

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL  
FACULDADE DE AGRONOMIA  
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

ELISA PICCININ FRANÇOIS

**EFEITOS DO TEMPO DE JEJUM PRÉ-ABATE E AFLATOXINAS NO FÍGADO DE  
FRANGOS DE CORTE**

Porto Alegre (RS), Brasil  
Março, 2020.

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Médica Veterinária

Dissertação apresentada como um dos  
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em Zootecnia na faculdade de  
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Rio Grande do Sul.  
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Orientador: Prof. Dr. Sergio Luiz Vieira

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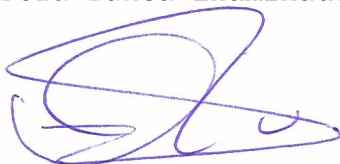
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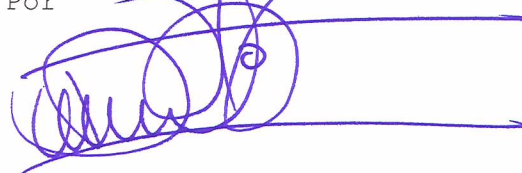
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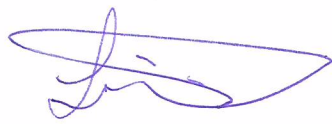
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# EFEITOS DO TEMPO DE JEJUM PRÉ-ABATE E AFLATOXINAS NO FÍGADO DE FRANGOS DE CORTE<sup>1</sup>

Autor: Elisa Piccinin François  
Orientador: Sergio Luiz Vieira

**RESUMO** – As aflatoxinas (AFLs) podem ser tóxicas para os animais, mesmo em concentrações relativamente baixas na ração, podendo levar à perda de peso corporal e à alterações na aparência do fígado. O jejum pré-abate é uma prática padrão de manejo, que também possui efeitos nas características do fígado e no desempenho dos frangos. O objetivo do presente estudo foi investigar os efeitos dos períodos de jejum alimentar que variaram de 0 a 12 h e os efeitos da ração contaminadas por AFLs em frangos de corte. Um total de 240 frangos de corte Cobb fêmeas de 1 d foi alimentado com uma ração pré-inicial a base de milho e soja não contaminada (1 a 7 d) e após esse período (8 a 28 d) dividido em um grupo controle com ração comercial (analisado sem AFLs) ou adicionado com 1 ppm de AFLs (792 ppb de aflatoxina B1, 35 ppb de aflatoxina B2, 219 ppb de aflatoxina G1 e quantidades indetectáveis de aflatoxina G2). Ao 28º dia, todas as aves foram pesadas e abatidas após três períodos de retirada das rações (0, 6 e 12h). O peso corporal, o peso do fígado e o conteúdo de gordura do fígado diminuíram à medida que o tempo de jejum pré-abate aumentava, enquanto a taxa de CA e o peso da vesícula biliar aumentava. AFLs diminuíram o peso corporal e o conteúdo de gordura no fígado, mas aumentaram o peso do fígado da vesícula biliar e a taxa de CA. Ambos os fatores independentes interagiram de tal forma que as AFLs exacerbaram os efeitos negativos do jejum sobre o peso corporal, a CA, bem como o aumento do peso do fígado e da vesícula biliar. A gordura do fígado foi reduzida pela retirada da ração, mas aumentou quando as aves foram alimentadas com AFLs. Fígados de frangos de corte sem jejum foram mais claros ( $L^*$ ) do que fígados de frangos de corte em jejum por 6 e 12h ( $L^* = 33,9, 29,5$  e  $23,9$ , respectivamente). A coloração amarelada ( $b^*$ ) foi maior nos fígados de frangos sem jejum quando comparado aos períodos de jejum de 6 ou 12h ( $b^* = 11,8, 8,97$  e  $10,54$ , respectivamente). Os valores de  $L^*$  e coloração avermelhada ( $a^*$ ) dos fígados de frangos de corte alimentados sem AFLs ( $L^* = 26,73$ ;  $a^* = 9,55$ , respectivamente) foram menores que os dos AFLs alimentados com aves ( $L^* = 29,54$ ;  $a^* = 10,74$ ). O aumento do tempo de jejum pré-abate diminui a luminosidade do fígado, enquanto a ingestão de AFL aumenta a coloração amarelada. Essas alterações podem confundir o serviço de inspeção e, eventualmente, levar à condenação errada do fígado nas plantas de processamento.

**Palavras chave:** aflatoxinas, fígado, jejum, frangos de corte

<sup>1</sup>Dissertação de Mestrado em Zootecnia – Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil (87 p.), Março 2020.

# PRE SLAUGHTER FEED WITHDRAWAL TIME AND INTAKE OF FEEDS CONTAMINATED WITH AFLATOXINS HAVE DIFFERENT VISUAL EFFECTS ON BROILER CHICKEN LIVERS<sup>1</sup>

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Advisor: Sergio Luiz Vieira

**ABSTRACT** - Aflatoxins (AFLs) can be extremely poisonous to animals, even at relatively low in feed concentrations, leading to body weight loss as well as altered liver appearance. Pre slaughter fasted is a standard management practice likewise have effects on the liver characteristics and broiler performance. The objective of the present study was to investigate the effects of feeding withdrawal periods ranging from 0 to 12 h in comparison to AFLs contaminated feeds. A total of 240 one-d-old female Cobb broiler chickens were fed non-contaminated corn-soy pre starters (1 to 7 d) and then (8 to 28 d) a commercial feed Control (analyzed without AFLs) or added with 1 ppm AFLs (792 ppb of aflatoxin B1, 35 ppb of aflatoxin B2, 219 ppb of aflatoxin G1 and undetectable amounts of aflatoxin G2). Day 28 all birds were weighted and slaughtered following 3 feed withdrawal time periods (0, 6 and 12h). Body weight, liver weight and liver fat content decreased as feed withdrawal lengthened whereas FCR and gall bladder weight increased. AFLs decreased body weight and liver fat content but increased FCR, liver and gall bladder weights. Both independent factors interacted such that AFLs exacerbated feed withdrawal negative effects on body weight, FCR, as well as increased liver and gall bladder weights. Liver fat was reduced by feed withdrawal but increased when birds were fed AFLs. Livers from non-fasted broilers showed greater lightness ( $L^*$ ) than livers of broilers fasted for 6 and 12h ( $L^*$ = 33.9, 29.5 and 23.9, respectively). Yellowness ( $b^*$ ) was greater in livers of non-fasted broilers when compared to those fasted for 6 or 12h ( $b^*$ =11.8, 8.97 and 10.54, respectively).  $L^*$  and redness ( $a^*$ ) values of livers of broilers fed without AFLs ( $L^*$ = 26.73;  $a^*$ = 9.55, respectively) were lower than those of AFLs fed birds ( $L^*$ = 29.54;  $a^*$ = 10.74). Prolonging preslaughter feed withdrawal increases liver darkness whereas AFLs intake increases yellowness. These changes may confound inspection service personnel and eventually can lead to wrong liver condemnation in processing plants.

**Key words:** aflatoxin, liver, feed withdrawal, broilers chicken

<sup>1</sup>Master of Science dissertation in Animal Science – Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. (87 p.), March 2020.

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## RELAÇÃO DE ABREVIATURAS

AFLs	Aflatoxinas
AFB1	Aflatoxina B1
AFB2	Aflatoxina B2
ALT	Alanina aminotransferase
AST	Aspartato aminotransferase
DNA	Ácido desoxirribonucleico
CA	Conversão alimentar
L*	Claridade/ palidez
a*	Vermelho
b*	Amarelo
AME	Apparent metabolizable energy
Av. P	Available phosphorus
BWG	Body weight gain
CIE	Comission Internationale de L'Eclairage
FCR	Feed conversion ratio

## CAPÍTULO I

## 1. INTRODUÇÃO

O Brasil é o segundo maior produtor mundial de carne de frango com 12,85 milhões de toneladas produzidas em 2018. Deste total mais de 4 milhões de toneladas são destinadas à exportação para diversos países, tornando o Brasil o maior exportador mundial (ABPA, 2019). Para garantir este cenário, o mercado de frango deve prezar pela excelência em qualidade de carne e garantir o status sanitário dos plantéis.

Problemas sanitários podem tanto comprometer índices zootécnicos como inviabilizar a cadeia produtiva, sendo de extrema importância o avanço no conhecimento do mecanismo de ação de vírus, bactérias, fungos, entre outros para melhor compreensão, seja do modo de ação ou dos prejuízos que causam na cadeia avícola. Aves com a saúde comprometida podem ter total ou parcialmente sua carcaça, partes dela e ou órgãos internos comprometidos e inviabilizados para o consumo. Dessa forma torna-se de fundamental importância para a cadeia o conhecimento das perdas que podem ser geradas pelos diferentes agentes nas variadas partes que compõem o rendimento de abatedouro das aves. Dentre os órgãos das aves, o fígado é um dos órgãos internos mais consumidos como alimento pelos seres humanos (VIEIRA, 2013). O fígado é um órgão polivalente, com envolvimento na secreção biliar e metabolismo de carboidratos, proteínas e lipídios, bem como várias outras funções metabólicas (ZAEFARIAN et al., 2019). Este órgão pode se adaptar facilmente a mudanças na alimentação e no meio ambiente. Alterações nas funções do fígado podem levar a mudanças nas características visuais desse órgão, incluindo tamanho e cor (VIEIRA, 2013) tornando este órgão visualmente fora dos padrões de consumo (OCAK, 2008). Pelo fato de o fígado ser importante no processo de desintoxicação do organismo, toxinas presentes nos alimentos, como as aflatoxinas (AFLs) podem causar alterações importantes neste órgão.

As AFLs fazem parte de um grupo de toxinas produzidas por fungos como metabólitos secundários, produzidos por *Aspergillus flavus*, *A. parasiticus* e *A. nomius*. As AFLs são uma das micotoxinas mais comuns em grãos e ração animal. Seus efeitos deletérios em frangos refletem no ganho de peso dos animais causando grandes

perdas econômicas na produção animal. Alguns relatos sugerem que as aflatoxinas podem ser responsáveis por mediar vários efeitos tóxicos e danificar os tecidos do fígado em frangos de corte como aumento da arquitetura hepática, degeneração gordurosa, hiperplasia biliar, fibrose periportal, vacuolização hepatocítica e necrose (BARRAUD et al., 2001; SURAI, 2002; LIU et al., 2008; UMARANI et al., 2008; ELLAKANY et al., 2011).

A legislação brasileira de inspeção sanitária de carne de aves - Regulamento Técnico da Inspeção Tecnológica e Higiênico-Sanitária de Carne de Aves (BRASIL, 1998) trata das regras da inspeção e avaliação desde o recebimento das aves, abate, avaliação visual de carcaças e órgãos internos, espostejamento, armazenamento e embalagem. A avaliação das carcaças geralmente é feita de forma visual e subjetiva, baseada nas características visuais da carcaça ou órgão. A aparência íntegra, uniforme e coloração padrão dos órgãos internos assume muita importância no aproveitamento destes miúdos para comercialização.

O fígado pode sofrer alterações em suas funções devido a presença de micotoxinas na ração e estas podem levar a mudanças de características visuais deste órgão, incluindo tamanho e cor (VIEIRA, 2013). Como estas características são utilizadas como critério para o julgamento subjetivo para a condenação ou não do órgão pelo serviço de inspeção oficial, torna-se extremamente importante estudos aprofundados sobre os diferentes aspectos do órgão quando submetidos aos procedimentos de manejo pré-abate, como por exemplo o jejum, que pode afetar o metabolismo de lipídios e açúcares, e as toxinas que comumente podem afetar este órgão. Segundo Bartov (1996), fígados de frangos de corte, na planta de processamento, podem eventualmente aparentar estarem aumentados, de coloração mais clara e parecem conter um excesso de lipídios. A coloração do fígado é um fator importante para a venda do produto. Fígados de coloração mais clara podem ser considerados inaptos para consumo, independentemente de quais causas possam ter contribuído para esta mudança de característica física.

Este trabalho foi desenvolvido com o intuito de observar o efeito do tempo do jejum e a contaminação do alimento por AFLs em diferentes aspectos do fígado de frangos de corte, aos 28 dias de idade, de maneira a avaliar seus efeitos sobre o órgão,

principalmente seus efeitos na mudança de coloração e concentração de lipídios em aves submetidas a diferentes tempos de jejum consumindo ração contaminada ou não por AFLs, buscando ser uma contribuição para o melhor entendimento das mudanças que ocorrem neste órgão, podendo contribuir como mais uma ferramenta ao serviço de inspeção.



## 2. REVISÃO BIBLIOGRÁFICA

### **2.1 Fígado**

Como nos mamíferos, o fígado das aves é responsável por uma série de funções metabólicas e homeostática, sendo considerado como uma fábrica bioquímica responsável pela maior parte da síntese, metabolismo, excreção e processos de desintoxicação. O fígado desempenha um papel importante na digestão e metabolismo, regulando a produção, armazenamento e liberação de lipídios, carboidratos e proteínas (ZAEFARIAN et al., 2019). O fígado está envolvido na síntese de proteínas, como as da coagulação, enzimas e hormônios além de diversos fatores imunológicos. Funciona como uma glândula endócrina e exócrina. A sua excelente condição é fundamental para manter uma ave saudável.

As funções do fígado são exercidas devido a sua composição por células distintas como as parenquimatosas que são os hepatócitos e não parenquimatosas como as células de Kupffer, satélites e sinusoidal endoteliais (MACARI & MAIORKA, 2017). Os hepatócitos ocupam cerca de 80% do volume total do fígado, e estão dispostos em placas que irradiam longitudinalmente umas às outras formando os lóbulos hepáticos, entre estes lóbulos existe o espaço da tríade portal que consiste em um ramo da artéria hepática, ramo da veia porta e o ducto biliar (ZAEFARIAN et al., 2019). A tríade portal recebe o sangue oxigenado da artéria hepática e o sangue da veia porta que contém os nutrientes e as toxinas provenientes do trato digestivo que serão metabolizados. O citoplasma dos hepatócitos é rico em organelas e possui também uma grande quantidade de glicogênio que serve como importante reserva de glicose para o organismo.

### **2.2 Metabolismo das gorduras**

O fígado desempenha um papel fundamental na lipogênese, fornecendo lipídios a serem utilizados por todos os tecidos. Nas aves a síntese de gordura é majoritariamente no fígado e limitada no tecido adiposo. As gorduras metabolizadas no fígado derivam de três principais fontes: gordura dietética, gordura de depósito e

gordura da síntese de ácidos graxos de novo (provenientes dos carboidratos de ração). Devido a um sistema linfático intestinal pouco desenvolvido nas aves, os ácidos graxos são drenados diretamente para o sistema sanguíneo portal (em vez do sistema linfático) como muitas lipoproteínas de baixa densidade que são denominadas de portomicrons. Do sistema sanguíneo portal, a maioria dos portomicrons passa pelo fígado antes de chegar ao resto da circulação. Esta característica única predispõe as aves ao acúmulo de gordura no fígado (ZAEFARIAN et al., 2019; MACARI & MAIORKA, 2017).

O fígado é a fonte da bile que é fundamental na digestão e absorção de gordura. A bile é produzida nos hepatócitos e secretada nos canalículos biliares. Canaliculi são os canais intracelulares entre os hepatócitos que servem para transporte da bile para armazenamento na vesícula biliar. A bile desempenha um papel fundamental na emulsificação dos lipídios da dieta, sendo influenciada pelo retorno dos sais biliares do intestino. Frangos de corte depois da terceira semana de idade já apresentam plena capacidade de emulsificar os lipídios da dieta e junto com a ação da lipase pancreática, que também aumenta seu potencial com a idade, melhoram a eficiência da absorção dos lipídios (MACARI & MAIORKA, 2017).

### ***2.3 Restrição alimentar***

Há muitos fatores durante o manejo pré-abate que têm o potencial de afetar a qualidade da carcaça e a lucratividade da produção, um destes fatores é a retirada da ração no período pré-abate. O período de jejum, dependendo do tempo, pode alterar o metabolismo da ave e afetar a qualidade do produto. Associado ao período de jejum também está relacionado o esgotamento do glicogênio hepático. Peebles et al. (2005) observaram redução do glicogênio hepático e aumento da glicemia quando submetidos a jejum de 24 horas, o que sustenta a hipótese de manutenção de alto conteúdo glicêmico pela glicólise.

Segundo Bartov (1996), a síntese de gorduras e a engorda são aumentadas em frangos de corte por rações com alta relação energia proteína. O jejum pré-abate é uma prática da indústria de frangos de corte sendo que este procedimento diminui a lipogênese depois de 2h e depois de um tempo maior também diminui o tamanho do

fígado e o teor de gordura em frangos de corte em idade de comercialização.

Bartov testou a retirada da alimentação por 10 ou 24 h, estes períodos diminuíram significativamente o peso do fígado e seu teor de gordura, independentemente do tipo de dieta utilizado (relação energia: proteína). Os valores observados após o jejum não foram afetados pelos fatores dietéticos avaliados. A composição dos ácidos graxos do fígado em pintinhos não submetidos ao jejum foi significativamente afetada pelo aumento da energia dietética (óleo de soja) e a relação energia: proteína da dieta.

Hickling et al. (1982), estudaram o nível de glicogênio no fígado de aves após diferentes períodos de retirada de alimento. Observou-se que dentro de 8 horas de retirada da ração, as concentrações em todos os casos haviam caído abruptamente para menos de 0,1%. A concentração foi recuperada para entre 0,6 e 0,99%, dentro de 32-56 horas após a retirada da ração e, posteriormente, diminuiu gradualmente novamente. O estudo mostrou uma concentração distinta de glicogênio hepático após a retirada da ração, que ocorreu mais rapidamente nas aves alimentadas com uma dieta rica em carboidratos.

Medições de cor do fígado no estudo de Buhr et al. (1998) indicaram que períodos de retirada de alimentos mais longos resultaram em alterações de coloração deste órgão além de alterações de peso e diâmetro de vísceras. Fígado de aves mais jovens (foram avaliados frangos de corte abatidos com 42, 44 e 48 dias), apresentaram perdas percentuais maiores no peso do fígado em relação ao peso corporal. No mesmo estudo, o tempo de retirada de ração alterou de forma linear os parâmetros de coloração do fígado na escala CIE (Comission Internationale de L'Eclairage) medidos pelos padrões de L\*, onde valores positivos relacionam-se a claridade palidez e negativos a colorações que tendem ao preto; a\*, onde valores positivos são relacionados com colorações mais avermelhadas e valores negativos esverdeadas e b\*, onde valores positivos são relacionados com coloração amarelada e negativos com colorações azuladas. Os resultados do estudo indicaram que, à medida que o tempo de retirada da ração aumentou, a superfície do fígado ficou mais escura, menos avermelhada e menos amarelada.

A coloração de fígados de aves abatidas sem jejum prévio foi medida pelo

sistema CIE e comparada com a coloração de fígados com tempos crescentes de jejum pré-abate. Quanto maior o tempo de jejum maior foi a diferença de coloração encontrada (NORTHCUTT et al., 1997). O autor concluiu que esta mudança pode ter sido referente a concentração de pigmentos hepáticos relacionada a diminuição do peso do órgão com o passar do tempo de jejum, apesar de não ter medido este parâmetro. Jensen et al. (1984) mostraram que as aves que receberam períodos inferiores a 8 horas de jejum não tiveram perda significativa do peso do fígado em relação ao peso corporal, mas aumentando o jejum de 12 para 16h causou uma perda significativa neste parâmetro (6,7 e 21% de perda, respectivamente).

Trampel et al. (2005) realizaram um experimento com períodos de jejum pré-abate de 6 a 12 h em frangos de corte utilizando um suplemento à base de maltodextrina durante as primeiras 6 h da retirada de ração. O objetivo foi manter o peso vivo antes do abate, fornecendo um tipo de nutrição que não contribuísse com nenhum resíduo no trato gastrointestinal. No primeiro tratamento foi utilizado maltodextrina e água por 6 h seguido por um período de 6 h sem alimentação e água. No momento do abate os fígados das aves tratadas foram mais claros do que os encontrados em frangos de corte que seguiram o mesmo período pré-abate, porém sem utilização de suplemento. O autor considerou que a coloração mais clara das aves suplementadas foi devido a retenção de glicogênio e lipídios no fígado (significativamente maior no grupo controle e numericamente maior no grupo suplementado com maltodextrina). A coloração mais clara dos fígados resultou em uma condenação maior destes órgãos pelos inspetores de carne do órgão fiscalizador na planta de processamento.

## **2.4 Aflatoxinas**

As AFLs foram identificadas pela primeira vez no início dos anos 1960 e desde então compõem o grupo de as micotoxinas mais estudadas. Sendo produzido principalmente por determinadas estirpes de *Aspergillus parasiticus*, *Aspergillus flavus* e *Aspergillus nomius*, essas micotoxinas são costumeiramente encontradas em produtos agrícolas nas regiões tropicais e subtropicais (LESZCZYNSKA et al., 2000). As AFLs estão frequentemente presentes nos alimentos para animais e podem causar alguns efeitos adversos que podem variar de vômito, perda de peso, necrose aguda de

células do parênquima a vários tipos de carcinoma e imunossupressão em grandes animais, animais de estimação e aves de corte (ROBENS & RICHARD, 1992; BBOSA et al., 2013).

Dentre todas as AFLs, a aflatoxina B1 (AFB1) e a ocratoxina são as mais prejudiciais para seres humanos. A AFB1 possui maior potencial carcinogênico sendo capaz de penetrar na membrana celular e se ligar ao DNA, onde faz alterações no genoma. A AFB1 é um composto lipossolúvel que pode ser absorvido no local de exposição e entrar na corrente sanguínea (AHMED et al., 2017).

Em frangos de corte, as AFLs afetam drasticamente quase todos os fatores de produção, incluindo ganho de peso, consumo de ração e taxa de conversão alimentar (CA), além de induzir imunossupressão, estando diretamente relacionada à eficácia reduzida de programas de vacinação, aumentando o do risco de infecção por doenças e alta mortalidade. Em aves de postura comercial, as aflatoxinas causam a diminuição na produção, tamanho e qualidade dos ovos (MUGHAL et al., 2017). Depois de absorvidas, as AFLs são distribuídas pelo organismo e podem ser encontradas nos músculos, rins e tecido adiposo, entretanto, as maiores concentrações destas toxinas são encontradas no fígado (JUNIOR et al., 2009). Como lesão macroscópica na necropsia das aves intoxicadas geralmente se observa um aumento aparente no tamanho do fígado bem como alteração na sua coloração (amarelado). As AFLs são importantes agentes imunossupressores para frangos e perus (JUNIOR et al., 2009).

No fígado ocorre a maior parte do processo de biotransformação das AFLs pelas enzimas microssomais do citocromo P-450, o sistema enzimático microssomal hepático é responsável pela metabolização das aflatoxinase, conseqüentemente, pela sua ativação no organismo. Esse sistema possui basicamente quatro mecanismos principais: epoxidação, hidratação, hidroxilação e  $\alpha$ -desmetilação (Figura 1).

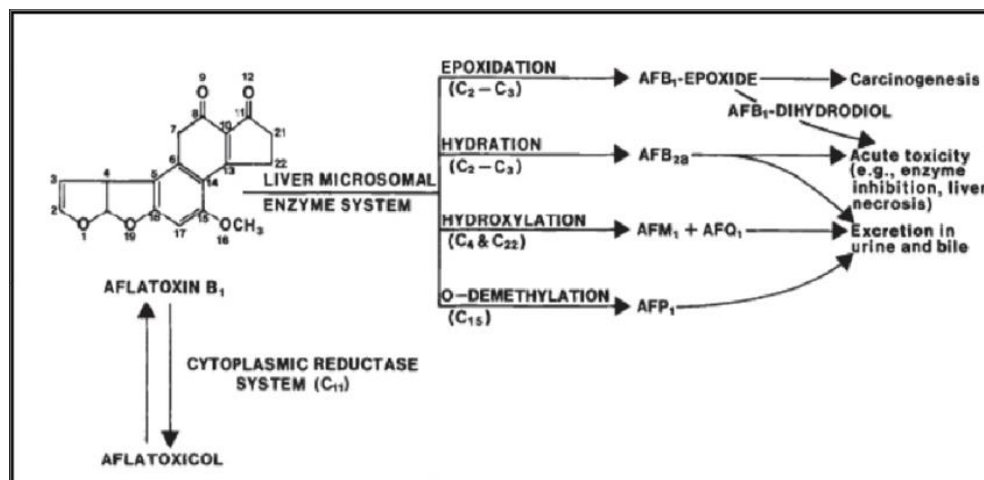


FIGURA 1 - Epoxidação, hidratação, hidroxilação e  $\alpha$ -desmetilação

Fonte: Omar, Hossam. *Mycotoxins-Induced Oxidative Stress and Disease*. 2013. Pages 63-92.

Sarkarati & Doustar (2012) estudaram a frequência de lesões hepáticas de carcaças de frangos de corte em um abatedouro no Irã e mostraram que 14,8% da avaliação patológica do fígado era hepatite tóxica. Uma das principais funções do fígado é decompor substâncias tóxicas e a maioria dos medicamentos em um processo de metabolismo destas drogas. Isso às vezes resulta em toxicidade, quando o metabólito é mais tóxico do que seu precursor (LUNA, 1968).

Um estudo feito por Franco et al. (2019) em fazendas dos estados de São Paulo e Santa Catarina no Brasil, a co-ocorrência de duas ou mais micotoxinas foi confirmada em 51% da ração a base de milho para animais. Os resultados indicam uma exposição considerável de animais de criação a micotoxinas na ração provenientes das fazendas estudadas. Dietas contendo AFB1 e Aflatoxina B2 (AFB2) podem induzir lesões patológicas nos fígados, alterar ligeiramente os parâmetros bioquímico séricos e danificar as funções antioxidantes hepáticas quando a inclusão de milho contaminado com AFB1 e AFB2 (YANG et al., 2012). A detecção os resíduos do metabolismo das AFLs nos tecidos dependem do quanto de AFLs na alimentação o animal foi exposto. Em um estudo de Hussain et al. (2016) não foram detectados resíduos de AFB1 no fígado e no músculo de aves após inclusão de 50 ppb e 50 e 100 ppb de AFB1 na ração respectivamente. Foram detectados resíduos apenas nos

tecidos hepáticos dos grupos alimentados com 400 ppb e 800 ppb de AFB1 na ração. As atividades da alanina aminotransferase (ALT) e aspartato aminotransferase (AST) nos grupos que receberam ração contaminada com AFLs foram significativamente maiores, e as concentrações totais de proteína e albumina foram significativamente menores em comparação aos controles, podendo ser estes parâmetros serem utilizados como indicação de um quadro de aflatoxicose crônica nestas aves.

## **2.5 Condenações**

A condenação de carnes e vísceras impróprias para o consumo visa zelar pela saúde pública, uma vez que a carne de frango e seus subprodutos, assim como todos os produtos de origem animal, são uma das mais importantes fontes de enfermidades transmitidas ao homem por alimentos. O fígado de frango *in natura* ou processado é passível de sofrer contaminação bacteriana devido à sua constituição orgânica, condições de obtenção e manipulação (OLIVEIRA et al., 2014)

Os critérios de condenação de fígados de frangos consideram o aspecto visual (cor, forma e tamanho), consistência e odor do órgão, de acordo com o que preconiza a Portaria 210/98 (BRASIL, 1998). É sabido que uma série de fatores podem contribuir em alterações anatômicas e na coloração deste órgão como contaminações bacterianas, fúngicas, parasitárias ou toxicológicas e até mesmo provocadas por alterações metabólicas na função do órgão e jejum alimentar (VIEIRA, 2013; BERCHIERI JUNIOR et al., 2009; OLIVEIRA et al., 2014). Segundo Vieira (2013), a alteração de cor mais comum em fígado de aves é a descoloração ou palidez, sendo esta característica frequentemente causa de condenação em frigoríficos.

Para evitar o abate de aves com repleção do trato gastrointestinal e, conseqüentemente, possíveis contaminações durante o processamento industrial as aves que chegarem ao abate, deverão cumprir a suspensão da alimentação por um período mínimo de 6 (seis) a 8 (oito) horas (BRASIL, 1998), não era descrito na legislação um período máximo, sendo este posteriormente definido em 12h para as aves em uma atualização posterior (BRASIL, 2018). Segundo Vieira (2013), períodos de jejum mais curtos podem produzir fígados de coloração mais esbranquiçadas, então os serviços de inspeção sanitária devem estar cientes das implicações do tempo de

jejum e suas possíveis alterações de coloração do fígado para evitar a condenação de aves saudáveis.

A inspeção interna das aves nos abatedouros deve ser realizada em duas linhas (BRASIL, 1998). Na linha A, a inspeção é realizada através da visualização da cavidade torácica e abdominal (pulmões, sacos aéreos, rins, órgãos sexuais), respeitando um tempo mínimo de 2 (dois) segundos por ave. Na linha B é visado o exame do coração, fígado, moela, baço, intestinos, ovários e ovidutos, no caso das poedeiras. Estes exames realizam-se através da visualização, palpação, conforme o caso, verificação de odores e ainda incisão. Neste exame dos órgãos verifica-se o aspecto (cor, forma, tamanho), a consistência, e em certas ocasiões, o odor. Também nesta linha deve ser respeitado o tempo mínimo de 2 (dois) segundos por ave.



### 3. HIPÓTESES E OBJETIVOS

#### 3.1 *Hipóteses*

Diferentes períodos de restrição alimentar anterior ao abate em frangos de corte podem alterar a coloração do fígado.

Consumo de dietas contaminadas com AFLs, associados com o tempo de jejum pré-abate podem a coloração e características fisiológicas do fígado de frangos de corte.

#### 3.2 *Objetivos*

Medir o desempenho zootécnico e a concentração lipídica e a coloração de fígados de frangos de corte de 8 a 28 d submetidos a diferentes períodos de jejum pré-abate, consumindo ou não ração contaminada com AFLs.

Determinar se o desempenho zootécnico e os parâmetros hepáticos avaliados serão alterados por diferentes períodos de retirada da ração e por consumo de ração inoculada com AFLs.

## CAPÍTULO II

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Artigo elaborado conforme as normas da revista Poultry Science (Apêndice1).

## RUNNING HEAD: FEED WITHDRAWAL, AFLATOXINS AND LIVER COLOR

**Pre slaughter feed withdrawal time and intake of feeds contaminated with aflatoxins have  
different visual effects on broiler chicken livers**

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## ABSTRACT

The objective of the present study was to investigate the effects of feeding withdrawal periods ranging from 0 to 12 h in comparison to aflatoxins (AFLs) contaminated feeds. Both factors potentially affect liver color and usually confound the Veterinary Inspection since effects on liver appearance are somewhat similar. However, whereas AFLs are poisonous to animals, even at relatively low feed concentrations, pre slaughter fasting is a demanded management practice targeting the reduction of fecal contamination of carcasses. A total of 240 one-d-old female Cobb broiler chickens were fed common non-contaminated corn-soy pre starters (1 to 7 d) and then a commercial feed Control (analyzed without AFLs) or added with 1 ppm AFLs (792 ppb of aflatoxin B1, 35 ppb of aflatoxin B2, 219 ppb of aflatoxin G1 and undetectable amounts of aflatoxin G2) from 8 to 28 d. On day 28 all birds were weighted and slaughtered following 3 feed withdrawal time periods (0, 6 and 12h). Body weight, liver weight and liver fat content decreased as feed withdrawal lengthened whereas FCR and gall bladder weight increased. AFLs reduced body weight and liver fat content while increasing FCR, liver and gall bladder weights. Both independent factors interacted such that AFLs exacerbated feed withdrawal negative effects on body weight, FCR, as well as lead to increases in liver and gall bladder weights. Liver fat was reduced as feed withdrawal was prolonged but increased when birds were fed AFLs. Livers from non-fasted broilers showed greater lightness ( $L^*$ ) than livers of broilers fasted for 6 and 12h ( $L^*= 33.9, 29.5$  and  $23.9$ , respectively). Yellowness ( $b^*$ ) was also greater in livers of non-fasted broilers when compared to those fasted for 6 or 12h ( $b^*=11.8, 8.97$  and  $10.54$ , respectively).  $L^*$  and redness ( $a^*$ ) values of livers of broilers fed without AFLs ( $L^*= 26.73$ ;  $a^*= 9.55$ , respectively) were lower than those of AFLs fed birds ( $L^*= 29.54$ ;  $a^*= 10.74$ ). Prolonging preslaughter feed withdrawal increased liver darkness whereas AFLs intake increase yellowness. These changes

may confound inspection service personnel and eventually can lead to wrong liver condemnation in processing plants. Since liver is a valuable organ, attention must be paid on these differences such that healthy livers are not condemned while public safety is guaranteed.

Key words: aflatoxin, liver, feed withdrawal, broiler chicken

## INTRODUCTION

Feed withdrawal prior to slaughter is required to reduce fecal contamination in processing plants. It has been demonstrated many years ago that fasting periods that minimize fecal contamination are in the range of 8 to 12 hours (Wabeck, 1972). These have been maintained up to the present in the integrations. Feed withdrawal leads to body weight loss averaging 0.25% per hour concurrently affecting liver weight (Gomes et al., 2008), but it also affects its paleness (Trampel et al., 2005). It has been demonstrated that liver weight of full-fed broilers was heavier when compared to those of fasted birds, but also that prolonging feed withdrawal increases liver darkness, which was correlated to organ depletion of glycogen (Northcutt et al., 1997; Ocak and Sivri, 2007; Trampel et al., 2005).

Aflatoxins (AFLs) are a group of toxins naturally produced by *Aspergillus flavus* and *A. parasiticus* extremely poisonous to animals, even at relatively low feed concentrations. They are known as the most important naturally occurring carcinogenics (Robens and Richard, 1992; Bbosa et al., 2013). AFLs content increases in plants under stress conditions, such as drought, heat or insect damage (Dorner, J.W. et al., 1989). Being of common occurrence in corn and other cereals, AFLs are frequently found in animal feeds (Bryden, 2012, Magnoli et al., 2011, Franco et al., 2019). Detoxification metabolism of birds ingesting AFLs occur mainly in the liver (Ma et

al., 2015; Qu et al., 2017; Nazarizadeh et al., 2019). Aflatoxicosis has clinical signs that include liver enlargement, fatty tissue degeneration, bile duct hyperplasia, periportal fibrosis, hepatocytic vacuolation, and necrosis (Barraud et al., 2001; Surai, 2002; Liu et al., 2008; Umarani et al., 2008; Ellakany et al., 2011). AFLs ingestion, at different concentrations in feeds, has also been reported to harm several immune functions, the intestinal gut lining and blood profile as well as inducing fat accumulation in broilers (Kermanshahi et al., 2007; Ghahri et al., 2010; Magnoli et al., 2011). AFLs reduce lipoprotein synthesis in birds (*f.i.* very low-density lipoproteins - VLDL), which, in turn, affects fat transport out of the liver (Bryden, 1981; 2012). Continuous intake of AFLs contaminated feeds, therefore, leads to fat accumulation and promotes color and morphometric changes in the liver (Merkley et al., 1987). In birds fed carotenoid containing feed ingredients, such as corn, liver fat accumulation becomes easily noted because of its increase in yellowness (Siloto et al., 2013).

Regardless of the concentration of AFLs in feeds, bird intoxication frequently contributes directly and indirectly to losses for the poultry industry. Whereas precise numbers are difficult to obtain, it has been estimated that AFLs inflict losses of at least \$143 million each year in the US poultry industry (Monson et al., 2015). It is well recognized that the presence of AFLs in corn is generally at levels that are usually below those considered harmful for broilers, *f.i.* in developed and developing countries, humans are exposed to dietary AFLs at levels lower than 1 ppb/kg body weight per day (World Health Organization, 2018). Low pathogenic doses of AFLs fed to poultry, however, lead to deleterious effects reflecting in the reduction of body weight gain as well as other negative parameters in overall live performance (Aikore et al., 2019; Monson et al., 2015).

The visual appearance of meat foods, including color, is a concern for consumers in the marketplace (Ocak and Sivri, 2007). Liver color is an important feature for poultry viscera inspection, because their color changes are indicative that on line processed poultry may not be acceptable for human consumption. Since online broiler sanitary inspection is visually oriented and because modern processing plants line speeds are very high, quick actions are demanded when making decisions on what is proper or not for consumption.

Condemnation of livers based on the routinely used visual veterinarian inspection procedures, frequently leads to mistakes due to the slight differences between paleness and yellowness, which potentially originate from fasting (lightness) and intoxication (yellowness). These factors can lead to confusion and condemnation of healthy organs in processing plants. The objective of the present study was to investigate the effects of feeding withdrawal periods ranging from 0 to 12 h in comparison to AFLs contaminated feeds fed to broilers.

## **MATERIALS AND METHODS**

### ***Birds, Housing and Feeds***

All procedures used in this study followed the procedures of the Commission of Ethics and Use of Animals of the Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.

A total of 240 one-d-old female Cobb 500 chickens were obtained from a commercial hatchery (BRF, Arroio do Meio, Brazil). Birds were randomly distributed into 60 battery cages, 4 birds per cage, 0.38 m<sup>2</sup> each, at the arrival in the experimental farm. Throughout the study all birds were fed a common pre-starter diet from 1 to 7 d (23.0% CP, 1.0% Ca, 0.5% Av.P. and 2,900 kcal/kg AME). Feeding treatments were provided in the starter from 8 to 28 d (20.7% CP, 0.95% Ca, 0.47% Av.P. and 2,980 kcal/kg AME). All diets were corn-soy all vegetable

formulated. A Control and an AFLs contaminated treatment (added 1 g/Ton of an AFLs inoculum containing 792 ppb of aflatoxin B1, 35 ppb of aflatoxin B2, 219 ppb of aflatoxin G1 and undetectable amounts of aflatoxin G2). Added AFLs was prepared following the method of Shotwell et al. (1966). A flow immunoassay was used to determine AFLs composition (Pereira et al. 2019), which was also used to analyze the diets fed to birds in the experiment.

All birds were slaughtered at 28 d following 3 feed withdrawal periods (0, 6, 12h). Feed consumption was measured at start of fasted period. Previously to slaughter, after each pre slaughter feed withdrawal time, all birds were individually weighed and immediately stunned using an electric stunner set at 45V for 3s and bled for 3 min after a jugular vein cut. Individual birds and their livers with full gallbladders were then weighed.

### ***Liver Collection***

Livers and their respective gallbladders were collected as a whole from all birds after slaughter. They were then immediately weighed and stored in individual container in a freezer (-18°C). Gall bladders were separated from the livers later to allow their assessment as a proportion of liver plus gall bladder.

### ***Lipid Content***

Total lipids from livers were quantified by the method of Folch and Stanley (1957). Samples were homogenized with a chloroform-methanol at a 2:1 ratio. After dispersion, the whole mixture was mixed during 20 min in an orbital shaker at room temperature. The homogenate was centrifuged to recover the liquid phase. The solvent was washed with 0.2 volume (4 mL for 20 mL) of 0.9% NaCl solution. After vortexing, the mixture was centrifuged at



2,000 rpm to separate the two phases and then the supernatant was siphoned and the lower chloroform phase containing lipids were evaporated under vacuum in a rotary evaporator. Lipid content, as a percentage, was calculated from the weight of the dried lipids (g of lipid/ g of liver  $\times$  100).

### ***Liver Color***

Livers collected immediately after slaughter were submitted to a color evaluation using the CIE (Comission Internationale de L'Eclairage) color values (Hunt et al., 1991). The CIE color scales representing lightness (L\*), redness (a\*), and yellowness (b\*), were measured using a HunterLab Labscan (HunterLab, Reston, VA) color meter. The instrument was calibrated using a white tile standard. A D65 light source with 2.54 cm opening was used for all measurements. Lightness (L\*) ranges from 100 (white) to 0 (black) whereas positive a\* values are measures of redness and negative a\* values are measures of greenness; positive b\* values are measures of yellowness and negative b\* values are measures of blueness. Liver color assessment was done at 3 random locations at the surface of each organ. Mean color values were then calculated (Folch and Stanley, 1957).

### ***Statistical Analysis***

All data in the present study were analyzed for normalcy and homoscedasticity prior to statistical analyses. The experiment was set in a completely randomized design of 6 treatments resulting from a 3 X 2 factorial arrangement of Feed Withdrawal X AFLs contaminated feeds. Data were submitted to a two-way analysis of variance using the GLM procedure of SAS (SAS Institute, 2001), with individual pens as experimental units. Means separation was done using

Tukey multiple-range test when the model effect was significant (Tukey, 1991). Statistical significance was considered at  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

The Control feed did not show any detectable amounts of AFLs, whereas the feed added with AFLs had 456 ppb of aflatoxin B1, 23 ppb of aflatoxin B2, 176 ppb of aflatoxin G1 and undetectable amounts of aflatoxin G2.

Overall growth performance results presented in Table 1 show that birds had reductions in BWG ( $P < 0.012$ ) and worsening in FCR as feed withdrawal advanced in time ( $P < 0.002$ ). The same was observed when birds were fed AFLs contaminated feeds ( $P < 0.014$  and  $P < 0.003$ , respectively for BWG and FCR). Both factors interacted such that AFLs fed birds had greater losses when feed withdrawal was of 12 h in comparison to 0 and 6 h ( $P < 0.036$  and  $P < 0.001$ , respectively BWG and FCR).

Liver characteristics are presented in Table 2 and show organ weights as well as their proportion of body weight, liver fat as well as its proportion of the liver, being reduced as feed withdrawal periods were prolonged (all had  $P < 0.001$ , except liver fat weight that had  $P < 0.043$ ). Gallbladder weights and their proportions of liver weights also increased as feed withdrawal was prolonged ( $P < 0.001$ ). Liver and gallbladder weights were significantly increased when birds were fed AFLs ( $P < 0.05$ ). Interactions between feed withdrawal and feed contamination were significant for liver weight as well as their percentage of body weight, but also for gall bladder weights as well as for the proportion their livers ( $P < 0.001$ ). These interactions showed liver weight decreases occurring only until 6 h feed withdrawal and, within the same withdrawal time period, birds fed AFLs had heavier livers. On the other hand, gall bladder weights, as well as their proportion of liver weights, did not present differences between Control and AFLs fed birds

at 0 h feed withdrawal. Gall bladder proportion was also not different at 6 h feed withdrawal between Control and AFLs fed, however, gall bladder weights were higher at 6 and 12 h feed withdrawal for the AFLs fed birds. The same occurred for the proportion of gall bladders at 12 h.

Liver color assessed with a colorimeter showed that  $L^*$  was gradually being reduced as feed withdrawal was longer ( $P < 0.001$ ) whereas  $b^*$  was reduced at the same proportion when non-fasted birds were compared to those fasted for 6 and 12 h ( $P < 0.001$ ). Birds fed AFLs were also paler than those fed the Control diet; however, differences were much smaller than those observed at each degree of lengthening of feed withdrawal (differences were in the range of 14 to 20% reduction per each increase of 6 h feed withdrawal, whereas it was of 13% between the Control and the AFLs treatment) ( $P < 0.001$ ). An interaction was observed between the Control and AFLs diets. This interaction demonstrates that, as feed withdrawal was prolonged, AFLs fed birds had paler livers inside the same feed withdrawal time ( $P < 0.001$ ).

It is widely known that AFLs cause loss of performance. In integrations this is a routine visual assessment done during necropsies, but which is also conducted at the inspection point in processing plants. In both of those moments, attention is usually paid to morphological and color changes in the liver because deviations from normal may be indicative of intoxication. Other macroscopic factors are related to harmful effects of AFLs in livers, such as enlarged, pale, and friable organs whereas more detailed inspection may show focal necrotic hepatocytes and hemorrhages (Monson, 2015). Added AFLs in the present study were in concentrations much higher than what is normally found in commercial poultry feeds. These were purposely chosen to exacerbate the effects of these contaminants allowing clearer separation of the effects between contaminated and non-contaminated feeds.

The research conducted by Trampel et al. (2005) demonstrated that liver lightness is related to glycogen depletion and this is due to the length of the fasting preceding slaughter. It is, therefore, clear from their study that the degree of glycogen depletion caused by fasting is a main reason leading to increased darkness of livers of processed chickens. Many logistic reasons and the moment of the last bird meal routinely lead to time variation in pre slaughter feed withdrawal and, therefore, changes in the degree of liver lightness are frequently expected.

AFLs are naturally produced under various circumstances as a group, from which Aflatoxin B1 (AFLB1) is the most harmful. Because it affects protein synthesis and leads to fat accumulation (including yellow carotenoids) in the liver (Smith and Hamilton, 1970; Siloto et al., 2013; Monson et al., 2015), changes in yellowness can be easily observed during veterinary inspection when birds were fed such toxins. Since sanitary inspection is done on line, condemnation of livers and in some cases, whole carcasses, occur as a preventative measure to avoid broiler meat with excessive levels of toxin metabolites reaching the market. Limits of AFLs in broiler meat are regulated in many countries, for instance in the USA the maximum allowed content of AFB<sub>1</sub> is 20 ppb, in the European Union the level for AFB<sub>1</sub> in feed ingredients is 50 ppb and lower levels for animal feed and young birds (10 and 20 ppb). AFLs analyses are time consuming and, obviously results of birds being processed are not known while they are going through processing. Such analysis is routinely conducted at arrival in import countries and can lead to rejection when levels surpass allowed legally acceptable contents.

Effects of feed withdrawal on body and liver as well as in gall bladder weights were as expected: reduction in liver weight, which was mainly due to the usage of stored nutrients in this organ while not fed and increase in gall bladder weight due to the accumulation of bile, which is

only released when feed passes through the duodenum (Siloto et al., 2013; Mughal et al., 2017). Worsening of FCR was a consequence of the lengthening of time without birds being fed.

The findings of the present study on the effects of feed withdrawal time on liver lightness are similar to those shown by Trampel et al. (2005). An important difference was demonstrated when organs were investigated from birds fed AFLs. These also showed an increase in lightness when compared to birds fed non-contaminated feeds. However, this effect was much less pronounced than the effects due to feed withdrawal. On the other hand, the degree of yellowness was significantly affected by AFLs fed birds as it was by feed withdrawal. Again, a major difference in this response was the degree of yellowness, which was much highly affected by AFLs.

The longer the feed withdrawal the lower the lipid contents in the liver at slaughter (1.62, 1.26 and 1.15 g per organ at 0, 6 and 12 h respectively) ( $P < 0.01$ ) (Table 2), therefore representing a 29 % loss after 12 h. The liver is the main site of lipid synthesis in the chicken (Leveille et al., 1975) and, as with glycogen (Northcutt et al., 1997), the prolongation of fasting leads to an important degree of energy depletion in this organ to support maintenance needs.

In the present study, feed withdrawal decreased liver weight, liver proportion of the whole body as well as the content of liver lipids, as has been previously shown by different authors (Bartov, 1996; Shapira et al., 1979). Rosa et al. (2012) showed that average of liver/BW (%) was not difference significant ( $P = 0.0911$ ) between broilers with fasting period of eight, ten and twelve hours ( $1.89 \pm 0.02$ ;  $1.85 \pm 0.02$ ;  $1.83 \pm 0.03$  %), similarly our results (Table 2). Similarly, Chen et al. (2014) showed that the relative liver weight of Pekin ducklings fed AFB<sub>1</sub> cultured on the growth tended to quadratically decrease with increasing AFB<sub>1</sub> concentration ( $p = 0.066$ ). Dixon et al. (1982) revealed that the apparent minimum effective doses of aflatoxin in young

chickens was dependent on the replications numbers, the number of birds per replication, the statistical approach employed, and the parameter measured. In addition, these authors observed that the linear-plateau mathematical models fit the relative weight of liver and pancreas, whereas quadratic polynomial models fit body weight and relative bursa weight. For this reason, in our study, the weight of liver of broilers fed Aflatoxin treat was higher than birds fed control treatment (Table 2). The observed changes in relative weight of liver may be due to the enlargement of the epithelium and to hyperanaemia in this organ, which is involved in detoxification and elimination of Aflatoxin partly via the liver, corroborating with Stoev et al. (2004) and Ringo et al. (2006).

Feed withdrawal times resulted in decreases in liver  $L^*$  color ( $P < 0.01$ ) (Table 3). Livers from broilers at 0 h feed withdrawal were paler ( $L^* = 34.0$ ,  $P < 0.0001$ ) than those from broilers in the treatments subjected to periods of 6 h and 12 h of feed withdrawal ( $L^* = 29.5$  and  $L^* = 24.0$ , respectively) however they had same redness ( $a^*$  values,  $P = 0.095$ ) (Table 3). Yellowness ( $b^*$  values) was numerically greater (11.81) in livers of without feed withdrawal broilers (0 h) than in those from fasted broilers of 6 or 12 hours (8.99 and 10.54, respectively), but the differences only were significant between 0 h and 6 h ( $P < 0.01$ ). These results indicate that as feed withdrawal time increased the surface of the liver appeared darker. However, Northcutt et al. (1997) and Buhr et al. (1998) observed a trend for redness for broilers liver subjected to feed withdrawal periods from 0 to 18 h. Similarly, Trampel et al. (2005) showed lightness values for livers from chickens without fasting were 38% higher ( $L^* = 54.41$ ) than those for livers from fasted broilers ( $L^* = 39.30$ ). The color intensity of livers of broilers in the Control treatment ( $L^* = 27.2$ ;  $a^*$  value = 9.9) was numerically less than the intensity of color in livers of broilers fed with AFLs treatment ( $L^* = 31.0$ ;  $a^*$  value = 11.3) (Table 3).

Liver color of broilers from the AFLs treatment does not show difference than liver of broilers from the Control treatment. Although, when we consider the interaction with preharvest feed withdrawal, L\* value and b\* value at 0 hours showed difference than others. The L\* value decreases with the increase of fasting (table 3), showed more decrease in broilers from the Control treatment.

Similarly in the current study, some reports have found that the effect of chronic exposure to aflatoxin diets on productivity parameters (BWG, feed consumption, and FCR) and the relative weights of livers did not show significant change compared with control diets (Oğuz et al., 2000; Magnoli et. al., 2011). However, Chen et. al. (2014) showed there was a 10.9, 31.7, and 47.4% ( $P < 0.05$ ) decrease in cumulative BWG with 0.11, 0.14, and 0.21 mg of AFB<sub>1</sub>/kg of diet of Pekin ducklings, respectively, but feed efficiency was not affected. Furthermore, lighter liver colors in full-fed broilers (0 hours) were associated with higher hepatic lipid concentrations (4.20%) and more total liver lipid (4.62 g/liver) than was found in broilers without feed for 12 h. The other hand, darker livers from fasted broilers had lower levels of lipid (4.42%) and less total lipid (2.68 g/liver) than the full-fed broilers (Trampel et al., 2005).

The results of this study showed that pre slaughter feed withdrawal and the intake of feeds contaminated with AFLs influence liver and gallbladder weight, liver color and liver lipid contents similarly such that sanitary inspection can be confounded if only relying on the visual aspects of processed carcasses. One important difference that can support the adequate condemnation of carcasses is related to an increase in darkness of livers as feed withdrawal is prolonged.

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Table 1. Growth performance of broilers submitted to varying pre slaughter feed withdrawal periods and fed aflatoxins (AFLs) contaminated feeds from 8 to 28 days of age.<sup>1</sup>

		Feed Intake	BWG <sup>1</sup>	FCR
		g		
Feed withdrawal, h	0	1.944	1.269 <sup>a</sup>	1.533 <sup>b</sup>
	6	1.946	1.246 <sup>ab</sup>	1.563 <sup>ab</sup>
	12	1.942	1.221 <sup>b</sup>	1.592 <sup>a</sup>
Feed contamination <sup>2</sup>	Control	1.935	1.255	1.542
	AFLs	1.953	1.235	1.582
<b>Interaction</b>				
0 h	Control	1.930	1.275 <sup>a</sup>	1.514 <sup>c</sup>
0 h	AFLs	1.959	1.263 <sup>ab</sup>	1.551 <sup>abc</sup>
6 h	Control	1.927	1.250 <sup>ab</sup>	1.542 <sup>bc</sup>
6 h	AFLs	1.964	1.241 <sup>ab</sup>	1.583 <sup>ab</sup>
12 h	Control	1.947	1.240 <sup>ab</sup>	1.570 <sup>abc</sup>
12 h	AFLs	1.937	1.201 <sup>b</sup>	1.613 <sup>a</sup>
SEM		0.008	0.007	0.007
<b>Probability &lt;</b>				
Feed withdrawal		0.267	0.012	0.002
Feed contamination		0.987	0.014	0.003
Feed withdrawal X feed contamination		0.729	0.036	0.001

<sup>a>b>c</sup> Means within a column without a common superscript differ significantly by Tukey test (P < 0.05).

<sup>1</sup> BW at 7 d was 161 ± 4 g.

<sup>2</sup> Feed formulations from 8 to 28 d had 20.7% CP, 0.95% Ca, 0.47% Av.P. and 2,980 kcal/kg AME). Analyzed Control feed had no detectable AFLs whereas AFLs contaminated feeds, formulated as the Control, contained 456 ppb of aflatoxin B1, 23 ppb of aflatoxin B2, 176 ppb of aflatoxin G1, but undetectable amounts of aflatoxin G2.

Table 2. Liver characteristics of broilers submitted to varying pre slaughter feed withdrawal periods and fed aflatoxins (AFLs) contaminated feeds from 8 to 28 days of age.<sup>1</sup>

		Liver <sup>2</sup>		Liver Fat		Gall Bladder	
		g	% of BW	g	% Liver	g	% Liver
Feed withdrawal, h	0	38.6 <sup>a</sup>	2.70 <sup>a</sup>	1.62 <sup>a</sup>	4.32 <sup>a</sup>	0.95 <sup>b</sup>	2.45 <sup>b</sup>
	6	32.3 <sup>b</sup>	2.29 <sup>b</sup>	1.26 <sup>b</sup>	4.19 <sup>b</sup>	1.74 <sup>a</sup>	5.38 <sup>a</sup>
	12	29.3 <sup>b</sup>	2.12 <sup>b</sup>	1.15 <sup>b</sup>	4.02 <sup>b</sup>	1.63 <sup>a</sup>	5.56 <sup>a</sup>
Feed contamination <sup>3</sup>	Control	31.8	2.24	1.27	4.12	1.32	4.29
	AFLs	35.0	2.50	1.41	3.96	1.56	4.63
<b>Interaction</b>							
0 h	Control	36.7 <sup>a</sup>	2.56 <sup>ab</sup>	1.53	4.27	0.90 <sup>c</sup>	2.45 <sup>c</sup>
0 h	AFLs	40.5 <sup>a</sup>	2.84 <sup>a</sup>	1.70	4.36	0.99 <sup>c</sup>	2.44 <sup>c</sup>
6 h	Control	29.6 <sup>d</sup>	2.10 <sup>c</sup>	1.16	4.16	1.58 <sup>b</sup>	5.34 <sup>b</sup>
6 h	AFLs	34.9 <sup>bc</sup>	2.49 <sup>ab</sup>	1.35	4.22	1.89 <sup>a</sup>	5.42 <sup>b</sup>
12 h	Control	29.0 <sup>d</sup>	2.07 <sup>c</sup>	1.12	3.95	1.47 <sup>b</sup>	5.07 <sup>b</sup>
12 h	AFLs	29.6 <sup>d</sup>	2.17 <sup>bc</sup>	1.17	4.08	1.79 <sup>a</sup>	6.05 <sup>a</sup>
<b>SEM</b>		0.53	0.03	0.19	0.46	0.05	0.16
<b>Probability &lt;</b>							
Feed withdrawal		0.001	0.001	0.001	0.043	0.001	0.001
Feed contamination		0.001	0.001	0.014	0.042	0.018	0.115
Feed withdrawal X feed contamination		0.001	0.001	0.995	0.808	0.001	0.001

<sup>a>b>c</sup> Means within a column without a common superscript differ significantly by Tukey test (P < 0.05).

<sup>1</sup> Values are representative of organs collected immediately after slaughter.

<sup>2</sup> Liver plus gall bladder.

<sup>3</sup> Feed formulations 8 to 28 d had 20.7% CP, 0.95% Ca, 0.47% Av.P. and 2,980 kcal/kg AME). Analyzed Control feed had no detectable AFLs whereas AFLs contaminated feeds, formulated as the Control, contained 456 ppb of aflatoxin B1, 23 ppb of aflatoxin B2, 176 ppb of aflatoxin G1, but undetectable amounts of aflatoxin G2.

Table 3. Liver color of broilers submitted to varying pre slaughter feed withdrawal periods and fed aflatoxins (AFLs) contaminated feeds from 8 to 28 days of age.

		L* <sup>2</sup>	a*	b*
Feed withdrawal, h	0	34.0 <sup>a</sup>	9.7	11.8 <sup>a</sup>
	6	29.5 <sup>b</sup>	9.9	9.8 <sup>b</sup>
	12	24.0 <sup>c</sup>	9.3	10.6 <sup>ab</sup>
Feed contamination <sup>1</sup>	Control	27.2	9.6	9.9
	AFLs	31.0	10.3	11.3
<b>Interaction</b>				
0 h	Control	32.9 <sup>ab</sup>	9.9	10.1 <sup>b</sup>
0 h	AFLs	35.0 <sup>a</sup>	9.5	13.5 <sup>a</sup>
6 h	Control	28.5 <sup>c</sup>	9.6	9.0 <sup>b</sup>
6 h	AFLs	30.5 <sup>bc</sup>	10.2	9.7 <sup>b</sup>
12 h	Control	20.3 <sup>d</sup>	9.3	10.5 <sup>b</sup>
12 h	AFLs	27.6 <sup>c</sup>	10.1	10.6 <sup>b</sup>
SEM		1.45	0.50	0.78
<b>Probability &lt;</b>				
Feed withdrawal		0.001	0.095	0.001
Feed contamination		0.001	0.061	0.005
Feed withdrawal X feed contamination		0.001	0.056	0.001

<sup>a>b>c</sup> Means within a column without a common superscript differ significantly by Tukey test (P < 0.05).

<sup>1</sup> Feed formulations from 8 to 28 d had 20.7% CP, 0.95% Ca, 0.47% Av.P. and 2,980 kcal/kg AME). Analyzed Control feed had no detectable AFLs whereas AFLs contaminated feeds, formulated as the Control, contained 456 ppb of aflatoxin B1, 23 ppb of aflatoxin B2, 176 ppb of aflatoxin G1, but undetectable amounts of aflatoxin G2.

<sup>2</sup> L\* (lightness) range from 100 (white) to 0 (black) whereas positive a\* values are measures of redness and negative a\* values are measures of greenness; positive b\* values are measures of yellowness and negative b\* values are measures of blueness.



### **CAPÍTULO III**

#### 4. CONSIDERAÇÕES FINAIS

Os resultados deste estudo mostraram que o jejum pré-abate junto com a ingestão de AFLS influenciou o peso do fígado e da vesícula biliar, coloração e quantidade de lipídios no fígado. Este resultado mostra a importância do conhecimento destas alterações influenciadas pelo jejum, uma vez que a retirada de alimentação é obrigatória para frangos de corte antes do carregamento e pode influenciar nas taxas de condenação deste órgão no momento da inspeção pós morte.

Um maior período de jejum pré-abate pode aumentar o metabolismo lipídico, enquanto a dieta contendo AFLS pode diminuir o metabolismo lipídico em frangos de corte. Os frangos de corte alimentados com dietas contendo AFLs tiveram o desempenho zootécnico inferior e o conteúdo lipídico total do fígado e peso do fígado e da vesícula biliar significativamente maiores quando comparados com a dieta controle, ao mesmo tempo o aumento do tempo de jejum reduziu significativamente o conteúdo lipídico total do fígado e o peso do fígado. A vesícula biliar, ao contrário do fígado, teve seu peso aumentado em maiores períodos de jejum.

Fígados de frangos não submetidos a jejum tiveram coloração mais clara (pálidos) do que a observada em fígados de frangos submetidos a tempos de jejum maiores (6 e 12 h) indicando que o tempo de jejum torna a coloração da superfície do fígado mais escura. Os fígados no tratamento com aflatoxinas apresentaram a coloração mais clara (esbranquiçada) e mais pálida (amarelada) do que fígados de frangos alimentados com dietas controle.

Este resultado indica que a ausência de jejum pré-abate pode resultar em fígados de coloração mais clara e retenção de lipídios no fígado, do mesmo modo os resultados indicam que a ingestão de aflatoxinas afeta a coloração do fígado provavelmente devido aos danos causados nas células hepáticas. O aumento de peso da vesícula biliar e seu percentual em relação ao peso do fígado em períodos maiores de jejum (6 e 12 h) pode estar relacionado com a contínua produção de bile apesar da ausência da ingestão de alimento que induz a sua liberação.

O prolongamento do jejum prévio ao abate leva a fígados mais escuros, do contrário, períodos mais curtos de jejum aumentam a probabilidade de fígados mais

brancos ou pálidos nos abatedouros. Períodos de jejum pré-abate mais altos podem aumentar o metabolismo lipídico do fígado, enquanto a dieta contendo AFLs pode diminuir o metabolismo lipídico em frangos de corte. Este resultado torna-se importante para balizar as decisões dos agentes de inspeção, uma vez que devido as variáveis de tempo de jejum realizado nas granjas, tempo de carregamento, transporte e espera para o abate no frigorífico podem influenciar diretamente estas características dentro de um mesmo lote.

A palidez também pode estar relacionada com concentração hepática de glicogênio (e tempo de jejum), sendo o fígado o maior órgão com maior concentração deste carboidrato no organismo do animal. Como sugestão para estudos futuros a medição do glicogênio hepático é um fator importante na coloração. Os resultados observados neste estudo também contribuíram para um maior conhecimento sobre as alterações causadas tanto pelo jejum quanto pelas aflatoxinas sobre as características visuais do fígado. Estes resultados mostram-se muito importantes no entendimento das diversas causas que afetam a coloração e características deste órgão e podem impactar no seu aproveitamento no momento do abate.

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## 6. APÊNDICES

**Apêndice 1:** Normas para publicação de artigos no periódico *Poultry Science*.

### **GUIDE FOR AUTHORS**

#### **SCOPE AND GENERAL INFORMATION**

##### **AIMS AND SCOPE**

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Symposium chair must decide whether or not the symposium is to be published and will inform the editor-in-chief of this decision at the January meeting. If the decision is not to publish the symposium, the individual authors retain the right to submit their papers for consideration for the journal as ordinary manuscripts. If publication is decided upon, all manuscript style and form guidelines of the journal shall be followed. If you are interested in publishing a symposium in Poultry Science, please contact the editor-in-chief for full guidelines.

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Invited papers are subject to review, and all manuscript style and form guidelines of the journal shall be followed. Invited papers are exempt from open access fee.

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Contemporary Issues will address critical issues facing poultry scientists and the poultry industry. As such, submissions to this section should be of interest to any poultry scientist, to the industry, to instructors and faculty teaching contemporary issues classes, and to undergraduate and graduate students. The section will consist of short papers (approximately 2 published pages) written in essay format and will include an abstract, appropriate subheadings, and references.

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A limited number of book reviews will publish in Poultry Science. Book reviews shall be prepared in accordance to the style and form requirements of the journal, and they are subject to editorial revision. No fees will be assessed.

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Note that there is no period after the corresponding author's e-mail address. The title page shall include the name and full address of the corresponding author. Telephone numbers and e-mail address must also be provided. The title page must indicate the appropriate scientific section for the paper (i.e., Animal Well-Being and Behavior; Genetics and Genomics; Immunology, Health and Disease; Metabolism and Nutrition; Molecular and Cellular Biology; Physiology and Reproduction; Processing and Products; Microbiology and Food Safety; Management and Production).

## Changes to authorship

Authors are expected to consider carefully the list and order of authors before submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only before the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the corresponding author: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors

that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.

Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors after the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

### **ABBREVIATIONS**

Author-derived abbreviations should be defined at first use in the abstract and again in the body of the manuscript. The abbreviation will be shown in bold type at first use in the body of the manuscript. Refer to the Miscellaneous Usage Notes for more information on abbreviations.

### **ABSTRACT**

The Abstract disseminates scientific information through abstracting journals and through convenience for the readers. The Abstract, consisting of not more than 325 words, appears at the beginning of the manuscript with the word ABSTRACT without a following period. It must summarize the major objectives, methods, results, conclusions, and practical applications of the research. The Abstract must consist of complete sentences and use of abbreviations should be limited. References to other work and footnotes are not permitted. The Abstract and Key Words must be on a separate sheet of paper.

### **KEY WORDS**

The Abstract shall be followed by a maximum of five key words or phrases to be used for subject indexing. These should include important words from the title and the running head and should be singular, not plural, terms (e.g., broiler, not broilers). Key words should be formatted as follows: Key words: ...

### **ARTICLE STRUCTURE**

#### **Introduction**

The Introduction, while brief, should provide the reader with information necessary for understanding research presented in the paper. Previous work on the topic should be summarized, and the objectives of the current research must be clearly stated.

#### **Materials and methods**

All sources of products, equipment, and chemicals used in the experiments must be specified parenthetically at first mention in text, tables, and figures [i.e., (model 123, ABC Corp., Provo, UT)]. Model and catalog numbers should be included. Information shall include the full corporate name (including division, branch, or other subordinate part of the corporation, if applicable), city, and state (country if outside the United States), or Web address. Street addresses need not be given unless the reader would not be able to determine the full address for mailing purposes easily by consulting standard references. Age, sex, breed, and strain or genetic stock of animals used in the experiments shall be specified. Animal care guidelines should be referenced if appropriate. Papers must contain analyzed values for those dietary ingredients that are crucial to the experiment. Papers dealing with the effects of feed additives or graded levels of a specific nutrient must give analyzed values for the relevant additive or nutrient in the diet(s). If products were used that contain different potentially active compounds, then analyzed values for these compounds must be given for the diet(s). Exceptions can only be made if appropriate methods



are not available. In other papers, authors should state whether experimental diets meet or exceed the National Research Council (1994) requirements as appropriate. If not, crude protein and metabolizable energy levels should be stated. For layer diets, calcium and phosphorus contents should also be specified.

When describing the composition of diets and vitamin premixes, the concentration of vitamins A and E should be expressed as IU/kg on the basis of the following equivalents:

#### *Vitamin A*

1 IU = 0.3 µg of all-trans retinol 1 IU = 0.344 µg of retinyl acetate

1 IU = 0.552 µg of retinyl palmitate

1 IU = 0.60 µg of β-carotene

#### *Vitamin E*

1 IU = 1 mg of dl-α-tocopheryl acetate 1 IU = 0.91 mg of dl-α-tocopherol

1 IU = 0.67 mg of d-α-tocopherol

In the instance of vitamin D3, cholecalciferol is the acceptable term on the basis that 1 IU of vitamin

D3 = 0.025 µg of cholecalciferol.

The sources of vitamins A and E must be specified in parentheses immediately following the stated concentrations.

- **Statistical analysis:** Biology should be emphasized, but the use of incorrect or inadequate statistical methods to analyze and interpret biological data is not acceptable. Consultation with a statistician is recommended. Statistical methods commonly used in the animal sciences need not be in detail, but adequate references should be provided. The statistical model, classes, blocks, and experimental unit must be designated. Any restrictions used in estimating parameters should be defined. Reference to a statistical package without reporting the sources of variation (classes) and other salient features of the analysis, such as covariance or orthogonal contrasts, is not sufficient. A statement of the results of statistical analysis should justify the interpretations and conclusions. When possible, results of similar experiments should be pooled statistically. Do not report a number of similar experiments separately. The experimental unit is the smallest unit to which an individual treatment is imposed. For group fed animals, the group of animals in the pen is the experimental unit; therefore, groups must be replicated. Repeated chemical analyses of the same sample usually do not constitute independent experimental units. Measurements on the same experimental unit over time also are not independent and must not be considered as independent experimental units. For analysis of time effects, use time sequence analysis.

- Usual assumptions are that errors in the statistical models are normally and independently distributed with constant variance. Most standard methods are robust to deviations from these assumptions, but occasionally data transformations or other techniques are helpful. For example, it is recommended that percentage data between 0 and 20 and between 80 and 100 be subjected to arc sin transformation prior to analysis. Most statistical procedures are based on the assumption that experimental units have been assigned to treatments at random. If animals

are stratified by ancestry or weight or if some other initial measurement should be accounted for, they should include a blocking factor, or the initial measurement should be included as a covariate.

- A parameter [mean ( $\mu$ ), variance ( $\sigma^2$ )], which defines or describes a population, is estimated by a statistic ( $\bar{x}$ ,  $s^2$ ). The term parameter is not appropriate to describe a variable, observation, trait, characteristic, or measurement taken in an experiment.

- Standard designs are adequately described by name and size (e.g., "a randomized complete block design with 6 treatments in 5 blocks"). For a factorial set of treatments, an adequate description might be as follows: "Total sulfur amino acids at 0.70 or 0.80% of the diet and Lys at 1.10, 1.20, or 1.30% of the diet were used in a 2 x 3 factorial arrangement in 5 randomized complete blocks consisting of initial BW." Note that a factorial arrangement is not a design; the term "design" refers to the method of grouping experimental units into homogeneous groups or blocks (i.e., the way in which the randomization is restricted).

- Standard deviation refers to the variability in a sample or a population. The standard error (calculated from error variance) is the estimated sampling error of a statistic such as the sample mean. When a standard deviation or standard error is given, the number of degrees of freedom on which it rests should be specified. When any statistical value (as mean or difference of 2 means) is mentioned, its standard error or confidence limit should be given. The fact that differences are not "statistically significant" is no reason for omitting standard errors. They are of value when results from several experiments are combined in the future. They also are useful to the reader as measures of efficiency of experimental techniques. A value attached by " $\pm$ " to a number implies that the second value is its standard error (not its standard deviation). Adequate reporting may require only 1) the number of observations, 2) arithmetic treatment means, and 3) an estimate of experimental error. The pooled standard error of the mean is the preferred estimate of experimental error. Standard errors need not be presented separately for each mean unless the means are based on different numbers of observations or the heterogeneity of the error variance is to be emphasized. Presenting individual standard errors clutters the presentation and can mislead readers.

- For more complex experiments, tables of subclass means and tables of analyses of variance or covariance may be included. When the analysis of variance contains several error terms, such as in split-plot and repeated measures designs, the text should indicate clearly which mean square was used for the denominator of each F statistic. Unbalanced factorial data can present special problems. Accordingly, it is well to state how the computing was done and how the parameters were estimated.

Approximations should be accompanied by cautions concerning possible biases.

- Contrasts (preferably orthogonal) are used to answer specific questions for which the experiment was designed; they should form the basis for comparing treatment means. Nonorthogonal contrasts may be evaluated by Bonferroni t statistics. The exact contrasts tested should be described for the reader. Multiple-range tests are not appropriate when treatments are orthogonally arranged. Fixed range, pairwise, multiple-comparison tests should be used only to compare means of treatments that are unstructured or not related. Least squares means are the correct means to use for all data, but arithmetic means are identical to least squares means unless the design is unbalanced or contains missing values or an adjustment is being made for a covariate. In factorial treatment arrangements, means for main effects should be presented when important interactions are not present. However, means for individual treatment combinations also should be provided in table or text so that future researchers may combine

data from several experiments to detect important interactions. An interaction may not be detected in a given experiment because of a limitation in the number of observations.

- The terms significant and highly significant traditionally have been reserved for  $P < 0.05$  and  $P < 0.01$ , respectively; however, reporting the P-value is preferred to the use of these terms. For example, use ". . . there was a difference ( $P < 0.05$ ) between control and treated samples" rather than ". . . there was a significant ( $P < 0.05$ ) difference between control and treated samples." When available, the observed significance level (e.g.,  $P = 0.027$ ) should be presented rather than merely  $P < 0.05$  or  $P < 0.01$ , thereby allowing the reader to decide what to reject. Other probability ( $\alpha$ ) levels may be discussed if properly qualified so that the reader is not misled. Do not report P-values to more than 3 places after the decimal. Regardless of the probability level used, failure to reject a hypothesis should be based on the relative consequences of type I and II errors. A "nonsignificant" relationship should not be interpreted to suggest the absence of a relationship. An inadequate number of experimental units or insufficient control of variation limits the power to detect relationships. Avoid the ambiguous use of  $P > 0.05$  to declare non significance, such as indicating that a difference is not significant at  $P > 0.05$  and subsequently declaring another difference significant (or a tendency) at  $P < 0.09$ . In addition, readers may incorrectly interpret the use of  $P > 0.05$  as the probability of a  $\beta$  error, not an  $\alpha$  error.

- Present only meaningful digits. A practical rule is to round values so that the change caused by rounding is less than one-tenth of the standard error. Such rounding increases the variance of the reported value by less than 1%, so that less than 1% of the relevant information contained in the data is sacrificed. Significant digits in data reported should be restricted to 3 beyond the decimal point, unless warranted by the use of specific methods.

## **Results and discussion**

Results and Discussion sections may be combined, or they may appear in separate sections. If separate, the Results section shall contain only the results and summary of the author's experiments; there should be no literature comparisons. Those comparisons should appear in the Discussion section. Manuscripts reporting sequence data must have GenBank accession numbers prior to submitting.

One of the hallmarks for experimental evidence is repeatability. Care should be taken to ensure that experiments are adequately replicated. The results of experiments must be replicated, either by replicating treatments within experiments or by repeating experiments.

## **Acknowledgements**

An Acknowledgments section, if desired, shall follow the Discussion section. Acknowledgments of individuals should include affiliations but not titles, such as Dr., Mr., or Ms. Affiliations shall include institution, city, and state.

**Appendix** A technical Appendix, if desired, shall follow the Discussion section or Acknowledgments, if present. The Appendix may contain supplementary material, explanations, and elaborations that are not essential to other major sections but are helpful to the reader. Novel computer programs or mathematical computations would be appropriate. The Appendix will not be a repository for raw data.

## **REFERENCES**

### ***Citations in Text***

In the body of the manuscript, refer to authors as follows: Smith and Jones (1992) or Smith and Jones (1990, 1992). If the sentence structure requires that the authors' names be

included in parentheses, the proper format is (Smith and Jones, 1982; Jones, 1988a,b; Jones et al., 1993). Where there are more than two authors of one article, the first author's name is followed by the abbreviation et al. More than one article listed in the same sentence of text must be in chronological order first, and alphabetical order for two publications in the same year. Work that has not been accepted for publication shall be listed in the text as: "J. E. Jones (institution, city, and state, personal communication)." The author's own unpublished work should be listed in the text as "(J. Smith, unpublished data)." Personal communications and unpublished data must not be included in the References section.

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To be listed in the References section, papers must be published or accepted for publication. Manuscripts submitted for publication can be cited as "personal communication" or "unpublished data" in the text.

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#### *Article:*

Bagley, L. G., and V. L. Christensen. 1991. Hatchability and physiology of turkey embryos incubated at sea level with increased eggshell permeability. *Poult. Sci.* 70:1412-1418.

Bagley, L. G., V. L. Christensen, and R. P. Gildersleeve. 1990. Hematological indices of turkey embryos incubated at high altitude as affected by oxygen and shell permeability. *Poult. Sci.* 69:2035- 2039.

Witter, R. L., and I. M. Gimeno. 2006. Susceptibility of adult chickens, with and without prior vaccination, to challenge with Marek's disease virus. *Avian Dis.* 50:354-365. doi:10.1637/7498-010306R.1

#### *Book:*

Metcalfe, J., M. K. Stock, and R. L. Ingermann. 1984. The effects of oxygen on growth and development of the chick embryo. Pages 205- 219 in *Respiration and Metabolism of Embryonic Vertebrates*. R. S. Seymour, ed. Dr. W. Junk, Dordrecht, the Netherlands.

National Research Council. 1994. *Nutrient Requirements of Poultry*. 9th rev. ed. Natl. Acad. Press, Washington, DC.

*Federal Register:*

Department of Agriculture, Plant and Animal Health Inspection Service. 2004. Blood and tissue collection at slaughtering and rendering establishments, final rule. 9CFR part 71. Fed. Regis. 69:10137-10151.

## Trade Publication

Wilgus, H. S. 1973. Temperature-programmed feeding schedules and other means of conserving protein in market turkey production. *Feedstuffs* 45(27):27-31.

*Other:*

Choct, M., and R. J. Hughes. 1996. Long-chain hydrocarbons as a marker for digestibility studies in poultry. *Proc. Aust. Poult. Sci. Symp.* 8:186. (Abstr.)

Dyro, F. M. 2005. Arsenic. WebMD. Accessed Feb. 2006. [http:// www.emedicine.com/neuro/topic20.htm](http://www.emedicine.com/neuro/topic20.htm).

El Halawani, M. E., and I. Rosenboim. 2004. Method to enhance reproductive performance in poultry. Univ. Minnesota, as- signee. US Pat. No. 6,766,767.

Hruby, M., J. C. Remus, and E. E. M. Pierson. 2004. Nutritional strategies to meet the challenge of feeding poultry without antibiotic growth promotants. *Proc. 2nd Mid-Atlantic Nutr. Conf.*, Timonium, MD. Univ. Maryland, College Park.

Luzuriaga, D. A. 1999. Application of computer vision and electronic nose technologies for quality assessment of color and odor of shrimp and salmon. PhD Diss. Univ. Florida, Gainesville.

Peak, S. D., and J. Brake. 2000. The influence of feeding program on broiler breeder male mortality. *Poult. Sci.* 79(Suppl. 1):2. (Abstr.)

**TABLES**

Tables must be created using the MS Word table feature and inserted in the manuscript after the references section. When possible, tables should be organized to fit across the page without running broadside. Be aware of the dimensions of the printed page when planning tables (use of more than 15 columns will create layout problems). Place the table number and title on the same line above the table. The table title does not require a period. Do not use vertical lines and use few horizontal

lines. Use of bold and italic typefaces in the table should be done sparingly; you must define such use in a footnote. Each table must be on a separate page. To facilitate placement of all tables into the manuscript file (just after the references) authors should use "section breaks" rather than "page breaks" at the end of the manuscript (before the tables) and between tables.

Units of measure for each variable must be indicated. Papers with several tables must use consistent format. All columns must have appropriate headings. Abbreviations not found on the inside front cover of the journal must be defined in each table and must match those used in the text. Footnotes to tables should be marked by superscript numbers. Each footnote should begin a new line. Superscript letters shall be used for the separation of means in the body of the table and explanatory footnotes must be provided [i.e., "Means within a row lacking a common

superscript differ ( $P < 0.05$ )."]; other significant P-values may be specified. Comparison of means within rows and columns should be indicated by different series of superscripts (e.g., a,b, . . . in rows; x-z . . . in columns) The first alphabetical letter in the series (e.g., a or A) shall be used to indicate the largest mean. Lowercase super- scripts indicate  $P \leq 0.05$ . Uppercase letters indicate  $P \leq 1.1$  or less.

Probability values may be indicated as follows: \* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ , and † $P \leq 0.10$ . Consult a recent issue of the journal for examples of tables.

Generally, results should be presented to the significant figure of the instrument used to collect the data. For example, results should not be presented to 5 digits when the instrument used only reads to 2 digits.

## **MISCELLANEOUS USAGE NOTES**

### **Abbreviations**

- Abbreviations shall not be used in the title, key words, or to begin sentences, except when they are widely known throughout science (e.g., DNA, RNA) or are terms better known by abbreviation (e.g., IgG, CD). A helpful criterion for use of abbreviation is whether it has been accepted into thesauri and indexes widely used for searching major bibliographic databases in the scientific field. Abbreviations

may be used in heads within the paper, if they have been first defined within the text. The inside back cover of every issue of the journal lists abbreviations that can be used without definition. The list is subject to revision at any time, so authors should always consult the most recent issue of the journal for relevant information. Abbreviations are allowed when they help the flow of the manuscript; however, excessive use of abbreviations can confuse the reader. The suitability of abbreviations will be evaluated by the reviewers and editors during the review process and by the technical editor during editing. As a rule, author-derived abbreviations should be in all capital letters. Terms used less than three times must be spelled out in full rather than abbreviated. All terms are to be spelled out in full with the abbreviation following in bold type in parentheses the first time they are mentioned in the main body of the text. Abbreviations shall be used consistently thereafter, rather than the full term.

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- Plural abbreviations do not require "s." Chemical symbols and three-letter abbreviations for amino acids do not need definition. Units of measure, except those in the standard *Poultry Science* abbreviation list, should be abbreviated as listed in the CRC Handbook for Chemistry and Physics (CRC Press, 2000 Corporate Blvd., Boca Raton, FL, 33431) and do not need to be defined.

- The following abbreviations may be used without definition in *Poultry Science*:

A adenine

ADG average daily gain

ADFI average daily feed intake

AME apparent metabolizable energy

AMEn nitrogen-corrected apparent metabolizable energy

ANOVA analysis of variance

B cell bursal-derived, bursal-equivalent derived cell bp base pairs

BSA bovine serum albumin  
BW body weight  
C cytosine  
cDNA complementary DNA  
cfu colony-forming units  
CI confidence interval  
CP crude protein  
cpm counts per minute  
CV coefficient of variation d day  
df degrees of freedom  
DM dry matter  
DNA deoxyribonucleic acid  
EDTA ethylenediaminetetraacetate  
ELISA enzyme-linked immunosorbent antibody assay  
EST expressed sequence tag  
g gram g gravity  
G guanine  
GAT glutamic acid-alanine-tyrosine  
G:F gain-to-feed ratio  
GLM general linear model  
h hour  
HEPES N-2-hydroxyethyl piperazine-N'-ethane-sulfonic acid  
HPLC high-performance (high-pressure) liquid chromatography  
ICU international chick units  
Ig immunoglobulin IL interleukin  
IU international units kb kilobase pairs kDa kilodalton  
L liter\*  
L:D hours light:hours darkness in a photoperiod (e.g., 23L:1D)  
m meter  
 $\mu$  micro M molar  
MAS marker-assisted selection  
ME metabolizable energy  
MEn nitrogen-corrected metabolizable energy  
MHC major histocompatibility complex  
mRNA messenger ribonucleic acid min minute  
mo month  
MS mean square  
n number of observations  
N normal  
NAD nicotinamide adenine dinucleotide  
NADH reduced nicotinamide adenine dinucleotide  
NRC National Research Council  
NS not significant  
PAGE polyacrylamide gel electrophoresis  
PBS phosphate-buffered saline  
PCR polymerase chain reaction pfu plaque-forming units  
QTL quantitative trait loci r correlation coefficient  
r<sup>2</sup> coefficient of determination, simple  
R<sup>2</sup> coefficient of determination, multiple  
RH relative humidity  
RIA radioimmunoassay

rpm revolutions per minute s second  
 SD standard deviation  
 SDS sodium dodecyl sulphate  
 SE standard error  
 SEM standard error of the mean  
 SRBC sheep red blood cells  
 SNP single nucleotide polymorphism  
 T thymine  
 TBA thiobarbituric acid  
 T cell thymic-derived cell  
 TME true metabolizable energy  
 TME<sub>n</sub> nitrogen-corrected true metabolizable energy  
 Tris tris(hydroxymethyl)aminomethane  
 TSAA total sulfur amino acids U uridine  
 USDA United States Department of Agriculture  
 UV ultraviolet  
 vol/vol volume to volume  
 vs. versus  
 wt/vol weight to volume  
 wt/wt weight to weight  
 wk week  
 yr year  
 \*Also capitalized with any combination, e.g.; mL.

### **International words and phrases**

Non-English words in common usage (defined in recent editions of standard dictionaries) will not appear in italics (e.g., *in vitro*, *in vivo*, *in situ*, *a priori*). However, genus and species of plants, animals, or bacteria and viruses should be italicized. Authors must indicate accent marks and other diacriticals on international names and institutions. German nouns shall begin with capital letters.

### **Capitalization**

Breed and variety names are to be capitalized (e.g., Single Comb White Leghorn). 2

### **Number style**

Numbers less than 1 shall be written with preceding zeros (e.g., 0.75). All numbers shall be written as digits. Measures must be in the metric system; however, US equivalents may be given in parentheses. *Poultry Science* requires that measures of energy be given in calories rather than joules, but the equivalent in joules may be shown in parentheses or in a footnote to tables. Units of measure not preceded by numbers must be written out rather than abbreviated (e.g., lysine content was measured in milligrams per kilogram of diet) unless used parenthetically. Measures of variation must be defined in the Abstract and in the body of the paper at first use. Units of measure for feed conversion or feed efficiency shall be provided (i.e., g:g).

### **Nucleotide sequences**

Nucleotide sequence data must relate to poultry or poultry pathogens and must complement biological data published in the same or a companion paper. If sequences are excessively long, it is suggested that the most relevant sections of the data be published in *Poultry Science* and



the remaining sequences be submitted to one of the sequence databases. Acceptance for publication is contingent

on the submission of sequence data to one of the databases. The following statement should appear as a footnote to the title on the title page of the manuscript. "The nucleotide sequence data reported in this paper have been submitted to Embank Submission (Mail Stop K710, Los Alamos National Laboratories, Los Alamos, NM 87545) nucleotide sequence database and have been assigned the accession number XNNNNN." Publication of the description of molecular clones is assumed by the editors to place them in the public sector. Therefore, they shall be made available to other scientists for research purposes. Nucleotide sequences must be submitted as camera-ready figures no larger than 21.6 x 27.9 cm in standard (portrait) orientation. Abbreviations should follow *Poultry Science* guidelines.

### **Gene and protein nomenclature**

Authors are required to use only approved gene and protein names and symbols. For poultry, full gene names should not be italicized. Gene symbols should be in uppercase letters and should be in italics. A protein symbol should be in the same format as its gene except the protein symbol should not be in italics. General usage

- Note that "and/or" is not permitted; choose the more appropriate meaning or use "x or y or both."
- Use the slant line only when it means "per" with numbered units of measure or "divided by" in equations. Use only one slant line in a given expression (e.g., g/d per chick). The slant line may not be used to indicate ratios or mixtures.
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Supplementary material such as applications, images and sound clips, can be published with your article to enhance it. Submitted supplementary items are published exactly as they are received (Excel or PowerPoint files will appear as such online). Please submit your material together with the article and supply a concise, descriptive caption for each supplementary file. If

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**Apêndice 2.** Desempenho zootécnico dos frangos de corte no período de 7 a 28 dias.

<b>TRATAMENTO</b>	<b>PESO IND 7-28</b>	<b>GP 7-28</b>	<b>CA 7-28</b>	<b>CONS IND 7-28</b>
CONTROLE	1.310	1.151	1.638	1.885
CONTROLE	1.490	1.329	1.536	2.040
CONTROLE	1.460	1.294	1.515	1.960
CONTROLE	1.455	1.295	1.509	1.955
CONTROLE	1.365	1.198	1.536	1.840
CONTROLE	1.350	1.195	1.695	2.025
CONTROLE	1.455	1.287	1.566	2.015
CONTROLE	1.475	1.316	1.462	1.925
CONTROLE	1.460	1.303	1.527	1.990
CONTROLE	1.375	1.216	1.625	1.975
CONTROLE	1.425	1.264	1.554	1.965
CONTROLE	1.455	1.289	1.572	2.025
CONTROLE	1.445	1.278	1.523	1.945
CONTROLE	1.453	1.291	1.581	2.041
CONTROLE	1.445	1.288	1.518	1.955
CONTROLE	1.445	1.286	1.540	1.980
CONTROLE	1.325	1.168	1.558	1.820
CONTROLE	1.395	1.240	1.593	1.975
CONTROLE	1.390	1.229	1.631	2.004
CONTROLE	1.380	1.221	1.557	1.902
CONTROLE	1.375	1.219	1.584	1.930
CONTROLE	1.345	1.190	1.568	1.865
CONTROLE	1.485	1.321	1.521	2.010
CONTROLE	1.320	1.166	1.579	1.840
CONTROLE	1.355	1.200	1.575	1.890
CONTROLE	1.410	1.251	1.615	2.020
CONTROLE	1.400	1.241	1.535	1.905
CONTROLE	1.465	1.308	1.530	2.000
AFLA	1.460	1.303	1.550	2.020
AFLA	1.400	1.244	1.471	1.830
AFLA	1.505	1.339	1.482	1.985
AFLA	1.405	1.236	1.521	1.880
AFLA	1.445	1.282	1.471	1.885
AFLA	1.450	1.291	1.495	1.930
AFLA	1.400	1.244	1.596	1.985
AFLA	1.340	1.183	1.539	1.820
AFLA	1.445	1.282	1.525	1.955
AFLA	1.510	1.345	1.495	2.010
AFLA	1.335	1.180	1.619	1.910
AFLA	1.420	1.264	1.451	1.835

AFLA	1.435	1.276	1.548	1.975
AFLA	1.440	1.281	1.479	1.895
AFLA	1.425	1.260	1.512	1.905
AFLA	1.460	1.297	1.503	1.950
AFLA	1.460	1.304	1.495	1.950
AFLA	1.375	1.216	1.555	1.890
AFLA	1.450	1.283	1.540	1.975
AFLA	1.470	1.313	1.512	1.985
AFLA	1.513	1.348	1.526	2.057
AFLA	1.390	1.223	1.566	1.915
AFLA	1.455	1.296	1.539	1.995
AFLA	1.380	1.218	1.552	1.890
AFLA	1.480	1.313	1.509	1.980
AFLA	1.495	1.328	1.480	1.965
AFLA	1.380	1.213	1.575	1.910
AFLA	1.390	1.232	1.486	1.830
AFLA	1.415	1.258	1.570	1.975
AFLA	1.480	1.325	1.472	1.950

**Apêndice 3.** Coloração do fígado dos frangos de corte no período imediato por abate com diferentes tempos de jejum.

<b>JEJUM</b>	<b>MICOTOXINA</b>	<b>MÉDIA L</b>	<b>MÉDIA a</b>	<b>MÉDIA b</b>
12H	CONTROLE	20.22	9.87	5.45
12H	CONTROLE	24.35	9.35	11.80
12H	CONTROLE	20.28	9.12	14.23
12H	CONTROLE	19.81	7.15	8.49
12H	CONTROLE	15.75	10.84	13.58
12H	CONTROLE	13.63	13.80	7.67
12H	CONTROLE	17.74	11.24	11.14
12H	CONTROLE	25.23	6.83	8.49
12H	CONTROLE	21.38	6.74	7.67
12H	CONTROLE	20.50	9.18	7.60
12H	CONTROLE	23.32	6.06	7.94
12H	CONTROLE	21.04	10.66	14.76
12H	CONTROLE	23.43	10.96	13.49
12H	CONTROLE	21.72	7.62	11.58
12H	CONTROLE	20.30	7.94	13.97
12H	CONTROLE	15.44	11.96	10.04
12H	CONTROLE	21.10	8.28	10.46
12H	CONTROLE	18.38	9.86	9.45
12H	CONTROLE	22.39	9.64	10.80

12H	CONTROLE	19.90	9.07	10.52
12H	AFLA	26.18	9.14	8.40
12H	AFLA	27.33	8.11	9.87
12H	AFLA	22.75	9.09	8.83
12H	AFLA	26.49	8.68	8.74
12H	AFLA	31.18	10.68	5.78
12H	AFLA	30.46	8.98	7.75
12H	AFLA	26.52	11.62	9.39
12H	AFLA	31.93	6.52	6.98
12H	AFLA	31.89	8.86	13.71
12H	AFLA	27.66	9.89	13.07
12H	AFLA	29.40	9.17	12.60
12H	AFLA	43.80	6.21	18.86
12H	AFLA	16.57	11.25	12.98
12H	AFLA	30.42	11.81	8.61
12H	AFLA	28.12	13.07	14.59
12H	AFLA	23.11	8.14	11.76
12H	AFLA	20.18	8.59	9.68
12H	AFLA	25.69	10.70	13.63
12H	AFLA	18.07	8.06	7.29
12H	AFLA	33.20	7.83	9.79
6H	CONTROLE	24.81	8.87	9.75
6H	CONTROLE	30.12	9.96	5.48
6H	CONTROLE	34.54	10.84	6.34
6H	CONTROLE	32.80	11.17	11.76
6H	CONTROLE	26.75	13.73	10.69
6H	CONTROLE	30.77	8.67	5.97
6H	CONTROLE	32.49	6.36	9.30
6H	CONTROLE	31.25	9.61	6.62
6H	CONTROLE	27.77	9.19	11.04
6H	CONTROLE	33.35	7.54	5.74
6H	CONTROLE	32.05	6.61	7.89
6H	CONTROLE	26.98	7.75	9.08
6H	CONTROLE	26.12	11.27	9.24
6H	CONTROLE	33.61	11.16	5.08
6H	CONTROLE	12.15	7.80	8.36
6H	CONTROLE	24.86	8.88	11.48
6H	CONTROLE	23.92	10.78	7.21
6H	CONTROLE	30.17	10.64	7.62
6H	CONTROLE	30.53	11.36	9.44
6H	CONTROLE	25.07	9.01	7.46
6H	AFLA	26.94	11.34	5.24
6H	AFLA	28.68	6.14	8.19
6H	AFLA	32.22	8.63	8.87
6H	AFLA	28.43	11.41	13.64

6H	AFLA	35.48	13.30	5.26
6H	AFLA	27.53	9.56	10.33
6H	AFLA	29.42	13.98	8.00
6H	AFLA	27.64	12.54	12.39
6H	AFLA	28.12	16.07	12.36
6H	AFLA	34.18	10.52	14.08
6H	AFLA	32.20	9.52	6.05
6H	AFLA	33.97	9.58	8.78
6H	AFLA	15.36	18.10	16.19
6H	AFLA	32.92	11.92	6.80
6H	AFLA	37.06	10.02	11.49
6H	AFLA	35.69	9.29	12.04
6H	AFLA	29.33	6.85	12.83
6H	AFLA	31.47	11.70	7.04
6H	AFLA	33.46	13.08	4.71
0H	CONTROLE	34.01	11.83	6.77
0H	CONTROLE	22.53	9.42	8.54
0H	CONTROLE	33.19	8.81	12.37
0H	CONTROLE	36.51	11.35	11.22
0H	CONTROLE	27.94	7.24	11.71
0H	CONTROLE	32.96	11.04	3.81
0H	CONTROLE	28.44	7.31	9.57
0H	CONTROLE	34.38	10.63	12.46
0H	CONTROLE	37.77	12.07	8.37
0H	CONTROLE	26.57	10.14	13.68
0H	CONTROLE	33.12	7.98	4.98
0H	CONTROLE	36.53	10.01	12.48
0H	CONTROLE	36.31	10.69	14.90
0H	CONTROLE	36.21	6.24	11.94
0H	CONTROLE	31.32	10.86	13.77
0H	CONTROLE	33.76	8.68	9.08
0H	CONTROLE	36.64	7.76	7.86
0H	CONTROLE	36.21	9.32	13.14
0H	CONTROLE	33.46	14.67	5.60
0H	CONTROLE	29.14	12.16	9.47
0H	AFLA	36.34	8.34	11.06
0H	AFLA	32.75	8.85	18.03
0H	AFLA	28.60	10.07	12.54
0H	AFLA	30.27	12.31	17.78
0H	AFLA	38.93	6.77	10.67
0H	AFLA	34.62	11.66	13.64
0H	AFLA	35.54	7.76	17.07
0H	AFLA	32.50	9.33	11.98
0H	AFLA	41.50	7.50	17.16
0H	AFLA	31.24	10.07	10.21

0H	AFLA	29.02	8.21	10.74
0H	AFLA	33.98	12.14	15.69
0H	AFLA	34.88	8.93	11.48
0H	AFLA	32.50	6.28	13.41
0H	AFLA	39.31	9.75	17.11
0H	AFLA	37.92	14.54	6.21
0H	AFLA	41.51	9.22	22.39
0H	AFLA	40.28	6.10	13.53
0H	AFLA	37.90	12.14	5.33
0H	AFLA	30.78	9.81	14.71

**Apêndice 4.** Pesos dos fígados com ou sem vesícula e da vesícula dos frangos de corte no período imediato por abate com diferentes tempos de jejum.

JEJUM	MICOTOXINA	C/ VESÍCULA LA g	SEM VESÍCULA g	VESÍCULA A g	% PESO FÍGADO COM VESÍCULA /PC	% PESO FÍGADO SEM VESÍCULA/ PC	% VESÍCULA/PC	% VESÍCULA/ FÍGADO
12H	CONTROLE	30.43	29.11	1.32	2.03	1.94	0.09	4.34
12H	CONTROLE	28.31	27.12	1.19	1.94	1.86	0.08	4.20
12H	CONTROLE	27.54	26.01	1.53	1.86	1.76	0.10	5.56
12H	CONTROLE	26.60	25.31	1.29	1.96	1.86	0.09	4.85
12H	CONTROLE	26.52	25.22	1.30	1.95	1.85	0.10	4.90
12H	CONTROLE	34.97	33.25	1.72	2.24	2.13	0.11	4.92
12H	CONTROLE	27.10	25.85	1.25	2.08	1.99	0.10	4.61
12H	CONTROLE	28.35	27.10	1.25	2.05	1.96	0.09	4.41
12H	CONTROLE	24.90	23.48	1.42	2.15	2.02	0.12	5.70
12H	CONTROLE	26.51	25.10	1.41	2.04	1.93	0.11	5.32
12H	CONTROLE	30.28	28.45	1.83	2.05	1.92	0.12	6.04
12H	CONTROLE	25.66	24.37	1.29	2.14	2.03	0.11	5.03
12H	CONTROLE	29.83	27.71	2.12	2.04	1.90	0.15	7.11
12H	CONTROLE	28.87	27.64	1.23	2.22	2.13	0.09	4.26
12H	CONTROLE	35.09	33.29	1.80	2.19	2.08	0.11	5.13
12H	CONTROLE	32.12	30.63	1.49	2.09	1.99	0.10	4.64
12H	CONTROLE	27.24	25.68	1.56	2.00	1.89	0.11	5.73
12H	CONTROLE	27.71	26.49	1.22	2.39	2.28	0.11	4.40
12H	CONTROLE	36.47	34.68	1.79	2.46	2.34	0.12	4.91
12H	CONTROLE	24.84	23.42	1.42	1.85	1.75	0.11	5.72
12H	AFLA	29.91	28.92	0.99	2.27	2.19	0.07	3.31
12H	AFLA	33.14	31.38	1.76	2.40	2.27	0.13	5.31
12H	AFLA	27.13	25.82	1.31	2.02	1.93	0.10	4.83
12H	AFLA	28.95	28.32	0.63	2.13	2.08	0.05	2.18
12H	AFLA	34.24	32.14	2.10	2.28	2.14	0.14	6.13
12H	AFLA	32.10	30.75	1.35	2.33	2.23	0.10	4.21



12H	AFLA	33.95	32.18	1.77	2.36	2.23	0.12	5.21
12H	AFLA	28.54	27.17	1.37	2.23	2.12	0.11	4.80
12H	AFLA	28.40	27.03	1.37	2.15	2.05	0.10	4.82
12H	AFLA	32.22	29.78	2.44	2.18	2.01	0.16	7.57
12H	AFLA	33.96	31.07	2.89	2.26	2.07	0.19	8.51
12H	AFLA	43.88	40.25	3.63	3.23	2.96	0.27	8.27
12H	AFLA	25.90	24.28	1.62	2.12	1.99	0.13	6.25
12H	AFLA	31.12	29.12	2.00	2.07	1.94	0.13	6.43
12H	AFLA	31.86	30.07	1.79	2.61	2.46	0.15	5.62
12H	AFLA	31.07	29.07	2.00	2.10	1.96	0.14	6.44
12H	AFLA	28.83	27.76	1.07	2.00	1.93	0.07	3.71
12H	AFLA	27.90	26.08	1.82	2.33	2.17	0.15	6.52
12H	AFLA	31.53	29.31	2.22	2.07	1.93	0.15	7.04
12H	AFLA	28.93	27.10	1.83	2.33	2.19	0.15	6.33
6H	CONTROLE	29.61	27.92	1.69	2.06	1.94	0.12	5.71
6H	CONTROLE	27.78	25.63	2.15	1.93	1.78	0.15	7.74
6H	CONTROLE	30.19	28.87	1.32	2.07	1.98	0.09	4.37
6H	CONTROLE	32.80	31.15	1.65	2.13	2.02	0.11	5.03
6H	CONTROLE	34.16	33.22	0.94	2.19	2.13	0.06	2.75
6H	CONTROLE	25.10	23.88	1.22	1.93	1.84	0.09	4.86
6H	CONTROLE	32.20	29.46	2.74	2.15	1.96	0.18	8.51
6H	CONTROLE	31.20	30.01	1.19	2.00	1.92	0.08	3.81
6H	CONTROLE	30.85	29.55	1.30	2.03	1.94	0.09	4.21
6H	CONTROLE	32.22	30.63	1.59	2.21	2.10	0.11	4.93
6H	CONTROLE	26.88	25.46	1.42	2.07	1.96	0.11	5.28
6H	CONTROLE	32.33	30.30	2.03	2.10	1.97	0.13	6.28
6H	CONTROLE	20.13	19.12	1.01	1.65	1.57	0.08	5.02
6H	CONTROLE	26.05	24.84	1.21	2.04	1.94	0.09	4.64
6H	CONTROLE	30.56	28.55	2.01	2.21	2.07	0.15	6.58
6H	CONTROLE	26.06	24.75	1.31	1.89	1.79	0.09	5.03
6H	CONTROLE	25.74	24.33	1.41	1.84	1.74	0.10	5.48
6H	CONTROLE	29.16	27.42	1.74	1.97	1.85	0.12	5.97
6H	CONTROLE	35.41	33.42	1.99	2.24	2.12	0.13	5.62
6H	CONTROLE	34.13	32.46	1.67	2.51	2.39	0.12	4.89
6H	AFLA	39.33	36.86	2.47	3.51	3.29	0.22	6.28
6H	AFLA	34.38	32.27	2.11	2.57	2.41	0.16	6.14
6H	AFLA	30.53	28.55	1.98	2.24	2.10	0.15	6.49
6H	AFLA	42.07	38.91	1.94	2.42	2.24	0.11	4.61
6H	AFLA	28.19	26.75	1.44	2.04	1.94	0.10	5.11
6H	AFLA	40.41	37.73	1.90	2.56	2.39	0.12	4.70
6H	AFLA	34.82	32.71	2.11	2.23	2.10	0.14	6.06
6H	AFLA	36.56	34.29	2.27	2.69	2.52	0.17	6.21
6H	AFLA	41.12	39.28	1.84	2.78	2.65	0.12	4.47
6H	AFLA	43.63	41.87	1.76	2.80	2.68	0.11	4.03
6H	AFLA	36.72	35.52	1.20	2.52	2.43	0.08	3.27

6H	AFLA	27.00	25.45	1.55	1.93	1.82	0.11	5.74
6H	AFLA	32.28	30.74	1.54	2.21	2.11	0.11	4.77
6H	AFLA	32.13	30.12	2.01	2.14	2.01	0.13	6.26
6H	AFLA	29.53	27.82	1.71	2.34	2.21	0.14	5.79
6H	AFLA	42.05	39.68	2.37	3.34	3.15	0.19	5.64
6H	AFLA	29.37	27.56	1.81	2.19	2.06	0.14	6.16
6H	AFLA	31.20	29.31	1.89	2.23	2.09	0.14	6.06
6H	AFLA	32.95	30.81	2.14	2.29	2.14	0.15	6.49
0H	CONTROLE	40.08	39.38	0.70	2.44	2.40	0.04	1.75
0H	CONTROLE	37.46	36.40	1.06	2.50	2.43	0.07	2.83
0H	CONTROLE	39.80	38.04	1.76	2.80	2.68	0.12	4.42
0H	CONTROLE	36.51	35.78	0.73	2.43	2.39	0.05	2.00
0H	CONTROLE	36.38	35.24	1.14	2.46	2.38	0.08	3.13
0H	CONTROLE	32.60	31.79	0.81	2.43	2.37	0.06	2.48
0H	CONTROLE	34.00	32.97	1.03	2.43	2.36	0.07	3.03
0H	CONTROLE	35.66	35.29	0.37	2.51	2.49	0.03	1.04
0H	CONTROLE	35.03	34.03	1.00	2.50	2.43	0.07	2.85
0H	CONTROLE	34.01	33.03	0.98	2.21	2.14	0.06	2.88
0H	CONTROLE	33.80	32.84	0.96	2.35	2.28	0.07	2.84
0H	CONTROLE	39.35	38.48	0.87	2.46	2.41	0.05	2.21
0H	CONTROLE	41.45	40.47	0.98	2.62	2.56	0.06	2.36
0H	CONTROLE	35.48	34.12	1.36	2.46	2.37	0.09	3.83
0H	CONTROLE	35.73	35.09	0.64	2.23	2.19	0.04	1.79
0H	CONTROLE	33.75	33.22	0.53	2.60	2.56	0.04	1.57
0H	CONTROLE	37.68	37.09	0.59	2.45	2.41	0.04	1.57
0H	CONTROLE	42.56	41.41	1.15	2.84	2.76	0.08	2.70
0H	CONTROLE	36.96	36.07	0.89	2.50	2.44	0.06	2.41
0H	CONTROLE	35.86	35.42	0.44	2.21	2.19	0.03	1.23
0H	AFLA	38.00	36.91	1.09	2.32	2.25	0.07	2.87
0H	AFLA	41.74	40.24	1.50	2.86	2.76	0.10	3.59
0H	AFLA	35.45	34.77	0.68	2.40	2.35	0.05	1.92
0H	AFLA	38.92	37.88	1.04	2.49	2.43	0.07	2.67
0H	AFLA	46.34	44.88	1.46	3.17	3.07	0.10	3.15
0H	AFLA	41.22	40.26	0.96	2.86	2.80	0.07	2.33
0H	AFLA	40.52	39.10	1.42	2.85	2.75	0.10	3.50
0H	AFLA	39.68	39.01	0.67	2.39	2.35	0.04	1.69
0H	AFLA	42.98	41.52	1.46	2.72	2.63	0.09	3.40
0H	AFLA	36.19	35.15	1.04	2.45	2.38	0.07	2.87
0H	AFLA	36.02	35.28	0.74	2.54	2.48	0.05	2.05
0H	AFLA	51.85	51.11	0.74	3.24	3.19	0.05	1.43
0H	AFLA	43.31	42.51	0.80	2.52	2.47	0.05	1.85
0H	AFLA	34.48	33.56	0.92	2.30	2.24	0.06	2.67
0H	AFLA	53.44	52.36	1.08	3.99	3.91	0.08	2.02
0H	AFLA	37.26	36.29	0.97	2.62	2.56	0.07	2.60
0H	AFLA	44.21	43.10	1.11	2.95	2.87	0.07	2.51

OH	AFLA	38.46	37.62	0.84	2.32	2.27	0.05	2.18
OH	AFLA	40.11	39.17	0.94	2.75	2.68	0.06	2.34
OH	AFLA	30.40	30.05	0.35	2.05	2.03	0.02	1.15

**Apêndice 5.** Quantidade de lipídio dos fígados dos frangos de corte no período imediato por abate com diferentes tempos de jejum.

JEJUM	TRT	Gordura(g) Inteiro	Gordura% Inteiro
0	AFLs	2.99	7.21
0	AFLs	1.70	4.35
0	AFLs	1.23	3.15
0	AFLs	2.20	5.12
0	AFLs	1.53	4.07
0	AFLs	0.93	2.07
0	AFLs	4.70	8.97
0	AFLs	1.83	5.19
0	AFLs	1.90	5.16
0	AFLs	1.95	4.84
0	AFLs	1.26	3.59
0	AFLs	0.93	3.09
0	AFLs	0.99	2.62
0	AFLs	1.25	3.74
0	AFLs	2.13	4.16
0	AFLs	1.36	3.33
0	AFLs	1.64	4.05
6	CONT	1.47	5.00
6	CONT	1.38	5.68
6	CONT	0.82	2.79
6	CONT	1.62	4.83
6	CONT	0.89	2.97
6	CONT	1.11	4.05
6	CONT	0.77	4.00
6	CONT	1.43	4.41
6	CONT	1.42	4.64
6	CONT	0.86	3.59
6	CONT	1.09	4.39
6	CONT	0.69	2.27
6	CONT	1.01	3.95
6	CONT	0.99	3.88
6	CONT	1.43	5.12
6	CONT	1.01	3.54

12	AFLs	0.66	2.34
12	AFLs	0.92	2.87
12	AFLs	1.77	6.54
12	AFLs	1.53	5.23
12	AFLs	1.95	6.55
12	AFLs	1.31	4.83
12	AFLs	1.40	4.37
12	AFLs	1.53	6.28
12	AFLs	1.54	5.00
12	AFLs	0.76	2.63
12	AFLs	1.65	5.32
12	AFLs	0.72	2.49
12	AFLs	1.90	6.33
12	AFLs	0.83	3.23
12	AFLs	0.45	1.63
12	AFLs	0.98	3.36
12	AFLs	0.85	3.12
12	AFLs	1.19	3.80
0	CONT	1.60	4.52
0	CONT	1.10	2.67
0	CONT	0.91	2.59
0	CONT	1.41	4.13
0	CONT	1.08	3.02
0	CONT	1.20	3.77
0	CONT	3.63	8.96
0	CONT	0.79	2.08
0	CONT	1.02	3.11
0	CONT	0.73	1.84
0	CONT	1.29	3.35
0	CONT	1.32	3.63
0	CONT	0.78	2.37
0	CONT	0.88	2.38
0	CONT	1.80	5.28
0	CONT	1.06	2.94
6	AFLs	1.90	5.54
6	AFLs	1.13	4.10
6	AFLs	1.18	3.01
6	AFLs	1.29	4.65
6	AFLs	0.86	3.37
6	AFLs	1.36	4.20
6	AFLs	1.66	3.97
6	AFLs	0.96	3.10
6	AFLs	1.69	5.15

6	AFLs	1.20	3.92
6	AFLs	1.05	2.79
6	AFLs	1.62	4.57
6	AFLs	1.20	3.25
6	AFLs	1.45	5.42
6	AFLs	0.85	2.98
12	CONT	0.88	3.42
12	CONT	1.24	4.85
12	CONT	1.43	4.11
12	CONT	0.48	1.77
12	CONT	1.38	3.96
12	CONT	0.67	2.64
12	CONT	1.27	3.80
12	CONT	0.62	2.40
12	CONT	0.88	3.49
12	CONT	0.81	2.94
12	CONT	1.08	4.59
12	CONT	1.52	6.23
12	CONT	0.84	3.10
12	CONT	0.60	2.57
12	CONT	0.88	3.19
12	CONT	1.12	3.94
12	CONT	1.16	3.97
12	CONT	1.28	3.84
12	CONT	0.73	2.38
12	CONT	0.40	1.58

## 7. VITA

Elisa Piccinin François, filha de Inez Ieda Piccinin François e Delmar François, nascida em 03 de junho de 1978, em Porto Alegre – RS. Completou o ensino médio no Colégio La Salle Nossa Senhora das Dores, localizado na cidade de Porto Alegre - RS em 1995. Em 1998, ingressou no curso de Medicina Veterinária na Universidade Federal do Rio Grande do Sul. No último semestre da faculdade foi Estagiária Nível Superior na Sadia S.A (atual BRF), em Chapeco SC, na área de produção de perus, sob supervisão de Nadir Jose Cervelin. Formou-se Médica Veterinária em janeiro de 2004 onde obteve Prêmio de Excelência em Zootecnia pelo desempenho acadêmico na área de Produção Animal. Após o estágio, continuou na empresa Sadia no programa de Trainees onde iniciou a vida profissional. Ao longo da vida profissional trabalhou em várias agroindústrias como Doux Frangosul, Seara e JBS. Em 2013 obteve diploma de Mestre em Economia pela UFRGS e no início de 2018 ingressou no programa de mestrado em Zootecnia sob orientação do professor PhD. Sergio Luiz Vieira. Foi submetida à banca de defesa de Dissertação em março de 2020.