The Influence of a Fructooligosaccharide Prebiotic Combined with Alfalfa Molt Diets on the Gastrointestinal Tract Fermentation, *Salmonella* Enteritidis Infection, and Intestinal Shedding in Laying Hens

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**ABSTRACT** Molting is a natural process, which birds undergo to rejuvenate their reproductive organs. The US poultry egg production industry has used feed withdrawal to effectively induce molt; however, susceptibility of *Salmonella* Enteritidis has encouraged the development of alternative methods. Previous research conducted in our laboratory showed that alfalfa is effective at molt induction and provides equivalent postmolt production numbers and quality when compared with feed withdrawal. In the attempt to further increase the efficacy of alfalfa molt diet and decrease the chicken susceptibility to *Salmonella* Enteritidis during molt, fructooligosaccharide (FOS) was added to a combination of 90% alfalfa and 10% layer ration in 2 levels (0.750 and 0.375%). Ovary and liver colonization by *Salmonella* Enteritidis in 3 and 2 of the 4 trials, respectively, were reduced (*P* ≤ 0.05) in hens fed FOS-containing diets compared with hens subjected to feed withdrawal. Significant decreases in cecal *Salmonella* Enteritidis counts were also observed in 2 of the 4 trials. In 3 of the 4 trials, the same diets did not affect (*P* > 0.05) the production of cecal total volatile fatty acids when compared with hens undergoing feed withdrawal. However, in all 3 alfalfa molt diets, the concentrations of lactic acid were greater (*P* ≤ 0.05) than hens with feed withdrawal, but no differences (*P* > 0.05) were observed among hens fed alfalfa combined with FOS and hens fed alfalfa/layer ration without FOS. Overall, given the similarities between hens fed 0.750% FOS (H) and 0.375% FOS (L), molt diets combined with the lower level of FOS should be sufficient.

**Key words:** *Salmonella* Enteritidis, molting, laying hen, alfalfa, fructooligosaccharide

**INTRODUCTION**

Frenzen et al. (1999) estimate the annual cost of foodborne *Salmonella* infection to be nearly 2.3 billion dollars. The 2 serotypes that cause the majority of the cases are *Salmonella* Enteritidis and *Salmonella* Typhimurium (Hedberg et al., 1993). *Salmonella* Enteritidis cases are generally believed to be derived from shell eggs from chickens (Humphrey, 1994; Mohle-Boetani et al., 1998; Guard-Petter, 2001). Management practices, such as feed withdrawal molting methods, increase *Salmonella* Enteritidis infection susceptibility in the hen, (Durant et al., 1999; Poppe, 1999; Holt, 2003; Ricke, 2003) as indicated by increased intestinal shedding and dissemination of *Salmonella* Enteritidis to organs such as the ovary, liver, spleen, and crop. Alternative molting approaches that limit *Salmonella* Enteritidis have involved dietary modification strategies that contain an excess of a particular compound such as zinc (Moore et al., 2004; Park et al., 2004a,b,c,d; Ricke et al., 2004). Feeding alternative feedstuffs such as wheat middlings (Seo et al., 2001) and alfalfa (Donalson et al., 2005; Woodward et al., 2005; Landers et al., 2005a,b; McReynolds et al., 2005,2006; Dunkley et al., 2007b) have also been recently examined for potential molt induction and *Salmonella* Enteritidis reduction. Alfalfa has proven to be an effective alternative molting diet, because it induces molt and produces comparable postmolt egg production and qualities when compared with feed withdrawal (FW; Landers et al., 2005a,b; Donalson et al., 2005). In addition to ameliorating physiological and immunological stress responses during molt (Landers et al., 2007), alfalfa diets
are desirable due to their high fermentation properties by Salmonella in vitro and Salmonella Enteritidis colonization in laying hens (Ricke, 2003; Woodward et al., 2005; McReynolds et al., 2005, 2006; Donalson et al., 2007a,b; Dunkley et al., 2007a,b,c).

The addition of prebiotics to diets has been shown to increase fermentation both in vitro (Rycroft et al., 2001) and in vivo (Xu et al., 2003). A common prebiotic compound used both in human as well as in animal diets is fructooligosaccharide (FOS) (Gibson and Roberfroid, 1995; Bomba et al., 2002). Due to the β-linkages possessed by FOS, it is able to resist enzymatic degradation and absorption in the upper gastrointestinal tract to reach the cecum, where the majority of fermentation occurs in chickens (Gibson and Roberfroid, 1995; Xu et al., 2003; Juskiewicz et al., 2004). Fermentation of prebiotics produces end products including short-chain fatty acids, which have been shown to modify the bacterial ecosystem in the ceca and inhibit the growth of enteric bacteria such as Salmonella, Escherichia coli, and Clostridium perfringens (Cummings et al., 2001; Cummings and Macfarlane, 2002). In addition to inhibiting the growth of enteric bacteria, FOS has been proven to serve as a fermentable substrate to promote the growth of beneficial microflora such as lactic acid bacteria and Bifidobacterium sp. (Allen et al., 1997; Cummings and Macfarlane, 2002; Juskiewicz et al., 2004).

The objective of this research was to examine the effects of the FOS combined with alfalfa molt diets on Salmonella Enteritidis colonization on internal organs, crop pH, volatile fatty acids (VFA) and lactic acid production.

**MATERIALS AND METHODS**

**Molting Procedure**

A total of 60 laying hens were obtained from a commercial laying facility. Cloacal swab samples were collected from each hen and examined for salmonellae by successive culturing in tetradionate broth (Difco Laboratories, Detroit, MI) and brilliant green agar (BGA; Difco Laboratories) plates as described by Andrews et al. (1992). Salmonella spp.-positive hens were eliminated from the study. Laying hens were placed in wire layer cages (1 hen per cage) and were provided free access to water and a balanced, unmedicated corn-soybean mash layer ration (Poultry Science Department, Texas A&M University, College Station, TX) that met or exceeded NRC requirements (1994). This diet was formulated to provide 2,818 kcal of ME/kg, 16.5% CP, 3.5% calcium, and 0.48% available phosphorus. Before use, 3 randomly selected 25-g samples of the feed were cultured successively in buffered peptone water, tetrathionate broth, and BGA as described by Andrews et al. (1992) and examined for salmonellae. The hens were allowed to acclimate for a minimum of 1 wk followed by complete random allocation to 5 treatment groups of 12 hens each, designated as follows: (1) feed withdrawal (molted, FW), (2) nonmolted control (full fed, FF), (3) 90% alfalfa/10% Texas A&M University (TAMU) layer ration (A90), (4) 90% alfalfa/10% TAMU layer ration plus 0.375% FOS (L), or (5) 90% alfalfa/10% TAMU layer ration plus 0.75% FOS (H). The hens were subsequently housed in approved facilities at the USDA-Agricultural Research Service, College Station, Texas, under a protocol approved by the USDA-Agricultural Research Service Animal Use and Care Committee.

On d 4 of each study, all hens in each treatment group were challenged by crop gavage with 1 mL of inoculum containing approximately 10⁸ cfu of a poultry isolate of Salmonella enterica serovar Enteritidis (phage type 13A, National Veterinary Services Laboratory, Ames, IA), which was selected for resistance to novobiocin and nalidixic acid at the USDA-Agricultural Research Service facility (College Station, TX). The challenge dosage approximated the 5.6 × 10⁶ cfu dose reported to be the mean infectious dosage for Salmonella Enteritidis in nonmolted hens (Holt et al., 1993). On d 9 of the study, 6 hens from each treatment group were euthanized, and the crop, ceca, liver, spleen, and ovary were aseptically excised. The crop, ceca, liver, spleen, and ovary of each hen were then cultured for Salmonella Enteritidis. After the molting period, the remaining 6 hens from each treatment group were placed on a maintenance diet and monitored for intestinal shedding of Salmonella Enteritidis.

**Crop Lactic Acid Concentrations and pH**

Crop lactic acid concentration and pH were determined as described by Durant et al. (1999). Crop pH was determined by insertion of a sterile glass pH electrode through an incision in the crop wall ensuring the electrode remained in contact with the crop mucosal surface. Each crop was aseptically excised, cut open, and blended with 10 mL of sterile Butterfield’s Buffer (Difco Laboratories, Sparks, MD) for 1 min in a Stomacher 80 blender (Seward Medical, London, UK). Samples of blended crop were collected and analyzed for lactic acid concentrations (Hohorst, 1974; Moore et al., 2004).

**Cecal VFA and Lactic Acid Concentrations**

Cecal content concentrations of VFA (acetic, propionic, butyric, isobutyric, valeric, and iso-valeric acids) were determined by gas-liquid chromatography as described by Corrier et al. (1990). The analysis was conducted with a gas chromatograph equipped with a flame ionization detector and peak profiles integration-quantification integrator (model 110 gas chromatograph, SRI Instruments, Torrence, CA). Each sample peak profile was integrated and quantified relative to an internal standard of methylbutyric acid placed in the same sample. Lactic acid concentrations were determined by an enzymatic method (Hohorst, 1974).

**Crop, Cecal, and Organ Colonization by Salmonella Enteritidis**

One milliliter of blended crop sample was transferred into 10 mL of Rappaport-Vassiliadis (RV) broth (EM Sci-
ence, Gibbstown, NJ) and incubated for 24 h at 42°C. One
cucm from each hen was cut into several pieces, placed
in 30 mL of RV broth, shaken vigorously, and incubated
for 24 h at 42°C. Liver, spleen, and ovary specimens were
minced with scissors and cultured for 24 h at 42°C in
RV broth. After incubation, the respective broths were
streaked onto BGA plates containing novobiocin (25
µg/mL) and nalidixic acid (20 µg/mL), incubated for an addi-
tional 24 h at 37°C, and examined for the presence of
Salmonella Enteritidis colonies. Suspect colonies were se-
erologically identified as Salmonella Enteritidis using,
Salmonella O antisera group D, factors 1, 9, and 12.

Salmonella Enteritidis Colony-Forming
Units per Gram of Crop
and Cecal Contents

The contents of the crop and 1 cecum from each hen
were serially diluted and spread-plated on novobiocin
and nalidixic acid-BGA plates at dilutions 10^1 through
10^8. The plates were incubated for 24 h at 37°C, after
which the number of colony-forming units of Salmonella
Enteritidis per gram of crop or cecal content was deter-
mained, and Salmonella Enteritidis colonies were sero-
logically confirmed as described in the previous section.

Intestinal Shedding
of Salmonella Enteritidis

Hens were monitored for intestinal shedding of Salmon-
ella Enteritidis on d 4, 10, 17, and 24 post-Salmonella
Enteritidis challenge for 6 hens per treatment group
(equivalent to d 8, 14, 21, and 28 after molt induction).
The birds were sampled using a modification of a procedure
described by Seo et al. (2001). Aluminum foil sheets were
placed under each hen for approximately 1 h, and the
secretions were collected. Approximately 0.5 mL of the
samples was weighed and added to dilution tubes con-
aining 4.5 mL of sterile Butterfield’s Buffer. The aliquots
were subsequently serially diluted at 10^1 through 10^8
dilutions and plated on novobiocin and nalidixic acid-BGA
plates. The plates were incubated for 24 h at 37°C, after
which the number of colony-forming units of Salmonella
Enteritidis per gram of intestinal shedding was deter-
mained, and Salmonella Enteritidis colonies were confirmed
by using Salmonella O antiserum group D, factors 1, 9,
and 12. The remaining samples were added to 25 mL of
RV broth for selective enrichment and incubated for 24 h
at 42°C, at which time they were plated on novobiocin
and nalidixic acid-BGA plates and incubated at 37°C for
another 24 h. The plates were subsequently examined for
the presence of suspect Salmonella Enteritidis colonies.
Suspect colonies were identified as Salmonella Enteritidis
serologically using Salmonella O antiserum group D, fac-
tors 1, 9, and 12.

Statistical Analysis

Chi-squared analysis was used to determine significant
differences among treatment groups for Salmonella Enter-
itidis incidences of crop, cecal, liver, spleen, and ovary
(Luginbuke and Schlotzhauer, 1987). Differences in VFA
and lactic acid concentrations were determined by AN-
OVA using the GLM procedures. Significant differences
were further separated using Duncan’s multiple range
test and commercial statistical analysis software (SAS In-
stitute, Cary, NC). All data were analyzed by an individ-
ual trial, and statistical analyses were considered signifi-
cant at P ≤ 0.05.

RESULTS AND DISCUSSION

Laying Hen Response to Treatments

Feed intake was greater (P ≤ 0.05) in full fed (FF) non-
molting hens (Table 1). No differences (P > 0.05) were

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Table 1. Effects of nonmolting and molting with and without alfalfa and fructooligosaccharide (FOS) on feed intake, body weight loss, and ovary weight of hens

<table>
<thead>
<tr>
<th>Treatment</th>
<th>A90</th>
<th>FF</th>
<th>FW</th>
<th>H</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed intake (g/hen daily)</td>
<td>11.39 ± 3.06</td>
<td>84.13 ± 3.57</td>
<td>NA</td>
<td>12.63 ± 1.00</td>
<td>11.32 ± 4.99</td>
</tr>
<tr>
<td>Body weight loss (%)</td>
<td>22.92 ± 1.97</td>
<td>3.10 ± 3.80</td>
<td>25.94 ± 1.27</td>
<td>25.40 ± 1.32</td>
<td>24.51 ± 6.86</td>
</tr>
<tr>
<td>Ovarian weight</td>
<td>0.69 ± 0.98</td>
<td>3.13 ± 0.27</td>
<td>0.86 ± 0.17</td>
<td>0.61 ± 0.11</td>
<td>0.65 ± 0.11</td>
</tr>
<tr>
<td>Feed intake (g/hen daily)</td>
<td>15.11 ± 6.05</td>
<td>93.69 ± 14.9</td>
<td>NA</td>
<td>21.06 ± 2.96</td>
<td>15.72 ± 0.79</td>
</tr>
<tr>
<td>Body weight loss (%)</td>
<td>25.38 ± 1.95</td>
<td>-1.07 ± 2.97</td>
<td>29.83 ± 1.09</td>
<td>23.99 ± 1.26</td>
<td>27.62 ± 2.31</td>
</tr>
<tr>
<td>Ovarian weight</td>
<td>0.44 ± 0.12</td>
<td>2.73 ± 0.69</td>
<td>0.63 ± 0.17</td>
<td>0.49 ± 0.11</td>
<td>0.85 ± 0.23</td>
</tr>
<tr>
<td>Feed intake (g/hen daily)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Body weight loss (%)</td>
<td>16.86 ± 1.18</td>
<td>0.24 ± 1.35</td>
<td>23.47 ± 0.77</td>
<td>17.62 ± 1.08</td>
<td>16.10 ± 0.84</td>
</tr>
<tr>
<td>Ovarian weight</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Feed intake (g/hen daily)</td>
<td>0.31 ± 0.94</td>
<td>3.11 ± 0.18</td>
<td>0.36 ± 0.06</td>
<td>0.38 ± 0.02</td>
<td>0.58 ± 0.08</td>
</tr>
<tr>
<td>Body weight loss (%)</td>
<td>22.11 ± 1.06</td>
<td>3.50 ± 2.90</td>
<td>29.56 ± 1.71</td>
<td>17.91 ± 1.66</td>
<td>22.95 ± 1.32</td>
</tr>
<tr>
<td>Ovarian weight</td>
<td>0.41 ± 0.22</td>
<td>2.13 ± 0.50</td>
<td>0.61 ± 0.07</td>
<td>0.40 ± 0.04</td>
<td>0.54 ± 0.12</td>
</tr>
</tbody>
</table>

*Means within a row with no common superscripts differ significantly (P < 0.05).

1A90 = 90% alfalfa/10% layer ration; FF = full fed, nonmolting; FW = feed withdrawal; H = 90% alfalfa/10% layer ration + 0.75% FOS; L = 90% alfalfa/10% layer ration + 0.375% FOS.
2As a percentage of body weight (ovary weight/body weight) × 100.
3NA = not applicable.
4ND = not done.
observed between any molting treatments in either trial. The decrease in feed intake of alfalfa molt diets could be attributed to low energy levels of alfalfa and reduced palatability (NRC, 1994; Biggs et al., 2004) or anorexia that hens voluntarily undergo at a natural molt (Mrosovsky and Sherry, 1980). Body weights also decreased markedly as a result of the decreased feed intake (Table 1). In trial 1 and trial 2, FF nonmolting hens exhibited lower (P ≤ 0.05) body weight losses compared with all molted treatments, which were not different (P > 0.05) from each other. Trial 3 and 4 hens exhibited a similar pattern, but the body weight losses of hens fed alfalfa were intermediate compared with FW- and FF-treated hens. In all trials, FF-treated hens exhibited greater (P ≤ 0.05) ovary weights typical of nonmolting hens, whereas all molted hens showed no significant differences among treatments (Table 1). We have previously observed a 5- to 6-fold decrease in ovary weights with alfalfa meal and pellets (Landers et al., 2005a; Woodward et al., 2005). Ovary regression is an important factor that influences both postmolt egg production and egg quality (Biggs et al., 2004). Overall, the addition of FOS at either level (H or L) to alfalfa diet did not significantly influence either feed intake or body and ovarian weights of the hens.

### Crop pH and Lactic Acid

Crop pH and lactic acid concentrations were taken from hens after the 9 d molt and are shown in Table 2. Lactobacillus spp. are the primary bacteria in the crop that produce lactic acid as their main fermentation product (Fuller, 1977; Rubio et al., 1998). No differences (P > 0.05) in lactic acid concentrations among any of the treatments were seen in trial 1 and 2. Alfalfa diet, however, supplemented with a higher level FOS (H) resulted in lactic acid production similar to that in FF hens in trial 3 and 4 and was significantly higher than hens with FW. In all 4 trials, H and L diets did not have any effect on the crop pH of hens when compared with hens fed A90. As expected, hens fed FF yielded lower (P ≤ 0.05) crop pH levels compared with hens with FW in trial 1, 3, and 4. The data agree with Durant et al. (1999), who established that FW reduce lactobacilli populations thus increasing crop pH.

### Table 2. Effects of nonmolting and molting with and without alfalfa and fructooligosaccharide (FOS) on crop pH and lactic acid concentrations

<table>
<thead>
<tr>
<th>Trial</th>
<th>Item</th>
<th>A90</th>
<th>FF</th>
<th>FW</th>
<th>H</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Crop pH</td>
<td>5.69 ± 0.21a</td>
<td>4.77 ± 0.12b</td>
<td>6.05 ± 0.29ab</td>
<td>5.60 ± 0.20a</td>
<td>6.12 ± 0.37a</td>
</tr>
<tr>
<td></td>
<td>Lactic acid (μmol/mL)</td>
<td>33.73 ± 10.94a</td>
<td>43.08 ± 12.96a</td>
<td>37.42 ± 17.43a</td>
<td>11.98 ± 1.67a</td>
<td>12.12 ± 2.97a</td>
</tr>
<tr>
<td>2</td>
<td>Crop pH</td>
<td>5.31 ± 0.11a</td>
<td>4.76 ± 5.59ab</td>
<td>5.59 ± 0.26a</td>
<td>5.33 ± 0.10b</td>
<td>5.68 ± 0.08a</td>
</tr>
<tr>
<td></td>
<td>Lactic acid (μmol/mL)</td>
<td>21.30 ± 5.45a</td>
<td>22.65 ± 4.27a</td>
<td>13.48 ± 1.80a</td>
<td>26.38 ± 11.0a</td>
<td>31.47 ± 10.24a</td>
</tr>
<tr>
<td>3</td>
<td>Crop pH</td>
<td>5.18 ± 0.18b</td>
<td>5.10 ± 0.08b</td>
<td>6.22 ± 0.29a</td>
<td>5.51 ± 0.26ab</td>
<td>5.84 ± 0.32ab</td>
</tr>
<tr>
<td></td>
<td>Lactic acid (μmol/mL)</td>
<td>9.33 ± 0.61b</td>
<td>47.52 ± 9.34a</td>
<td>8.37 ± 0.35a</td>
<td>39.17 ± 17.33a</td>
<td>8.63 ± 1.83b</td>
</tr>
<tr>
<td>4</td>
<td>Crop pH</td>
<td>5.31 ± 0.28ab</td>
<td>4.61 ± 0.22b</td>
<td>5.78 ± 0.29a</td>
<td>5.60 ± 0.16a</td>
<td>4.98 ± 0.36ab</td>
</tr>
<tr>
<td></td>
<td>Lactic acid (μmol/mL)</td>
<td>11.41 ± 0.28b</td>
<td>68.82 ± 22.38a</td>
<td>11.08 ± 0.65b</td>
<td>45.05 ± 19.08a</td>
<td>27.38 ± 5.96b</td>
</tr>
</tbody>
</table>

*Means within a row with no common superscripts differ significantly (P < 0.05).

### Table 3. Effects of nonmolting and molting with and without alfalfa and fructooligosaccharide (FOS) on Salmonella enterica serovar Enteritidis (Salmonella Enteritidis) crop colonization of hens

<table>
<thead>
<tr>
<th>Treatment</th>
<th>A90</th>
<th>FF</th>
<th>FW</th>
<th>H</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive hens per total</td>
<td>1/6 (17%)a</td>
<td>0/6 (0%)b</td>
<td>0/6 (0%)b</td>
<td>0/6 (0%)b</td>
<td>1/6 (17%)a</td>
</tr>
<tr>
<td>Log10 cfu/g</td>
<td>0.74 ± 0.57bc</td>
<td>0.00 ± 0.00b</td>
<td>0.95 ± 0.00a</td>
<td>0.65 ± 0.49ab</td>
<td>0.61 ± 0.45bc</td>
</tr>
<tr>
<td>Trial 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive hens per total</td>
<td>2/6 (33%)ab</td>
<td>0/6 (0%)b</td>
<td>4/6 (83%)ab</td>
<td>1/6 (17%)ab</td>
<td>2/6 (33%)ab</td>
</tr>
<tr>
<td>Log10 cfu/g</td>
<td>1.07 ± 0.71ab</td>
<td>0.00 ± 0.00b</td>
<td>1.96 ± 0.31a</td>
<td>0.68 ± 0.68ab</td>
<td>1.03 ± 0.71ab</td>
</tr>
<tr>
<td>Trial 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive hens per total</td>
<td>0/6 (0%)a</td>
<td>0/6 (0%)a</td>
<td>2/6 (33%)ab</td>
<td>1/6 (17%)ab</td>
<td>0/6 (0%)a</td>
</tr>
<tr>
<td>Log10 cfu/g</td>
<td>0.00 ± 0.00b</td>
<td>0.54 ± 0.54ab</td>
<td>1.20 ± 0.76a</td>
<td>1.27 ± 0.58b</td>
<td>0.00 ± 0.08ab</td>
</tr>
<tr>
<td>Trial 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive hens per total</td>
<td>0/6 (0%)a</td>
<td>0/6 (0%)a</td>
<td>3/6 (50%)ab</td>
<td>2/6 (33%)ab</td>
<td>1/6 (17%)ab</td>
</tr>
<tr>
<td>Log10 cfu/g</td>
<td>0.00 ± 0.00b</td>
<td>0.00 ± 0.00b</td>
<td>2.37 ± 1.46a</td>
<td>0.86 ± 0.55ab</td>
<td>1.10 ± 1.10ab</td>
</tr>
</tbody>
</table>

**Means within a row with no common superscripts differ significantly (P < 0.05).

1. Hens were challenged by crop gavage with 10^7 cfu of Salmonella Enteritidis on d 4 of molt and cultured for Salmonella on d 9 of molt.

2. A90 = 90% alfalfa/10% layer ration; FF = full fed, nonmolted; FW = feed withdrawal; H = 90% alfalfa/10% layer ration + 0.75% FOS; L = 90% alfalfa/10% layer ration + 0.375% FOS.
Colonization of the Crop and Ceca

Salmonella Enteritidis

The crop serves as a major site for Salmonella Enteritidis, and colonization is known to increase during feed withdrawal for broilers and laying hens (Ramirez et al., 1997; Durant et al., 1999). The same trend was observed in 3 of the 4 trials in our study as well (Table 3). When percentage of Salmonella Enteritidis colonization in the crop of hens fed H and L were compared with A90 and FF treatments, no significant differences (P > 0.05) were observed in trial 2, 3, and 4. Upon the examination of colony-forming units per gram counts, FW hens yielded higher counts than FF hens, and all other molted treatments (A90, H, L) were not found significantly different from each other in 3 of the 4 trials. Salmonella Enteritidis colonization of the ceca is shown in Table 4. Greater Salmonella Enteritidis colonization (% P ≤ 0.05) occurred in FW-treated hens compared with hens from all other treatments, which were not different (P > 0.05) from each other in trial 1. The results from trials 2, 3, and 4 showed similar patterns with FW-treated hens having higher percentage of Salmonella Enteritidis (P ≤ 0.05) cecal colonization than FF birds but not with birds in the other molted treatments. In general, FF hens exhibited less (P ≤ 0.05) Salmonella Enteritidis per gram, whereas FW hens yielded greater (P ≤ 0.05) colony-forming units per gram and all other molted treatments appeared to be intermediate. Similar results were seen by Woodward et al. (2005) and Moore et al. (2004) with FW molted hens resulting in greater Salmonella Enteritidis colony-forming units per gram counts than nonmolted hens.

Salmonella Enteritidis in the Liver, Spleen, and Ovaries

In general, the number of Salmonella Enteritidis-positive hens with respect to liver, spleen, and ovaries increased in FW birds compared with FF birds (Table 5). The increase in organ invasion of FW hens could be due to a decrease of peristalsis muscle contractions and mucin production (Sturkie, 1965), which can facilitate the bacte-

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### Table 4. Effects of nonmolting and molting with and without alfalfa and fructooligosaccharide (FOS) on Salmonella enterica serovar Enteritidis (Salmonella Enteritidis) cecal colonization of hens

<table>
<thead>
<tr>
<th>Trial</th>
<th>Item</th>
<th>A901</th>
<th>FF2</th>
<th>FW2</th>
<th>H2</th>
<th>L2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1</td>
<td>Positive hens per total</td>
<td>1/6 (17%)b</td>
<td>0/6 (0%)b</td>
<td>6/6 (100%)a</td>
<td>2/6 (33%)b</td>
<td>2/6 (33%)b</td>
</tr>
<tr>
<td>Trial 2</td>
<td>Positive hens per total</td>
<td>5/6 (83%)a</td>
<td>0/6 (0%)b</td>
<td>5/6 (83%)a</td>
<td>3/6 (50%)a</td>
<td>3/6 (50%)a</td>
</tr>
<tr>
<td>Trial 3</td>
<td>Positive hens per total</td>
<td>2/6 (33%)ab</td>
<td>0/6 (0%)b</td>
<td>4/6 (67%)a</td>
<td>1/6 (17%)ab</td>
<td>1/6 (17%)ab</td>
</tr>
<tr>
<td>Trial 4</td>
<td>Positive hens per total</td>
<td>2/6 (33%)ab</td>
<td>0/6 (0%)b</td>
<td>4/6 (67%)a</td>
<td>2/6 (33%)ab</td>
<td>2/6 (33%)ab</td>
</tr>
</tbody>
</table>

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### Table 5. Effects of nonmolting and molting with and without alfalfa and fructooligosaccharide (FOS) on Salmonella enterica serovar Enteritidis (Salmonella Enteritidis) colonization of the liver, spleen, and ovary of hens

<table>
<thead>
<tr>
<th>Trial</th>
<th>Item</th>
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<th>FF2</th>
<th>FW2</th>
<th>H2</th>
<th>L2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1</td>
<td>Liver</td>
<td>0/6 (0%)b</td>
<td>0/6 (0%)b</td>
<td>6/6 (100%)a</td>
<td>2/6 (33%)b</td>
<td>2/6 (33%)b</td>
</tr>
<tr>
<td>Trial 2</td>
<td>Liver</td>
<td>2/6 (33%)b</td>
<td>0/6 (0%)b</td>
<td>6/6 (100%)a</td>
<td>3/6 (50%)a</td>
<td>3/6 (50%)a</td>
</tr>
<tr>
<td>Trial 3</td>
<td>Liver</td>
<td>3/6 (50%)a</td>
<td>0/6 (0%)b</td>
<td>4/6 (67%)a</td>
<td>3/6 (50%)a</td>
<td>3/6 (50%)a</td>
</tr>
<tr>
<td>Trial 4</td>
<td>Liver</td>
<td>0/6 (0%)a</td>
<td>0/6 (0%)b</td>
<td>2/6 (33%)b</td>
<td>1/6 (17%)a</td>
<td>0/6 (0%)a</td>
</tr>
</tbody>
</table>

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Means within a row with no common superscripts differ significantly (P < 0.05).

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1Hens were challenged by crop gavage with 10⁵ cfu of Salmonella Enteritidis on d 4 of molt and cultured for Salmonella on d 9 of molt.

2A90 = 90% alfalfa/10% layer ration; FF = full fed, nonmolted; FW = feed withdrawal; H = 90% alfalfa/10% layer ration + 0.375% FOS; L = 90% alfalfa/10% layer ration + 0.75% FOS.

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### FRUCTOOLIGOSACCHARIDE PREBIOTIC AND ALFALFA MOLT DIETS

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**Salmonella Enteritidis Colonization of the Crop and Ceca**

The crop serves as a major site for *Salmonella Enteritidis*, and colonization is known to increase during feed withdrawal for broilers and laying hens (Ramirez et al., 1997; Durant et al., 1999). The same trend was observed in 3 of the 4 trials in our study as well (Table 3). When percentage of *Salmonella Enteritidis* colonization in the crop of hens fed H and L were compared with A90 and FF treatments, no significant differences (*P > 0.05*) were observed in trial 2, 3, and 4. Upon the examination of colony-forming units per gram counts, FW hens yielded higher counts than FF hens, and all other molted treatments (A90, H, L) were not found significantly different from each other in 3 of the 4 trials. *Salmonella Enteritidis* colonization of the ceca is shown in Table 4. Greater *Salmonella Enteritidis* colonization (％ *P ≤ 0.05*) occurred in FW-treated hens compared with hens from all other treatments, which were not different (％ *P > 0.05*) from each other in trial 1. The results from trials 2, 3, and 4 showed similar patterns with FW-treated hens having higher percentage of *Salmonella Enteritidis* (％ *P ≤ 0.05*) cecal colonization than FF birds but not with birds in the other molted treatments. In general, FF hens exhibited less (％ *P ≤ 0.05*) *Salmonella Enteritidis* per gram, whereas FW hens yielded greater (％ *P ≤ 0.05*) colony-forming units per gram and all other molted treatments appeared to be intermediate. Similar results were seen by Woodward et al. (2005) and Moore et al. (2004) with FW molted hens resulting in greater *Salmonella Enteritidis* colony-forming units per gram counts than nonmolted hens.

**Salmonella Enteritidis in the Liver, Spleen, and Ovaries**

In general, the number of *Salmonella Enteritidis*-positive hens with respect to liver, spleen, and ovaries increased in FW birds compared with FF birds (Table 5). The increase in organ invasion of FW hens could be due to a decrease of peristalsis muscle contractions and mucin production (Sturkie, 1965), which can facilitate the bacte-
Table 6. Effects of nonmolting and molting with and without alfalfa and fructooligosaccharide (FOS) on Salmonella enterica serovar Enteritidis (Salmonella Enteritidis) intestinal shedding (trial 1 and 2)

<table>
<thead>
<tr>
<th>Trial</th>
<th>Day</th>
<th>Item</th>
<th>A90</th>
<th>FF</th>
<th>FW</th>
<th>H</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1</td>
<td>Day 8</td>
<td>Positive hens per total</td>
<td>5/6 (83%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3/6 (50%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5/6 (83%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4/6 (67%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3/6 (50%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>Log&lt;sub&gt;10&lt;/sub&gt; cfu/g</td>
<td>0.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Day 21</td>
<td>Positive hens per total</td>
<td>1/6 (17%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/6 (0%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1/6 (17%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/6 (0%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1/6 (17%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Day 28</td>
<td>Positive hens per total</td>
<td>1.34 ± 0.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.67 ± 0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.16 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.33 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Trial 2</td>
<td>Day 8</td>
<td>Positive hens per total</td>
<td>1/4 (25%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2/5 (40%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2/5 (40%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1/5 (20%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2/5 (40%)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>Positive hens per total</td>
<td>3/5 (60%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/6 (0%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0/6 (0%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0/6 (0%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0/6 (0%)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Day 21</td>
<td>Positive hens per total</td>
<td>1/4 (25%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/6 (0%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/6 (0%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/6 (0%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/6 (0%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Day 28</td>
<td>Positive hens per total</td>
<td>0.24 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Means within a row with no common superscripts differ significantly (P < 0.05).

<sup>1</sup>Hens were challenged by crop gavage with 10<sup>7</sup> cfu of Salmonella Enteritidis on d 4 of molt and cultured for Salmonella on d 9 of molt. Days represent number of days postinitiation of molt.

<sup>2</sup>A90 = 90% alfalfa/10% layer ration; FF = full fed, nonmolting; FW = feed withdrawal; H = 90% alfalfa/10% layer ration + 0.75% FOS; L = 90% alfalfa/10% layer ration + 0.375% FOS.

intestinal colonization by pathogens (Holt and Porter, 1992; Holt et al., 1993). The lack of feed in the gastrointestinal tract also alters the normal intestinal microflora and pH, which can create a microenvironment favoring pathogen development (Ricke, 2003). In our study, FOS-containing diets reduced (P < 0.05) ovary and liver colonization by Salmonella Enteritidis in 3 and 2 of the 4 trials, respectively, when compared with hens subjected to FW. The Salmonella Enteritidis colonization of liver, spleen, and ovary of hens fed FOS diets, however, was not found to be different from that of hens fed A90. Seo et al. (2001) reported that by providing some form of bulk in the gastrointestinal tract, hens can clear an infection more readily than if the gut was empty. By providing alfalfa in the diet, the Salmonella Enteritidis was unable to fully colonize and was therefore possibly cleared from the tract. In our study, FOS addition to alfalfa-containing diets did not further prevent invasion by Salmonella Enteritidis in the organs of hens.

Table 7. Effects of nonmolting and molting with and without alfalfa and fructooligosaccharide (FOS) on Salmonella enterica serovar Enteritidis (Salmonella Enteritidis) intestinal shedding (trial 3 and 4)

<table>
<thead>
<tr>
<th>Trial</th>
<th>Day</th>
<th>Item</th>
<th>A90&lt;sup&gt;2&lt;/sup&gt;</th>
<th>FF&lt;sup&gt;2&lt;/sup&gt;</th>
<th>FW&lt;sup&gt;2&lt;/sup&gt;</th>
<th>H&lt;sup&gt;2&lt;/sup&gt;</th>
<th>L&lt;sup&gt;2&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>Trial 3</td>
<td>Day 8</td>
<td>Positive hens per total</td>
<td>2/6 (33%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1/6 (17%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/6 (0%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/6 (0%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>Positive hens per total</td>
<td>0.58 ± 0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.98 ± 0.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Day 21</td>
<td>Positive hens per total</td>
<td>1/5 (20%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Day 28</td>
<td>Positive hens per total</td>
<td>0.42 ± 0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Trial 4</td>
<td>Day 8</td>
<td>Positive hens per total</td>
<td>0.41 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.56 ± 0.95&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>Positive hens per total</td>
<td>0.16 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Day 21</td>
<td>Positive hens per total</td>
<td>0.83 ± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Day 28</td>
<td>Positive hens per total</td>
<td>1/4 (25%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1/5 (20%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.67 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Means within a row with no common superscripts differ significantly (P < 0.05).

<sup>1</sup>Hens were challenged by crop gavage with 10<sup>7</sup> cfu of Salmonella Enteritidis on d 4 of molt and cultured for Salmonella on d 9 of molt. Days represent number of days postinitiation of molt.

<sup>2</sup>A90 = 90% alfalfa/10% layer ration; FF = full fed, nonmolting; FW = feed withdrawal; H = 90% alfalfa/10% layer ration + 0.75% FOS; L = 90% alfalfa/10% layer ration + 0.375% FOS.
Figure 1. Effects of nonmolting and molting diets with and without alfalfa and fructooligosaccharide (FOS) on cecal volatile fatty acids (VFA; μmol/mL). A–C Means within trial 1 (striped bars) without a common letter differ significantly ($P < 0.05$). a,b Means within trial 2 (white bars) without a common letter differ significantly ($P < 0.05$). A–C Means within trial 3 (black bars) without a common letter differ significantly ($P < 0.05$). a–c Means within trial 4 (gray bars) without a common letter differ significantly ($P < 0.05$). A90 = 90% alfalfa/10% layer ration; FF = full fed, nonmolted; FW = feed withdrawal; H = 90% alfalfa/10% layer ration + 0.75% FOS; L = 90% alfalfa/10% layer ration + 0.375% FOS.

Cecal VFA Profile

The data on the total VFA and the specific volatile compounds are presented in Figure 1 and Figure 2. In half of the trials, FF hens yielded greater ($P \leq 0.05$) acetic acid concentrations in the ceca than hens undergoing FW. Likewise, Woodward et al. (2005) reported higher acetic acid production in FF hens than in FW- and alfalfa-fed hens. The A90, H, and L diets did not differ from each other by acetate production in contrast to the study of Kass et al. (1980), in which cecum and colon production of acetate in growing swine were stimulated by the addition of 40% alfalfa to swine diets. There were no significant ($P \leq 0.05$) differences in propionic acid concentrations (Figure 1b) in the ceca of alfalfa-molted hens in any of the 4 trials as well. Concentrations of isobutyric acid (Figure 1c) were significantly higher in H- and L-treated hens when compared with FW hens in trial 1. Compared with alfalfa-molted birds, isobutyric acid concentrations in trial 4 were higher ($P \leq 0.05$) in L-fed hens than in A90 birds but not different ($P > 0.05$) from any other treatment.
Although no differences in the concentrations of isobutyric acid in FF and FW hens were observed by Moore et al. (2004), Woodward et al. (2005) reported high variability in isobutyric concentrations in the different treatments among different trials. Butyric acid concentrations (Figure 1d) were lower ($P \leq 0.05$) in FW hens when compared with all other treatments in trial 1. Although relatively inconsistent, our butyric acid results followed the trend observed by Woodward et al. (2005), in which nonmolted birds in half of the trials were characterized with higher production of cecal butyric acid when compared with molted and alfalfa-fed chickens. The H-treated hens in our study yielded greater ($P \leq 0.05$) butyric acid concentrations in the ceca compared with FW- and FF-treated hens in 1 of the trials but were not significantly different from any other alfalfa-molted hens.

No differences ($P > 0.05$) between any treatments were seen when 2-methylbutyric acid concentrations were measured in any of the 4 trials (Figure 1e). Similarly, there were no differences ($P > 0.05$) in isovaleric acid concentrations between any treatments in trial 1, 3, or 4 (Figure 2a). Concentrations of valeric acid (Figure 2b) were lower ($P \leq 0.05$) in the ceca of FW-treated hens than in the ceca of all alfalfa molt-treated hens in trial 1. However, in trial 2, valeric acid concentrations in the ceca of FF hens were greater ($P \leq 0.05$) than valeric acid concentrations in the ceca of FW hens. Alfalfa-molted hens did not exhibit differences ($P > 0.05$) in valeric acid concentration when compared with either FW- or FF-treated hens. No differences ($P > 0.05$) in valeric acid concentrations were seen between any treatments in trial 3 or 4.

Total VFA concentrations (Figure 2c) were lower ($P \leq 0.05$) in the ceca of FW hens than in all alfalfa molt treatments but not FF-treated hens in trial 1. Hens from trials 2 and 4 were opposite of each other when total VFA concentrations were estimated. The total VFA concentrations in the ceca of FF hens were greater ($P \leq 0.05$) than all molt treatments in trial 2 and greater ($P \leq 0.05$) than all other treatments except H in trial 4. Similar to individual VFA concentrations in trial 3, there were no differences ($P > 0.05$) among treatments when total VFA were quantified. The results from trial 1 were similar to the results seen by Moore et al. (2004), in which no differences were found in total VFA in molted and nonmolted treated hens, whereas the results of trial 2 and 4 corresponded to the observations of Woodward et al. (2005) that FF hens produced greater total VFA concentrations than hens molted by alfalfa or FW hens.

Concentrations of lactic acid (Figure 2d) were significantly greater in birds fed all 3 alfalfa molt diets compared with FF or FW hens in trials 1 and 3. In trial 2 only FW-treated hens exhibited lower ($P \leq 0.05$) lactic acid concentrations than the hens fed the 3 alfalfa molt diets. This trend was consistent with the findings of Woodward et al. (2005), who observed an approximately 2-fold increase of lactic acid concentration in alfalfa molt diets when compared with FW treatments. The alfalfa results
are not surprising, because lactate is considered a predominant end product of a successful fermentation of alfalfa silage (Owens et al., 2002). In contrast to our study, the dominant end product of a successful fermentation are not surprising, because lactate is considered a pre-

In summary, in half of the trials in our study, FOS-containing diets significantly reduced Salmonella Enteriti-

ACKNOWLEDGMENTS

This research was supported by Hatch grant H8311 administered by the Texas Agricultural Experiment Station, USDA-National Research Initiative grant number 2002-02614, and US Poultry and Egg Association grant number 485. Lisa Donalson was partially supported by the Maurice Stein Fellowship Award. We thank Maurice Connell and Albert Blanks (USDA-Agricultural Research Service, College Station, TX) for their assistance. We also like thank Encore Technologies (Plymouth, MN) for donating the FOS.

REFERENCES


