Effects of increasing dietary concentrations of fish oil on lamb performance, ruminal fermentation, and leptin gene expression in perirenal fat

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ABSTRACT - The objective of this study was to evaluate the effects of four levels of fish oil on lamb performance, carcass yield, ruminal fermentation, and leptin gene expression in perirenal fat. Thirty-two lambs (24.10±2.15 kg, Katahdin × Pelibuey) were used in a completely randomized experimental design. The lambs were assigned to one of four dietary treatments (n = 8 lambs/treatment), expressed as g/kg DM basis: 0 fish oil and 300 corn; 10 fish oil and 250 corn; 20 fish oil and 205 corn; and 30 fish oil and 170 corn. The lambs were weighed on consecutive days at the beginning (days 0 and 1) and at the end (days 55 and 56) of the trial. Ruminal fluid samples were collected on day 56 to evaluate the ruminal fermentation pattern. The lambs were slaughtered on day 56; perirenal adipose tissue samples were collected and the carcass yields were recorded. Volatile fatty acids, ammonia N, and leptin mRNA expression were not affected by the dietary treatments. However, the dry matter intake, average daily gain, final body weight, and the hot carcass yield showed either increased linear or quadratic responses as the proportion of fish oil increased in the ration; the estimated optimal level obtained of fish oil levels for average daily gain was 11.2±0.21 g/kg and 12.8±4.67 g/kg for feed conversion. Additionally, feed efficiency and backfat thickness had an increment, showing quadratic response as the proportion of fish oil increased in the diet. Increasing the fish oil concentration in the diet does not affect leptin messenger ribonucleic acid expression. The lamb performance can be improved with 12 g/kg fish oil in diets of finishing lambs.

Key Words: finishing lambs, oils, carcass quality

Introduction

Fish oil has been incorporated into lamb diets at concentrations of 20-30 g/kg, based on forage or concentrate, with no effect on intake or daily gain (Marinova et al., 2007; Toral et al., 2009; Annett et al., 2011). Other studies have partially replaced vegetable oils with fish oil and attempted to avoid the negative effects of the omega-3 polyunsaturated fatty acids, which are present in high concentrations in fish oil, on lamb performance. Ferreira et al. (2014) evaluated fish oil concentrations from 2.5 to 7.5 g/kg in lamb finishing diets with 40 g/kg total oil and found no effect on intake, daily gain, or feed efficiency.

Nutrigenomics (the study of the bidirectional interactions between genes and diet) is a rapidly developing field that is changing research on nutrition (Zeisel and da Costa, 2009).

Leptin is a hormone primarily secreted by white adipocytes (Chilliard et al., 2005) and is a protein involved in the central and/or peripheral regulation of body homeostasis, energy intake, storage and expenditure, fertility, and immune functions (Ingvartsen and Boisclair 2001; Tokuda et al., 2000). The role of leptin is well documented in rodents and humans, increasing lipolysis and decreasing lipogenesis in the adipose tissues or in the liver (Havel et al., 2004; Sadri et al., 2011), but it is more poorly understood in ruminants (Chilliard et al., 2005). Chilliard et al. (2005) suggested that fatty acids, particularly linoleic, could stimulate leptin expression in ruminants as well as in rodents. However, in ruminants, the effects of fat ingestion on leptin blood concentrations have not been consistently demonstrated and the effects would likely depend on the amount of fat consumed or on other dietary interactions. Lambs supplemented with a high amount of rumen-
protected fat increased leptin blood concentrations (Yildiz et al., 2003). Chilliard et al. (2001) found that leptin plasma concentrations were lower in a group fed a diet with moderate energy content compared with a group fed a high-energy diet. Similarly, leptin messenger ribonucleic acid (mRNA) expression increased in lactating goats fed sunflower seed oil and natural grassland hay, but not when fed an oil and corn silage diet (Bonnet et al., 2009).

It is important to identify the optimal concentration of fish oil in diet dry matter (DM), below the 30 g/kg limit that allows for improved gain and feed conversion. We hypothesized that performance variables would show a quadratic response to fish oil in the diet of lambs, associated with changes in leptin expression. Therefore, the objective of the current study was to evaluate lamb performance, ruminal fermentation characteristics, and the expression of the leptin gene in lambs fed a high-concentrate diet supplemented with varying concentrations of fish oil.

**Material and Methods**

All procedures were approved by the local Animal Care and Use Committee. The experiment was conducted in Huitzilac, Morelos, Mexico.

Thirty-two male lambs (24.10±2.15 kg, Katahdin × Pelibuey), housed in individual pens (0.80 × 1.75 m), were used in a completely randomized experimental design, where response variables were measured once; therefore, animal nested within treatment was the experimental unit. The lambs were dewormed with closantel sodium (5 mg/kg body weight); vaccinated for *Clostridium perfringens* types C and D, *Clostridium novyti*, *C. sordelii*, *C. chauvoei*, and *C. septicum* (Ultrabac 7), and dosed with vitamins A, D, and E (Vigantol 2 mg/lamb). The lambs were assigned to one of four dietary treatments (n = 8 lambs/treatment). The lambs had continuous access to water and were weighed on two consecutive days starting from the sixth day, the lambs were gradually adapted to the experimental diet, replacing 200 g/kg of forages by the experimental diet every three days. Lambs had continuous access to and were weighed on two consecutive days at the beginning (day 0 and day 1) and at the end of the trial (day 55 and day 56). Feed was provided at 08.00 and 15.00 h. All the lambs had free access to feed, ensuring 100 g refusal per kg from the daily quantity offered, and daily intake was calculated by weighing refusals daily.

Samples of the feed and the refusals were collected daily and composited every 14 days. The dry matter and total nitrogen content of the diets were analysed according to the AOAC (2000). The cell wall analyses were conducted according to the method of Van Soest et al. (1991). Rumen fluid (50 mL) was extracted on day 56 at 07.00 h by esophageal tube obtained before morning feeding with a flexible Tygon tube. Rumen fluid was obtained using an electric vacuum pump and before being strained through a cheesecloth layer, acidified with 1 mL of sulphuric acid (300 g/L), and stored in a freezer (−20 °C) for further analyses. The volatile fatty acids were measured using gas chromatography of samples prepared with metaphosphoric acid (Erwin et al., 1961) and the ammonia N was colorimetrically analysed (McCullough, 1967).

The performance variables were daily feed intake, average daily gain, feed efficiency (ratio of kg gained per kg feed intake), hot carcass yield, and ultrasound back fat of the *Longissimus dorsi* muscle area. The hot carcass yield was obtained following slaughter of the lambs on day 56 and the ultrasound back fat was assessed one day before slaughter (Silva et al., 2005).

| Table 1 - Ingredients and nutritional composition of diets (dry matter basis) offered to finishing lambs |
|----------------------------------------------------------|---------------|--------------|--------------|---------------|
| Ingredient                                              | 0             | 10           | 20           | 30           |
| Fish oil                                                | 0             | 10           | 20           | 30           |
| Corn                                                    | 300           | 250          | 205          | 170          |
| Sorghum                                                 | 300           | 300          | 300          | 300          |
| Corn gluten                                             | 50            | 50           | 50           | 50           |
| Soybean meal                                            | 0             | 20           | 15           | 20           |
| Cane molasses                                           | 110           | 110          | 110          | 110          |
| Corn stover                                             | 210           | 230          | 270          | 290          |
| Urea                                                    | 10            | 10           | 10           | 10           |
| Buffer                                                  | 10            | 10           | 10           | 10           |
| Mineral premix                                            | 10            | 10           | 10           | 10           |
| **Chemical composition (g/kg)**                          |               |              |              |              |
| **Dry matter**                                           | 905.4         | 908.0        | 911.1        | 918.5        |
| **Organic matter**                                       | 862.3         | 859.5        | 862.3        | 870.8        |
| **Crude protein**                                        | 140.5         | 142.2        | 159.9        | 162.1        |
| **Neutral detergent fiber**                              | 308.1         | 313.0        | 314.8        | 321.4        |
| **Ether extract**                                        | 23.7          | 31.3         | 40.1         | 49.1         |
| **Metabolizable energy (Mcal/kg)**                       | 2.26          | 2.25         | 2.23         | 2.23         |

1 Commercial buffer (Acid Bufl) containing CaCO₃ (750 g) and MgCO₃ (190 g).
2 Commercial mix (Vitisal Engorda Ovinos Plus) containing: Ca, 270 g; P, 30 g; Mg, 7.5 g; Na, 65.5 g; Cl, 100 g; K, 0.5 g; S, 42 mg; lasalocid, 2000 mg; Mn, 2000 mg; Fe, 978 mg; Zn, 3000 mg; Se, 20 mg; Cu, 15 mg; vitamin A, 35,000 IU; vitamin D, 150,000 IU; and vitamin E, 150 IU.

1 Estimated with NRC tables values.
Samples from the perirenal adipose deposits were removed via biopsy techniques within 4 min after slaughter to determine expression of leptin mRNA (Kumar et al., 1998) and total RNA was isolated from the fat by homogenization of tissue (Tissue-Tearor™, Daigger®, Daigger & Company Inc.) in Tri Reagent® (Sigma Chemical Co. Ltd.). Total RNA was used for reverse transcription and Leptin mRNA expression was quantified with real-time polymerase chain reaction (PCR) using the forward and reverse primers, 5’-ACAACACACGATGGAAGCAT-3’ and 5’- ACCCCAAAAAGCCTGGAAAA-3, and total number of cDNA copies was estimated as described by Tricarico et al. (2002).

Data were tested for the Shapiro–Wilk test of normality and results were analysed according to a completely randomized design and were tested for linear and quadratic effects of fish oil concentration (Steel et al., 1997) and gene expression results were log-transformed prior to analysis. Data were analysed with the following model:

\[ Y_{ij} = \mu + \tau_i + a_{ij0} + e_{ij} \]

in which \( \mu \) is the mean value, \( \tau_i \) is the treatment effect (fixed), \( a_{ij0} \) is the effect of animal nested within each treatment, and \( e_{ij} \) is the error term. If quadratic effect was significant, the optimal concentration for lamb performance variables were estimated using the derived quadratic equation (-b\(_1\)/2 b\(_{11}\)) from the equation \( Y = b_0 + b_1 X + b_{11} X^2 \) (Draper and Smith, 1998), in which \( Y \) is the response variable and \( X \) is the fish oil concentration. The response variables were also tested for simple correlations. Data were analysed with the JMP7 software (Sall et al., 2012).

In each experimental treatment, costs per kg of live weight and the partial net income were estimated per lamb, considering incomes (sales value) and expenditures (value of direct costs such as cost of animal, feed, veterinary costs, etc.), as described by Mendoza et al. (2015).

### Results

As expected, the variables related to growth and fattening showed a quadratic response that was used to estimate the highest final body weight (39.81 kg), which occurred with 11.6 g/kg fish oil, and the highest carcass weight (19.78 kg), which occurred with 10.3 g/kg fish oil. The maximum average daily gain (275 g/d) was estimated to occur with 11.2±0.21 g/kg fish oil and the best feed conversion ratio (4.10) with 12.8±4.67 g/kg (the best feed efficiency estimated with 13.1±0.15 g/kg fish oil). Final body weight was positively correlated with average dry matter intake \( (r = 0.74, P<0.0001) \), average daily gain \( (r = 0.84, P<0.0001) \), and feed efficiency \( (r = 0.66, P<0.0001) \). Although there were changes in back fat and gain-to-feed ratio, leptin gene expression was not affected by the treatments (Table 2) and leptin mRNA was not correlated with other variables.

The molar proportions of acetate and butyrate decreased linearly as fish oil concentration increased, whereas propionate increased. The total volatile fatty acid concentrations also tended to decrease in a linear manner (Table 3). There were no differences \( (P>0.10) \) in the ammonia N concentration among the treatments (Table 3).

Economic evaluation showed that inclusions up to 20 g/kg of fish oil concentration were profitable, but higher amounts caused economic losses (Table 4).

### Discussion

Consistent with the results of Ferreira et al. (2014), this experiment demonstrated a positive response in average daily gain with the lowest concentration of fish oil in finishing lamb rations. Overall, in studies in which fish oil concentrations were approximately 10 g/kg, final weight,

<table>
<thead>
<tr>
<th>Item</th>
<th>Fish oil concentration (g/kg)</th>
<th>SEM</th>
<th>P-value</th>
<th>Contrast</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
<td>10</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Initial weight (kg)</td>
<td>23.88</td>
<td>24.31</td>
<td>24.33</td>
<td>23.80</td>
</tr>
<tr>
<td>Final weight (kg)</td>
<td>37.17</td>
<td>39.50</td>
<td>38.68</td>
<td>33.03</td>
</tr>
<tr>
<td>Dry matter intake (DMI) (g/d)</td>
<td>1122</td>
<td>1146</td>
<td>1080</td>
<td>898</td>
</tr>
<tr>
<td>Average daily gain (g)</td>
<td>237</td>
<td>271</td>
<td>256</td>
<td>165</td>
</tr>
<tr>
<td>G:F (kg of gain/kg of DMI)</td>
<td>0.176</td>
<td>0.201</td>
<td>0.194</td>
<td>0.156</td>
</tr>
<tr>
<td>Hot carcass weight (kg)</td>
<td>18.66</td>
<td>19.51</td>
<td>18.83</td>
<td>16.02</td>
</tr>
<tr>
<td>Hot carcass yield (%)</td>
<td>54.49</td>
<td>54.08</td>
<td>51.77</td>
<td>52.22</td>
</tr>
<tr>
<td>Back fat (mm)</td>
<td>1.87</td>
<td>2.50</td>
<td>2.87</td>
<td>2.00</td>
</tr>
<tr>
<td>Leptin[^{5}]</td>
<td>8.83</td>
<td>8.59</td>
<td>8.87</td>
<td>9.03</td>
</tr>
</tbody>
</table>

\[^{1}\] Dry matter basis.
\[^{2}\] Standard error of the mean; \( n = 8 \).
\[^{3}\] P-value for the F-Test of the mean for the dietary treatment.
\[^{4}\] P-value for linear and quadratic functions in response to increasing fish oil.
\[^{5}\] Log. No. copies of leptin mRNA expressed.
average daily gain, and feed efficiency were unaffected
(Toral et al., 2009; Annett et al., 2011; Ferreira et al., 2014),
but when the fish oil concentrations were above 30 g/kg,
the intake and average daily gain were reduced (Kitessa
et al., 2001; Wachira et al., 2002; Marinova et al. 2007).
Annett et al. (2011) observed that with 35 g/kg fish oil, the
subcutaneous fat depth over the leg area in lambs increased
slightly and Marinova et al. (2007) reported that a diet with
10 g/kg fish oil mixed with 20 g/kg sunflower oil reduced
the subcutaneous fat in the loin, but intramuscular fat was
higher in the shoulder.

Results from other experiments have indicated that
changes in the ruminal fermentation pattern are related to
the amount of fish oil; reductions in acetate and increases
in propionate were reported at concentration of 40 g/kg fish
oil (Wachira et al., 2002; Fievez et al., 2003); and acetate
was only reduced at concentrations between 2.5 and 10 g/kg
(Toral et al., 2009; Ferreira et al., 2014). However, the forage
proportion can modify the response. Chikunya et al. (2004)
used 50 g/kg fish oil in a forage-based diet and did not detect
changes in molar proportions of volatile fatty acids. Results
of the response of butyrate to fish oil have been inconsistent
and the only change was observed in an experiment with
10 g/kg fish oil (Toral et al., 2009). Marinova et al. (2007)
suggested that changes in propionate and acetate are related
to the depression of acetate-producing bacteria, which
are inhibited by polyunsaturated fatty acids. However, in
high-grain diets, these bacteria do not grow (Russell et al.,
2009); therefore, the changes in propionate and acetate
could be related to effects on the rumen protozoa, because
polyunsaturated fatty acids are also toxic to rumen protozoa
(Oldick and Firkins, 2000). The microbial defaunation by
high-grain diets have similar effects on the fermentation
pattern (Mendoza et al., 1993). The total concentration of
volatile fatty acids must be carefully evaluated, because
the process of absorption is a function of the pH and the
concentration gradient. Fievez et al. (2003) reported no
changes in total volatile fatty acid concentrations, but Toral
et al. (2009) observed a reduction in the volatile fatty acid
concentration. The ruminal ammonia N concentrations
were not affected by the concentrations of fish oil used in
this experiment, which is in contrast with other studies that
reported increases in this metabolite in sheep (Toral et al.,
2009) and cattle with 50 g/kg fish oil in high-grain diets
(Kook et al., 2002).

Leptin mRNA has been positively correlated with the
adipocyte volume in steers (Yang et al., 2003) and blood
leptin concentrations have shown positive relationships
with body fat content in sheep across a broad range of
ages and body weights (Altmann et al., 2005). Chilliard
et al. (2001) found that leptin plasma concentrations were
lower in a group fed a diet with a moderate energy content
compared with a group fed a diet with high-energy content.
However, in this study, the differences in energy intake did
not change the expression of the leptin gene.

Decreases in feed intake in finishing ruminants have
been associated with an increase in leptin, which is involved
in the homeostatic regulation of body energy by regulating
appetite (Ban-Tokuda et al., 2008). Higher concentrations
of fish oil reduced intake and tended to show the highest
leptin gene expression; although gene expression was not

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<td></td>
<td>0</td>
<td>10</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Acetate (%)</td>
<td>66.75</td>
<td>64.01</td>
<td>61.72</td>
<td>59.39</td>
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<tr>
<td>Propionate (%)</td>
<td>20.07</td>
<td>24.71</td>
<td>30.72</td>
<td></td>
</tr>
<tr>
<td>Butyrate (%)</td>
<td>13.16</td>
<td>11.26</td>
<td>9.88</td>
<td></td>
</tr>
<tr>
<td>Total volatile fatty acids (mMol/L)</td>
<td>48.09</td>
<td>49.85</td>
<td>33.45</td>
<td></td>
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<tr>
<td>N-NH₃ (mg/dL)</td>
<td>13.91</td>
<td>12.00</td>
<td>12.55</td>
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</table>

Table 3 - Effect of fish oil dietary concentration on ruminal fermentation pattern and ammonia N

<table>
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<tr>
<th>Item</th>
<th>Fish oil concentration (g/kg)</th>
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<tr>
<td></td>
<td>0</td>
<td>10</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Duration of fattening days</td>
<td>56</td>
<td>56</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>Total weight gain (kg)</td>
<td>13.27</td>
<td>15.17</td>
<td>14.33</td>
<td></td>
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<tr>
<td>Cost per kg feed (US$)</td>
<td>0.50</td>
<td>0.513</td>
<td>0.514</td>
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<tr>
<td>Total feed intake (as feed) (kg)</td>
<td>73.92</td>
<td>75.60</td>
<td>70.00</td>
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<tr>
<td>Feed costs (US$)</td>
<td>36.96</td>
<td>38.80</td>
<td>35.99</td>
<td></td>
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<tr>
<td>Weight sold (depleted) (kg)</td>
<td>35.42</td>
<td>37.21</td>
<td>37.26</td>
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<tr>
<td>Initial cost of animal (US$)</td>
<td>63.68</td>
<td>64.82</td>
<td>64.93</td>
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<tr>
<td>Other costs per animal (US$)</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
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<tr>
<td>Selling price (US$/kg)</td>
<td>2.91</td>
<td>2.91</td>
<td>2.91</td>
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</tr>
<tr>
<td>Total sales revenues (US$)</td>
<td>106.24</td>
<td>112.90</td>
<td>108.70</td>
<td></td>
</tr>
<tr>
<td>Partial net income per animal (US$)</td>
<td>+2.34</td>
<td>+6.02</td>
<td>+4.50</td>
<td></td>
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</table>

Table 4 - Economic analysis of the inclusion of fish oil concentration in diets of finishing lambs

1 Dry matter basis.
2 Standard error of the mean; n = 8.
3 P-value for the F test of the mean for the dietary treatment.
4 P-value for linear and quadratic functions in response to increasing fish oil.

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</table>
significantly affected, the logarithmic scale should be taken into consideration. Puglisi et al. (2011) concluded that the n-3 fatty acids present in fish oil could increase the expression of leptin in the adipocytes via PPAR gamma. The PPAR isotypes in ruminants are activated by long-chain fatty acids (Bionaz et al., 2013).

Although fish oil is an expensive ingredient, its inclusion reduces the amount of grain and improves the efficiency of feed utilization, which results in increased partial net income per animal. The results were similar to those reported in Mexico for lamb feedlot rations with exogenous enzymes (Mendoza et al., 2105). Inclusion levels will depend on the cost, since future demand for fishmeal will be higher by sectors such as aquaculture, livestock feed, pharmaceutical production, and cosmetic production (FAO, 2012).

Conclusions

Lamb performance can be improved in finishing diets with the inclusion of 12.0 g/kg fish oil dry matter. However, care must be taken when feeding fish oil to finishing lambs at higher concentrations because of the observed impairment of all performance variables. The concentrations of fish oil evaluated do not affect the expression of the leptin gene in the perirenal adipose tissue.

Acknowledgments

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