.2	1.485	7.16	80.4	53.9	47.8	1.0861
Avizyme									
None			93.9	1.494	7.75	80.8	53.7	47.6	1.0862
Pullet only			97.6	1.494	7.61	80.0	53.9	47.4	1.0859
Pullet + Layer			97.7	1.513	7.50	80.2	54.1	48.1	1.0861
Probabilities									
M.E.			NS	0.04	0.02	NS	NS	NS	NS
Avizyme			0.10	NS	NS	NS	NS	NS	NS
M.E. x Avizyme			NS	NS	NS	NS	0.01	NS	NS

[a]Coefficient of variation for body weight within a replicate pen.

data was conducted using Proc Mixed procedure from SAS testing for main effects of diet ME, enzyme supplementation and the interaction effect of diet M.E. by enzyme supplementation.

Results

Table 2 shows the average pullet body weight every 2 weeks, the coefficient of variation for body weight and the average feed intake from 0-16 weeks of age. Both dietary M.E. level and supplementation of diets with Avizyme 1500 had effects on pullet growth rate. Pullet chicks on the High M.E. diet had a greater rate of growth beginning at 6 weeks. Supplementation of enzyme helped bring the pullets on the low M.E. diet to a rate of growth equivalent to hens on high M.E. diet throughout the study. There were times when the high M.E. diet benefitted from Avizyme 1500 supplementation, but the effect was not as consistent as it was in the low M.E. diets. Coefficient of variation of body weight did not indicate any significant effects of diet on pullet body weight uniformity. Average feed intake showed increased in pullets on the low M.E. diets compared to those fed the high level of M.E. with no effect of Avizyme.

Table 3 shows the egg production data averaged from 16 to 40 weeks of age. In this Table, you will see a total of 6 dietary treatments reflecting the addition of Avizyme to layer diets. Addition of Avizyme to pullet and/or layer diets indicated some increase in feed intake by about 4 grams/day. This may have resulted in an increase in average body weight for the pullets fed Avizyme in both the pullet and layer stages, especially for the hens on the low M.E. diets. Hens fed the higher level of diet M.E. weighed more that hens fed the low M.E. diets, but had a greater coefficient of variation for pen uniformity of body weight. Dietary M.E. and enzyme supplementation had no effect on egg production. A significant interaction effect was seen for egg weight, such that hens fed the lower M.E. diets had increased egg weights when supplemented with Avizyme 1500. This positive effect of Avizyme 1500 was not seen in the high M.E. diets. There were no significant differences in egg mass or specific gravity as affected by diet.

Nutrient retention was measured in pullets at 12 weeks of age and in laying hens at 40 weeks of age (Table 4). There were some trends towards improved nutrient retentions with the addition of Avizyme 500 to pullet diets. Phosphorus retention improved in the high ME diets with the addition of Avizyme during the pullet stage. Retention of N also improved with the addition of Avizyme 1500 in both levels of M.E. diets. The positive effect of Avizyme 1500 on N retention carried over into the layer stage, especially in the low ME diets. This improvement in N retention is likely due to an improved digestion of the cell walls of soybeans and corn rendering not only more energy available but also better protein and amino acid digestibility. Digestible energy (ME) of both the high and low M.E. diets improved with enzyme

Table 4. Avizyme 1500 in pullet and layer diets — nutrient retention and tibia ash.

M.E.	Avizyme 1500		Nutrient Retention Ca	P	N	M.E.	Tibia Ash
Pullets	**Pullets**		(%)				(%)
High	No		23.3	9.7	30.3	2779	57.7
High	Yes		30.8	18.1	36.7	2923	57.3
Low	No		28.7	21.9	30.7	2881	57.4
Low	Yes		29.8	10.4	35.6	2823	57.2
Probabilities							
M.E.			NS	NS	NS	NS	NS
Avizyme			NS	0.09	0.10	NS	NS
M.E. x Avizyme			NS	NS	NS	NS	NS
Layers	**Pullets**	**Layers**					
High	No	No	50.8	7.4	31.3	2867	60.8
High	Yes	No	53.8	8.9	35.0	2916	60.9
High	Yes	Yes	56.5	10.9	37.4	2916	60.9
Low	No	No	48.7	12.1	36.8	2744	60.1
Low	Yes	No	49.2	11.6	38.6	2741	60.9
Low	Yes	Yes	50.2	12.4	35.2	2792	60.9
Probabilities							
M.E.			0.02	0.10	0.02	0.0001	NS
Avizyme			NS	NS	0.05	0.004	NS
M.E. x Avizyme			NS	NS	0.01	0.10	NS

supplementation during the layer stage, more so in the high energy diets than the low energy diets in both the pullet and layer stages. Calcium retention decreased in the low M.E. diets while phosphorus retention increased when lower M.E. diets were fed. The cause of this effect is not completely understood. Dietary treatments had no effects on tibia ash content in either pullets or layers in this study.

Cost savings in the formulation of diets "specified down" compared to the normal diet costs based on March, 2003 commodity prices (corn at $2.42/bushel, soybean meal at $172.00/ton, wheat midds at $70.00/ton and tallow at 14 cents/lb) were as follows: pullet starter - $4.49/ton; pullet grower - $5.55/ton; pullet developer - $1.39/ton and layer - $2.74/ton. These savings were due to less energy supplementation to the ration. They do not include costs of the enzyme. Actual savings will vary dependent on ratio of corn:soybean meal and added fat in the ration.

Summary

Supplementation of Avizyme 1500 to low M.E. diets improved rate of growth in the pullet and layer stage. Egg production was equal in the layer stage regardless of Avizyme 1500 supplementation or diet M.E. level. Improvements were seen in N retention and availability of energy in pullet and layer diets supplemented with Avizyme 1500. A "specified down" approach to formulation rations with Avizyme 1500 will result in diet cost savings; however the more conservation approach of "over the top" formulation with Avizyme 1500 can also be of benefit to some egg producers formulating on a lower margin of safety for protein and M.E. content.

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[1]Sheila E. Scheideler, Professor and Extension Poultry Specialist, and Trish Weber, Agricultural Research Technician, Animal Science, Lincoln.

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