The demand for meat with a lower percentage of fat in broilers has increased in the last years. Fatness in fowl is a heritable and highly variable secondary trait that is often assessed by indirect measurements (Ricard and Rouvier 1967, 1969). Information related to the allelic variation of DNA may improve the efficiency of selection for lean birds. Fatty acid synthase is a key enzyme of lipogenesis and may play a crucial role in the weight variability of the abdominal adipose tissue. The locus coding for Fatty-acid Synthase (named FASN by GenBank, accession number J02839 and for Swissprot: locus FAS_CHICK, accession P12276) enzyme has been identified within the linkage group E31E21C25W12 (Pitel et al. 1998). The objective of this study was to search for polymorphism of a chromosomal region coding for Fatty-acid Synthase in chickens.

The study was carried out on 44 (F1) broilers originating from a cross of two slowly growing bird stocks (Free-Range). The male stock originated from a crossbreed of two different strains of Plymouth Rock and the female from Cornish and Rhode Island Red crosses. The selection carried out on this original population was such that birds without coloured plumage, with sanitary defects and low live weight were excluded. These populations were raised at the INTA experimental station, EEA Pergamino, Buenos Aires, Argentina. Forty-four 81-day chickens raised together were slaughtered. All the blood was extracted and from these samples, genomic DNA was isolated by phenolic extraction. Forward and reverse primers were designed complementary to the flanking exons of the last intron of the chicken FASN gene. The choice of the last intron of the FASN gene was due to its high polymorphism characteristic of the non-coding regions and because its appropriate length for the PCR reaction. The forward primer position was at 643 to 663 and the reverse was at 103 to 126.
2048 to 2069 bp, respectively (GenBank accession number J02839). The region of interest described by Kastury et al. (1988) was amplified from the DNA of twelve broilers. These broilers were six with the highest and six with the lowest fat percentage. The PCR amplified a 1427 bp product. Primer sequences:

**FASN** Forward 5' GCTGAAGGCTGCTGACAAGTA 3',

**FASN** Reverse: 5' AACACCATCTCCCTCCAATAAG 3'.

PCR conditions: the amplification of the intron was carried out in 20 μl reaction containing 10 pmoles of each primer, 0.5 U Taq polymerase (Promega®) with its buffer, 2 mM of each dNTP, 1.5 mM of Cl₂Mg and 50 ng of DNA. Genomic DNA was denatured for 90 s at 94°C, and PCR run for 40 cycles at 94°C for 30 s, 61°C for 30 s for annealing, and the extension was at 72°C for 90 s. Sequencing of the last intron from the twelve birds was done from the PCR product using an ABI 373 DNA Automated Sequencer. Restriction conditions for the three enzymes: in a reaction of 20 μl, 160 ng of the PCR product were digested with 1U of the appropriate restriction enzyme with the buffer supplied by manufacturer (New England Biolab®). After 60 min of incubation at 37°C, the fragments were resolved on a 3% agarose gel (Figure 1).

Two mutations were found from the analysis of the sequences of the 12 animals. The sequences presented a G/A substitution located at base 459 of the PCR product (and at base 1222 of GenBank number J02839) resulting in a site that was recognised by the restriction enzymes *Hae* III and *Ava* II. The genotypic identification was carried out following the restriction fragment pattern obtained from the digestion of the PCR product (Figure 1). The use of two restriction enzymes recognizing the same mutation may be utilized as a control of partial digestion in identifying genotypes of different animals. The sequence also presented

![Figure 1. Reverse image of ethidium bromide stained 3% agarose gel, indicating the genotypes of twelve Campero broilers for Hae III. Lanes N and M: Molecular weight marker. The A/A genotype is represented by animals 42, 32, and 31. The A/G genotype is represented by animals: 69, 52, 33 and 28. The G/G genotype is represented by animals: 64, 61, 59, 30 and 29. Lane A: undigested PCR product.](image)

<table>
<thead>
<tr>
<th>Restriction Enzyme</th>
<th><em>Hae</em> III (at position 459 bp)</th>
<th><em>Ava</em> II (at position 459 bp)</th>
<th><em>Pst</em> I (at position 603 bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fragments for allele A (frequency)</td>
<td>780, 340 and 305 (0.48)</td>
<td>850, 330, 150, and 95 (0.48)</td>
<td>1170 and 256 (0.14)</td>
</tr>
<tr>
<td>Fragments for allele G (frequency)</td>
<td>780, 340, 240 and 65 (0.52)</td>
<td>945, 330 and 150 (0.52)</td>
<td>702, 468 and 256 (0.86)</td>
</tr>
<tr>
<td>Frequency of genotype A/A</td>
<td>0.23</td>
<td>0.23</td>
<td>0</td>
</tr>
<tr>
<td>Frequency of genotype A/G</td>
<td>0.5</td>
<td>0.5</td>
<td>0.27</td>
</tr>
<tr>
<td>Frequency of genotype G/G</td>
<td>0.27</td>
<td>0.27</td>
<td>0.73</td>
</tr>
</tbody>
</table>
ents a G/A substitution (located at base 603 of the PCR product, and No. 1366 of the J02839 GenBank accession) resulting in a site that is recognised by the restriction enzyme Pst I. The PCR-RFLP was employed for the genotyping of the other individuals. Allelic and genotype frequencies for the three enzymes and for 44 broilers are described in Table 1.

Many mechanisms may be involved in the genetic determinism of fat deposition in poultry. Leclercq et al. (1990) presented results especially in poultry, pointing out that hepatic lipogenic enzymes and apoprotein genes may be interesting candidates since they explain some of fat deposition variability. Douaire et al. (1992) found a positive correlation in chickens among the abdominal fat pad and the mRNA quantity of some lipogenic enzymes, such as acetyl-CoA carboxylase, Fatty-acid Synthase, malic enzyme, stearoyl-coenzyme A desaturase, AI and B apoproteins. Sourdioux et al. (1996), working on a turkey commercial line and the Msp I restriction endonuclease for the FASN gene, found associations in leaner genotypes. An analysis of the average effects of gene substitution for the same mutation, carried out by Sourdioux et al. (1999) confirmed the association between leanness and one allele of the fatty acid synthase polymorphism.

This study shows two SNPs in the last intron of the FASN gene in chickens. Data collection from families having an adequate number of individuals will be necessary in order to detect possible associations between this polymorphism and fatness traits.

Acknowledgements. This work was supported by ProHuerta, FONCyT 1998-2001 and UBACyT 1998-2001 grants.

REFERENCES


