British Poultry Science Volume 50, Number 2 (March 2009), pp. 207–212

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Using different ratios of bitter vetch (*Vicia ervilia*) seed for moult induction and post-moult performance in commercial laying hens

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Abstract 1. The applicability of different ratios of bitter vetch seed as a new method for moult induction in laying hens was studied. The effectiveness of bitter vetch seed on post-moult production and post-moult egg quality was also investigated.

2. A total of 120 Single Comb White Leghorn hens, 78 weeks of age, were used in this study. The hens were randomly assigned to 5 treatment groups of 24 birds each. The treatments were 30% bitter vetch seed (BV30) diet, 60% bitter vetch seed (BV60) diet, 90% bitter vetch seed (90BV) diet, feed withdrawal method (FW) and full-fed non-moulted control (CON).

3. Egg production ceased first in FW and BV90 treated hens and last in BV30 treated hens. As the percentage of bitter vetch seed increased in the moulting ration, feed intake decreased and body weight loss increased during the 10-d moult induction period. Time to first egg production was significantly greater in hens exposed to the FW and BV90 diets.

4. FW and BV90 treatment hens had significantly higher hen-d egg production than non-moulted control hens. Egg weight was significantly higher in BV30 and BV90 treatments. There were no differences in egg mass, feed intake and mortality among experimental treatments during the post-moult period.

5. No significant improvements were observed in exterior or interior egg quality in moulted hens, except for Haugh units, which were significantly higher in moulted hens when compared to the non-moulted control hens.

6. In conclusion, the present study showed *ad libitum* feeding of a layer ration with 90% of bitter vetch seed for 10 d proved to be effective for inducing moult, increasing post-moult egg production and improving some internal egg quality parameters.

INTRODUCTION

Forced moulting of laying hens can be an important management tool to increase the profitability in the second year of egg production. The main purpose of moulting is to increase egg production and egg quality (Webster, 2003). The most common procedure for moult induction is removal of feed until a hen loses between 15 and 25% of body weight. However, in recent years use of feed withdrawal has been declining due to animal stress and susceptibility to *Salmonella enteritidis* infections (Holt, 2003). As a consequence, non-feed-withdrawal methods for moult induction have been explored. These alternative methods include dietary manipulation of minerals (Keshavarz, 1995; Bell, 2003), use of anti-ovulatory drugs (Burke and Attia, 1994) and use of feed ingredients with low nutritional value (Vermaut *et al.*, 1998; Keshavarz and Quimby, 2002; Donalson *et al.*, 2005).

Another strategy for reducing body weight and induction of forced moulting involves supplementing laying hen diets with components that provide adequate nutrition but decrease feed intake. One such feed source is the bitter vetch (*Vicia ervilia*) seed which is known for its high nutritional value, capacity for nitrogen

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Accepted for publication 2nd September 2008.

ISSN 0007–1668(print)/ISSN 1466–1799 (online)/09/020207–6 © 2009 British Poultry Science Ltd
DOI: 10.1080/00071660902773691

fixation and ability to grow in poor soils (López-Bellido, 1994). It is grown in low and medium rainfall environments in northern Africa, Asia, southern Europe and the USA. Bitter vetch seed is a good source of crude protein and energy containing up to 285 g/kg crude protein, 18·2 MJ/kg gross energy and 12·97 MJ/kg metabolisable energy (Farran *et al.*, 2001; Sadeghi *et al.*, 2008). However, it also contains anti-nutritional factors including L-canavanine (Sadeghi *et al.*, 2004), trypsin inhibitors (Berger *et al.*, 2003), catechin (Aletor *et al.*, 1994) and a lectin (Fornestedt and Porath, 1975) that have been associated with decreased feed intake in poultry. Several studies have found that supplementing feed with more than 15% bitter vetch seed decreases feed intake in broiler chickens (Halaby, 1997; Sadeghi *et al.*, 2004) and laying hens (Ergun *et al.*, 1993; Farran *et al.*, 2005). Feeding a diet with greater proportions (60%) of raw bitter vetch seed resulted in reduced feed intake and egg production in laying hens and cessation of egg production within 2 weeks (Halaby, 1997; Sadeghi, unpublished data).

Because of its potential as a feed source for moult induction, there is a need to determine the optimum proportion of bitter vetch seed to add to regular poultry feed that will achieve the desired goal of moult induction. The objective of the study was to investigate the applicability of different ratios of bitter vetch seed as a new forced moulting method and to compare this with a conventional feed withdrawal method.

MATERIALS AND METHODS

Experimental design

A total of 120 Single Comb White Leghorn hens (78 weeks of age) were obtained from a commercial laying facility. The birds were selected on the basis of having similar rates of egg production and body weight. The birds were divided into 30 groups (4 per cage) and housed at the Isfahan University of Technology Poultry Science Research Centre. The hens were given a 2-week adaptation period prior to the initiation of the experiment. During this time, the birds were given a complete layer ration (Table 1) *ad libitum* and allowed full access to water. After the adaptation period, the hens were allocated randomly to 5 experimental groups with 24 birds (6 replicates of 4 hens) per treatment group. The experimental treatments were as follows: full-fed non-moulted control (CON), feed withdrawal (FW), 30% bitter vetch seed diet (BV30), 60% bitter vetch seed diet (BV60) and 90% bitter vetch seed diet (BV90). The control diet was formulated to meet NRC (1994) requirements, and in the BV diets corn, soybean and barley were replaced by bitter vetch seed (Table 1). Throughout the adaptation period and the experimental phase of the study the birds were allowed *ad libitum* access to water and their respective diets. The treatment diets were fed to the hens for 10 d. Each hen's weight and feed intake was measured by weighing the birds and their feed consumption before and after the moult period. After the moult period, all the hens received a complete layer ration diet until termination of the study at 96 weeks of age.

Table 1. *Ingredients and composition (as-fed basis) of the diets (g/kg)*

Components	Layer ration (CON)[1]	BV30	BV60	BV90
Bitter vetch seed meal[2]	0	300	600	900
Maize meal	643	300	0·00	0·00
Soybean meal	190	0·00	0·00	0·00
Barley meal	56	303	303	0·00
Oyster shell	87	87	87	87
Alfalfa meal	10	0·00	0·00	0·00
Base mix[3]	14	10	10	13
Calculated composition				
AME_N (MJ/kg)	11·13	11·48	11·05	11·37
Crude protein (g/kg)	157·7	144·0	196·2	243·4
Lysine (g/kg)	8·0	6·2	9·7	12·6
Methionine (g/kg)	4·2	2·7	3·2	3·8
Methionine + cystine (g/kg)	7·1	4·6	5·5	6·5
Calcium (g/kg)	35·8	34·2	34·6	35·0
Available phosphorus (g/kg)	1·7	1·4	1·4	1·4
Sodium (g/kg)	1·2	1·2	1·2	1·2

[1]CON = full-fed non-moulted control; BV30 = 30% bitter vetch seed; BV60 = 60% bitter vetch seed; BV90 = 90% bitter vetch seed.
[2]The bitter vetch seed contained 12·55 MJ/kg AME_N, 265 g/kg crude protein, 4 g/kg crude fat, 1·6 g/kg calcium, 3 g/kg phosphorus, 74·2 g/kg lysine and 11·1 g/kg methionine.
[3]The base mix contained 5·44 MJ/kg metabolisable energy, 300 g/kg crude protein, 50 g/kg calcium, 38·7 g/kg available phosphorus, 34 g/kg lysine, 39·5 g/kg methionine, and vitamin and trace mineral premixes that provided per kg of diet: vitamin A (retinyl acetate) 3·9 mg, vitamin D (cholecalciferol) 0·083 mg, vitamin E (alpha-tocopherol) 27 mg, vitamin B_1 (thiamin mononitrate) 2 mg, vitamin B_2 (riboflavin) 4 mg, vitamin B_6 (pyridoxine hydrochloride) 2 mg, D-pantothenic acid (calcium D-pantothenate) 9 mg, niacin 25 mg, choline chloride 100 mg, folic acid 0·7 mg, biotin 0·02 mg, cyanocobalamin 0·015 mg, Fe 70 mg, Zn 100 mg, Mn 100 mg, Cu 5 mg, I 0·8 mg, Se 0·2 mg.

On d 1 (the initiation of feed withdrawal or feeding moult diets), the daily photoperiod was reduced to 10·5 h to ensure a more complete and rapid moult (Andrews *et al.*, 1987). On d 22 and 31 the daily photoperiod was increased to 12 and 13 h, respectively. The photoperiod was then increased by 30 min per week until a photoperiod of 16 h was reached.

Egg production and quality parameters

Production parameters and egg quality were measured for 12 weeks after moulting. Egg production (hen-d production) and mortality were measured daily. Feed intake was recorded at 2-week intervals and 3 d of eggs were collected from the end of each 2-week period and weighed. Egg mass was calculated using hen-d production and average egg weight. Shell weight, shell thickness, shell strength, albumen height and yolk colour were measured on 12 eggs from each treatment (2 eggs per cage) every 14 d. The eggshell thickness was measured in millimetres using an FHK Eggshell Thickness Gauge and the eggshell strength was measured in kg/cm^2 using an FHK Eggshell Force Gauge (FHK, Fujihira Industry Co. Ltd, Tokyo, Japan). Haugh units were calculated as an indicator of interior egg quality. Yolk colour was compared to the Roche Yolk Colour Fan, which ranges from a pale yellow at score 1 to a dark orange at score 15 (Vuilleumier, 1969).

Statistical analysis

Data were pooled within cage by treatment group and statistically analysed using the GLM procedure. Test of mean differences was computed using Duncan's Multiple Range test (SAS Institute, 2001). The level of significance used in all analyses was $P < 0{\cdot}05$.

RESULTS AND DISCUSSION

Feed intake and body weight loss

During the moulting period, hens receiving the CON or BV30 diet had significantly greater ($P < 0{\cdot}05$) feed intake than did hens fed on either the BV60 or BV90 diets (Table 2). Hens given BV60 and BV90 averaged 67 and 75% reductions of feed intake, respectively, when compared with the feed intake of hens given the CON diet. The BV60 and BV90 hens also consumed less feed than hens fed on the BV30 diet. These findings are consistent with a study by Farran *et al.* (2005) who found that 47-week-old laying hens given a diet containing 60% bitter vetch seed experienced significantly reduced daily feed intake.

As noted earlier, the reduction in feed intake in hens fed with bitter vetch seed may be attributable to the presence of anti-nutritional factors, especially canavanine and lectin. Reduction in feed consumption may be due to toxic factors in the digestive tract or an unpleasant taste caused by these factors in the diet. Previous work has shown that supplementation of broiler diets with canavanine depressed feed intake by 30% in relation to the control diet (Michelangeli and Vargas, 1994). D'Mello *et al.* (1990) suggested that a canavanine–arginine interaction may be occurring which parallels the well-established lysine–arginine antagonism that depresses feed intake. Another possibility for the effects of canavanine may be its association with the inhibition of nitric oxide formation (Hrabak *et al.*, 1994) and the effects that nitric oxide has on affecting the feed intake response (D'Mello, 1995). Regardless of the specific mechanism, it appears certain that high levels of BV lead to feed intake reduction resulting in BV being an effective method for inducing moult.

Body weight loss is a major factor contributing to the moult induction and affects on the post-moult performance (Baker *et al.*, 1983). Non-moulted hens had the least ($P < 0{\cdot}05$) body weight loss compared with the moulted hens (Table 2). Hens fed on diets BV60, BV90 and FW underwent greater weight loss than those receiving BV30 (Table 2). The weight loss among the BV-fed hens was likely to have been due primarily to feed intake reduction. However, some of the weight depression from consuming bitter vetch seed diets may also have been due to the some antimetabolic activities of anti-nutritional factors in bitter vetch seed. Its arginine-like structure enables canavanine to bind many enzymes that usually interact with arginine and it also incorporates into polypeptide chains, resulting in structurally aberrant canavanine-containing proteins (Rosenthal, 1977). Canavanine may inhibit L-arginine Na^+-dependent transport across the

Table 2. *The effects of bitter vetch seed layer ration and feed withdrawal moult diets on feed and protein consumption and body weight parameters during moulting and egg production parameters during and after moulting*

Parameters	CON	FW	BV30	BV60	BV90	SEM
Feed intake (g/bird)	93·4^{a}	–	80·8^{a}	38·0^{b}	22·8^{b}	6·46
Initial body weight (g)	1745·8	1742·5	1765·0	1749·6	1760·1	17·97
Body weight at the end of moult induction (g)	1702·7^{a}	1493·0^{b}	1624·7^{ab}	1462·0^{b}	1446·3^{b}	13·91
Body weight loss (%)	2·47^{c}	14·30^{a}	7·96^{b}	16·38^{a}	17·56^{a}	0·869
Days from start of treatments to egg arrest	–	4·3^{b}	7·1^{a}	5·6^{ab}	5·1^{b}	0·32
Days from egg arrest to first egg production (rest)	–	21·3^{a}	16·1^{b}	18·0^{b}	21·8^{a}	0·89
Days to return to at least 50% egg production	–	35·5	33·2	39·6	38·0	1·38

CON = full-fed non-moulted control; FW = feed withdrawal; BV30 = 30% bitter vetch seed diet; BV60 = 60% bitter vetch seed diet; BV90 = 90% bitter vetch seed diet.

a–c Means within rows with no common superscript differ significantly ($P < 0{\cdot}05$).

enterocyte apical membrane in intestinal brush border membrane vesicles (Rueda *et al.*, 2003), and it also interferes with protein synthesis in avian liver (Kessler *et al.*, 1990). In addition, it has been shown that lectins reduce the digestive and absorptive capacity of the small intestine and therefore the lectin-fed birds lost weight as a result of malabsorption of dietary nutrients.

Though the non-moulted hens lost significantly less weight than the moulted ones, they did experience measurable weight loss. This weight loss could be explained by the reduced photoperiod (Donalson *et al.*, 2005). Periods of reduced light exposure causes an inhibition of reproductive hormones and subsequent weight loss associated with regression of the ovary and oviduct (Berry, 2003). Body weight at the end of the experimental period was not significantly different between moulted and non-moulted hens (Table 2). This shows the rebuilding of body organs in moulted hens during the post-moult period. Feed intake was also measured following the moult and then for 12 weeks of production. Intake of standard laying ration did not differ among treatment groups during this period (Table 3).

Egg production parameters and mortality

The egg arrest time and rest period (days to first egg production) were significantly ($P<0{\cdot}05$) different among the moulting treatment conditions. Egg production ceased first in the FW and BV90 treated hens and last in BV30 treated hens (Table 2). Farran *et al.* (2005) similarly reported cessation in egg production within 14 d after feeding a diet containing 60% bitter vetch seed. The rest period was significantly longer ($P<0{\cdot}05$) in hens exposed to FW and BV90-fed hens than those fed the BV30 and BV60 diets. This result suggests that suppression of feed intake in hens fed with 90% of bitter vetch seed negatively impacted their egg laying capacity. All moulting groups returned to 50% egg production at a similar time (Table 2).

Egg production in FW and BV90 hens was significantly higher ($P<0{\cdot}05$) than in the non-moulted control hens (Table 3). The lower egg production of the BV30 hens after 12 weeks post moult is likely due to an incomplete moult, with higher feed intake and lower weight loss than the other BV-fed groups. Of the bitter vetch seed diets, BV90 was comparable with the FW treatment for post-moult egg production. Brake (1992) showed higher body weight loss results in higher post-moult production. Hens in the FW and BV90 groups stayed out of production for a longer period than others and that resulted in greater egg production. In the weekly analysis of egg production (Figure), it appears that all moulted hens increased egg production up to 96 weeks of age. Hens moulted by the 90% bitter vetch diet rebounded more quickly in the first weeks after moult induction than other moulted hens. However, un-moulted control hens maintained the low egg production rate for up to 96 weeks.

All moulted hens regained their weight loss; there were no significant differences among treatment groups in the final body weight measurements (Table 3). There were also no differences in overall egg mass among experimental treatments. The bitter vetch seed diets did not result in any mortality during the induction period or later (Table 3), which concurs with previous work (Farran *et al.*, 2005).

Interior and exterior egg quality

Egg weight was significantly higher ($P<0{\cdot}05$) in the BV30 and BV90 treatments than in the FW treated hens, but the egg weights of non-moulted hens were not ($P>0{\cdot}05$) different from those of moulted hens (Table 3). This finding

Table 3. *The effects of feed withdrawal and bitter vetch seed layer moult diets on egg production performance during post-moult period (85 to 96 weeks of age)*

Parameters	CON	FW	BV30	BV60	BV90	SEM
Hen-d egg production (%)	53·62^{b}	65·70^{a}	61·08^{ab}	60·30^{ab}	64·64^{a}	1·626
Egg mass (g/hen-d)	43·19	43·32	44·10	39·40	45·47	1·153
Feed intake (g/hen-d)	111·3	113·2	106·5	108·0	110·2	2·138
Mortality (%)	0·30	0·26	0·23	0·17	0·33	0·097
Final body weight (g)	1956·2	1872·2	1889·2	1907·4	1949·7	16·810
Egg weight (g)	69·89^{ab}	67·64^{b}	71·74^{a}	69·67^{ab}	70·56^{a}	0·398
Shell weight (g)	6·08	6·26	6·52	6·20	6·63	0·071
Shell thickness (mm)	0·419	0·420	0·410	0·406	0·419	0·0024
Shell strength (kg/cm^2)	4·10	4·17	3·76	4·03	3·95	0·059
Haugh units	60·13^{b}	69·43^{a}	65·63^{ab}	72·85^{a}	72·1^{a}	1·147

CON = full-fed non-moulted control; FW = feed withdrawal; BV30 = 30% bitter vetch seed diet; BV60 = 60% bitter vetch seed diet; BV90 = 90% bitter vetch seed diet.

[a,b]Means within rows with no common superscript differ significantly ($P<0{\cdot}05$).

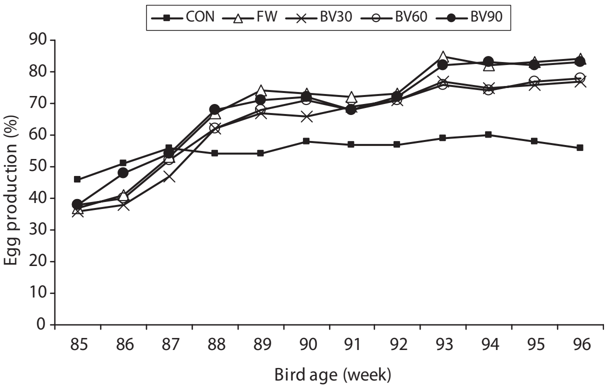

Figure. *Weekly post-moult egg production from weeks 85 to 96. CON = full-fed non-moulted control; FW = feed withdrawal; BV30 = 30% bitter vetch seed diet; BV60 = 60% bitter vetch seed diet; BV90 = 90% bitter vetch seed diet.*

is in agreement with those of Christmas *et al.* (1985), Alodan and Mashaly (1999) and Wu *et al.* (2007) who found that different induced-moulting programmes did not significantly affect egg weight when compared to the non-moulted hens. Shell weight, shell thickness and shell strength did not differ among treatments. BV60, BV90 and FW moulting treatments resulted in higher ($P < 0.05$) Haugh units during the post-moult period (Table 3). This finding is in agreement with those of Alodan and Mashaly (1999), who found a higher Haugh unit in moulted hens when compared to the non-moulted control hens. Egg yolk colour was not affected by feeding treatments (data not shown).

Based on the results of this study, BV90 appears to be the best alternative to the FW moulting method and yields comparable results. The BV60 treatment may also be a viable alternative; however, moult induced by the BV60 treatment may not be sufficiently complete due to higher feed intake in this group. Further research is necessary to determine an optimal level of bitter vetch seed in layer ration intermediate to the BV60 and BV90 diets, for maximising moult induction and post-moult egg quality. Use of a layer ration with 90% of bitter vetch seed proved to be effective for inducing moult, increasing post-moult egg production and improving internal egg quality when compared with non-moulted hens. Moult diets containing 90% of bitter vetch seed increased egg weight and resulted in comparable post-moult egg production and egg quality when compared with a standard FW method. The body weight loss in bitter vetch treatment hens in this study was lower than the 25 to 30% that is suggested in the literature to result in better post-moult eggshell quality and egg numbers. Therefore, more research is needed to examine longer periods (more than 10 d) to moult induction by bitter vetch seed diets. More research is also required to determine the mechanism of action of bitter vetch in limiting feed intake and whether it is welfare friendly.

ACKNOWLEDGEMENT

The authors are grateful to Dr Kenneth J. Gruber at the University of North Carolina at Greensboro for his critical and constructive reading of the manuscript.

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